

1 Turkey B cell Transcriptome Profile During Turkey

2 Hemorrhagic Enteritis Virus (THEV) Infection Highlights

3 Upregulated Apoptosis and Breakdown Pathways That May

4 Mediate Immunosuppression

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6 Abraham Quaye, Brett E. Pickett, Joel S. Griffitts, Bradford K. Berges, Brian D. Poole\*

7 Department of Microbiology and Molecular Biology, Brigham Young University

8 \*Corresponding Author

9 **Corresponding Author Information**

10 brian\_poole@byu.edu

11 Department of Microbiology and Molecular Biology,

12 4007 Life Sciences Building (LSB),

13 Brigham Young University,

14 Provo, Utah

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16 **Abstract**

17 Infection with Turkey Hemorrhagic Enteritis Virus (THEV) can cause hemorrhagic enteritis, which affects  
18 young turkeys. This disease is characterized by bloody diarrhea and immunosuppression (IMS), which is  
19 attributed to apoptosis of infected B cells. Secondary infections due to IMS exacerbates economic losses.  
20 We performed the first transcriptomic analysis of a THEV infection to elucidate the mechanisms mediat-  
21 ing THEV-induced IMS. After infecting and sequencing mRNAs of a turkey B-cell line, trimmed reads were  
22 mapped to the host turkey genome and gene expression was quantified with StringTie. Differential gene  
23 expression analysis was followed by functional enrichment analyses using gprofiler2 and DAVID from NCBI.  
24 RT-qPCR of select genes was performed to validate the RNA-seq data. A total of 2,343 and 3,295 differ-  
25 entially expressed genes (DEGs) were identified at 12-hpi and 24-hpi, respectively. The DEGs correlated  
26 with multiple biological processes including apoptosis, ER unfolded protein response, and cell maintenance.  
27 Multiple pro-apoptotic genes, including *APAF1*, *BMF*, *BAK1*, and *FAS* were upregulated. Genes that play  
28 a role in ER stress-induced unfolded protein response including *VCP*, *UFD1*, *EDEM1*, and *ATF4* were also  
29 upregulated and may contribute to apoptosis. Our data suggest that several biological processes and path-  
30 ways including apoptosis and ER response to stress are important aspects of the host cell response to  
31 THEV infection. It is possible that interplay between multiple processes may mediate apoptosis of infected  
32 B-cells, leading to IMS.

33 **Keywords**

34 Turkey hemorrhagic enteritis virus (THEV), Adenovirus, RNA sequencing, Apoptosis, Immunosuppression,  
35 ER stress, B cell.

36 **1. Introduction**

37 Turkey hemorrhagic enteritis virus (THEV) belongs to genus *Siadenovirus*, family *Adenoviridae*, and in-  
38 fects turkeys, chickens, and pheasants (1, 2). THEV is transmitted via the fecal-oral route and causes  
39 hemorrhagic enteritis (HE) in turkeys, a debilitating disease affecting predominantly 6-12-week-old turkey  
40 pouls characterized by immunosuppression (IMS), lack of vitality, splenomegaly, intestinal lesions leading  
41 to bloody diarrhea, and up to 80% mortality (3-6). The clinical disease usually persists in affected flocks for  
42 7-10 days, causing death and economic losses. However, secondary bacterial infections may extend the  
43 duration of illness and increase mortality for an additional 2-3 weeks due to the immunosuppressive nature  
44 of the virus, exacerbating the economic losses (5, 7). Naturally occurring low pathogenic (avirulent) strains  
45 of THEV have been isolated; these strains cause subclinical infections but retain the immunosuppressive  
46 effects. Since its isolation from a pheasant spleen, the Virginia Avirulent Strain (VAS) has been effectively  
47 used as a live vaccine despite the immunosuppressive side-effects. However, the vaccinated birds are  
48 rendered more susceptible to opportunistic infections and death than unvaccinated cohorts (4, 5, 8-10).

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

65 Bulk mRNA sequencing (RNA-seq), a next generation sequencing approach to transcriptomic studies, is a  
66 versatile, high throughput, and cost-effective technology that allows a broad survey of the entire transcrip-  
67 tome of a cell population, thereby uncovering the active genes and molecular pathways and processes.

68 This technology has been leveraged in an ever-increasing number of studies to elucidate active cellular  
69 processes under a wide range of treatment conditions, including viral infections (12–16). In RNA-seq stud-  
70 ies, differentially expressed genes (DEGs) identified by contrasting pairs of different experimental conditions  
71 are key to unlocking the interesting biology or mechanism. Identified DEGs are typically used for functional  
72 enrichment analyses in large curated knowledgebases such as gene ontology (GO) and Kyoto Encyclo-  
73 pedia of Genes and Genomes (KEGG) pathways which connect genes to specific biological processes,  
74 functions, and pathways, shedding light on the biological question under study (17, 18).

75 To our knowledge, no study has used RNA-seq to elucidate the molecular mechanisms and pathways  
76 leading to THEV-induced IMS. To effectively counteract the immunosuppressive effect of the vaccine, it is  
77 essential to unravel the host cell processes/pathways influenced by the virus to bring about IMS. In this  
78 study, we present the first transcriptomic profile of THEV-infected cells using paired-end bulk RNA-seq in  
79 a turkey B-cell line (MDTC-RP19), highlighting key host genes, cellular/molecular processes and pathways  
80 affected during a THEV time course infection. We specifically focus on cellular processes related to cell  
81 survivability that can elucidate THEV-induced IMS.

82 **2. Materials and Methods**

83 **Cell culture and Infection**

84 The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete  
85 Leibovitz's L-15/McCoy's 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5%  
86 tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100 $\mu$ g/mL Strepto-  
87 mycin), at 41°C in a humidified atmosphere with 5% CO<sub>2</sub>. Infected cells were maintained in 1:1 serum-  
88 reduced Leibovitz's L15/McCoy's 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic  
89 solution. A commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain).  
90 The stock virus was titrated using an in-house qPCR assay with titer expressed as genome copy number  
91 (GCN)/mL, similar to Mahshoub *et al* (19). Cells were THEV-infected or mock-infected in triplicates or du-  
92 plicates, respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41°C for 1 hour, and  
93 washed three times with phosphate buffered saline (PBS) to remove unattached virus particles. At each  
94 time point (4-, 12-, 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were  
95 harvested for total RNA extraction.

96 **RNA Extraction and Sequencing**

97 Total RNA was extracted from infected cells using the ThermoFisher RNaseous™-4PCR Total RNA Iso-  
98 lation Kit (which includes a DNase I digestion step) per manufacturer's instructions. Agarose gel elec-  
99 trophoresis was performed to check RNA integrity (data not shown). The RNA quantity and purity was  
100 initially assessed using Nanodrop, and RNA was used only if the A260/A280 ratio was 2.0 ± 0.05 and the  
101 A260/A230 ratio was >2 and <2.2. Extracted total RNA samples were sent to LC Sciences, Houston TX for  
102 poly-A-tailed mRNA sequencing. RNA integrity was checked with Agilent Technologies 2100 Bioanalyzer  
103 High Sensitivity DNA Chip and samples with an RNA integrity number (RIN) < 7 were excluded. Poly(A)  
104 RNA-seq library was prepared following Illumina's TruSeq-stranded-mRNA sample preparation protocol.  
105 Paired-end sequencing, generating 149 bp reads was performed on the Illumina NovaSeq 6000 sequenc-  
106 ing system. The paired-end 149 bp sequences obtained during this study and all expression data have  
107 been submitted to the Gene Expression Omnibus database, under accession no GSE286211

108 **Quality Control and Mapping Process**

109 Sequencing reads were processed following a well-established protocol described by Pertea *et al* (20),  
110 using Snakemake - version 7.32.4 (21), a popular workflow management system to drive the pipeline.

111 Briefly, raw sequencing reads were trimmed with Cutadapt - version 1.10 (22) and the quality of trimmed  
112 reads evaluated using the FastQC software, version 0.12.1 (Bioinformatics Group at the Babraham  
113 Institute, Cambridge, United Kingdom; [www.bioinformatics.babraham.ac.uk](http://www.bioinformatics.babraham.ac.uk)), achieving an overall Mean  
114 Sequence Quality (PHRED Score) of 36. Trimmed reads were mapped the reference turkey (*Meleagris gallopavo*) genome file GCF\_000146605.3\_Turkey\_5.1\_genomic.fna.gz from NCBI (genome build:  
115 melGal5) ([https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF\\_000146605.3\\_Turkey\\_5.1/](https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/))  
116 with Hisat2 - version 2.2.1 (20) using the accompanying gene transfer format (GTF) annotation file  
117 (GCF\_000146605.3\_Turkey\_5.1\_genomic.gtf.gz) to build a genomic index. Samtools - version 1.21 was  
118 used to convert the output Sequence Alignment Map (SAM) file to the Binary Alignment Map (BAM)  
119 format. The StringTie (v2.2.1) software (20), set to expression estimation mode was used to generate  
120 normalized gene expression estimates from the BAM files for genes in the reference GTF file after which  
121 the prepDE.py3 script was used to extract read count information from the StringTie gene expression files,  
122 providing an expression-count matrix for downstream DEG analysis.

#### 124 **DEG Analysis and Functional Enrichment Analysis**

125 DEG analysis between mock- and THEV-infected samples was performed using DESeq2 (23), which em-  
126 ploys a Negative Binomial distribution model for determining statistical significance when comparing read  
127 counts from multiple replicates. Genes with (FDR)-adjusted P-value  $\leq 0.05$  were considered as differen-  
128 tially expressed. The sequencing data (FASTQ files), expression-count matrix, and DEG analysis results  
129 from DESeq2 are deposited at NCBI Gene Expression Omnibus under accession number GSE286211. The  
130 functional profiling of DEGs (GO and KEGG analyses) were performed based on GO databases and KEGG  
131 databases using DAVID and the R package gprofiler2 (24) with *M. gallopavo* as the reference organism.  
132 Results with P<sub>adjusted</sub>-value  $\leq 0.05$  were included as functionally enriched. All visualization plots were made  
133 using ggplot2, pheatmap, and ggvenn R packages (25–27).

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

135 The gene expression levels of representative DEGs (*APAF1*, *BMF*, *FADD*, *PDCD4*, *MADD*, *VCP*, *UFD1*,  
136 *EDEM1*, *EIF3D*, *EIF3M*, *RPL8*, *RPL10A*) were validated by quantification of relative mRNA levels with  
137 turkey *GAPDH* mRNA levels as the control gene. Briefly, the cells were infected and RNA extracted as  
138 described for the RNA sequencing samples with three biological replicates at 12- and 24-hpi each for both  
139 THEV-infected or mock-infected samples. First-strand cDNA synthesis of total RNA was performed with an  
140 oligo-dT primer to amplify poly-A-tailed mRNA using SuperScript™ IV First-Strand Synthesis System. The

141 parent RNA was digested using RNase H after cDNA synthesis was complete to ensure that only cDNA  
142 remained as the template for the RT-qPCR quantification. The RT-qPCR was performed with the PowerUp™  
143 SYBR™ Green master mix from Applied Biosystems with primers designed manually in the SnapGene  
144 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**  
145 **Table S1.** Relative mRNA levels were calculated by  $2^{-\Delta\Delta CT}$  method (28).

147 **Statistical Analysis**

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

151 **3. Results**

152 **3.1.1 Sequencing Results**

153 To identify the host transcriptomic profile during THEV infection, we infected MDTC-RP19 cells with THEV  
154 or no virus (mock) in triplicates or duplicates, respectively, and harvested at total RNA at 4-, 12-, 24-, and  
155 72-hours post infection (hpi). mRNAs extracted from mock- or THEV-infected cells were sequenced on the  
156 Illumina platform, yielding a total of **776.1** million raw reads (149 bp in length) across all samples (see **Table**  
157 **1** for sequencing statistics). After trimming low-quality reads, the remaining **742.8** million total paired-end  
158 trimmed reads (approximately, **34.7-47.9** million reads per sample) were mapped to the reference genome  
159 of *M. gallopoavo* obtained from the National Center for Biotechnology Information (NCBI). The percentage of  
160 reads that mapped to the host genome across all samples ranged from **32.4** to **89.2%**. We observed that  
161 the fraction of reads that mapped to the host genome decreased while those mapping to the virus genome  
162 increased over the course of the infection as the viral infectious cycle progressed. Despite excellent quality  
163 scores at all time points (**Table 1**), DEGs identified at 4- and 72-hpi did not yield any statistically significant  
164 results in the downstream functional enrichment analyses (GO term and KEGG pathway analysis) and they  
165 were excluded from all subsequent analyses. In the remaining 12- and 24-hpi samples, a high consistency  
166 was observed between biological replicates (**Figure 2A** and **B**).

167 **3.1.2 DEGs of THEV-infected Versus Mock-infected Cells**

168 We quantified gene expression levels with the StringTie software (20) in Fragments per kilobase of transcript  
169 per million (FPKM) units. The differential expression analysis of DEGs was performed with the DESeq2 R  
170 package (23) which employs a negative binomial distribution model for determining statistical significance.  
171 Using a false discovery rate (FDR)-adjusted P-value cutoff  $\leq 0.05$  as the inclusion criteria, **2,343** and **3,295**  
172 genes from THEV-infected samples were identified as differentially expressed relative to their time-matched  
173 mock-infected samples at 12-hpi and 24-hpi, respectively. The results from the DEG analyses at 12- and  
174 24-hpi have been deposited in NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under  
175 accession number GSE286211 with files named total\_12hrsDEGs.csv.gz and total\_24hrsDEGs.csv.gz, re-  
176 spectively. We compared THEV-infected samples relative to their time-matched mock-infected samples in  
177 identifying the significant DEGs and in the functional enrichment analyses. At 12-hpi (THEV-infected ver-  
178 sus mock-infected), we found **1,079** upregulated genes and **1,264** downregulated genes, whereas **1,512**  
179 genes were upregulated and **1,783** genes downregulated at 24-hpi (THEV-infected versus mock-infected)  
180 (**Figure 2C**, and **Figure 3A-C**). The log<sub>2</sub>fold-change (FC) values at 12-hpi ranged between **-1.4** and **+1.7**  
181 for **TMEM156** (Transmembrane Protein 156) and **LIPG** (Lipase G), respectively. At 24-hpi, the log<sub>2</sub>FC val-  
182 ues ranged between **-2.0** and **+2.6** for **C1QTNF12** (C1q And TNF Related 12) and **KCNG1** (Potassium

183 Voltage-Gated Channel Modifier Subfamily G Member 1), respectively.

184 **3.1.3 Functional Enrichment Analyses (GO and KEGG pathway Analyses)**

185 Gene ontology (GO) enrichment analysis was performed for the DEGs determined at the 12- and 24-hpi  
186 timepoints with the DAVID (Database for Annotation, Visualization and Integrated Discovery; version 2021)  
187 online resource (29) and the gprofiler2 R package – version **0.2.3** (24), which outputs results according  
188 to the three branches of the GO directed acyclic graph – cellular components (CP), biological processes  
189 (BP), and molecular functions (MF). We compared THEV-infected samples relative to their time-matched  
190 mock-infected samples for each timepoint. Results with  $P_{adjusted}$ -value  $\leq 0.05$  were considered functionally  
191 enriched. The GO enrichment analyses results at 12-hpi and 24-hpi showed significant overlaps among  
192 all three GO categories. At both time points, cellular breakdown processes were upregulated while cellular  
193 maintenance processes and structures were downregulated in all three GO categories (**Table 2A-B** and  
194 **Table 3A-B**).

195 For upregulated DEGs at 12-hpi, we observed that the GO terms annotated under the BP category broadly  
196 cluster into: apoptosis and autophagy, cellular metabolism (catabolic processes), sterol biosynthesis, re-  
197 sponse to stimuli, and protein processing (**Figure 4A** and **Table 2A**). In the CC category, the GO terms  
198 relate primarily with cytoplasmic vacuolation, while in the MF category, they broadly fit under protein  
199 binding and kinase activity (**Table 2A**). For downregulated DEGs at 12 hpi, the GO terms in BP cate-  
200 gory generally fell under translation, protein biosynthesis and folding, ribosome biogenesis, nitrogen com-  
201 pound metabolism, nucleic acid synthesis, repair, metabolism, processing, and replication, and energy  
202 metabolism. Also, immunoglobulin production and isotype switching were downregulated (**Figure 4C** and  
203 **Table 2B**). In the CC category GO terms broadly grouped into ribosome, mitochondria, respirosome, nu-  
204 cleus, and spliceosome, while in the MF category, they generally belong to translation regulator activity,  
205 protein folding chaperone, catalytic activity (acting on a nucleic acids), and ATP hydrolysis activity (**Table**  
206 **2B**).

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

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

221 KEGG pathway analysis on the DEGs was also performed using both the gprofiler2 R package (24) and the  
222 DAVID online resource. Both resources gave similar results, but the results from DAVID (**Table 4**) included  
223 more information than the gprofiler2 results (**Table S2**). The results from the KEGG pathway analysis  
224 were consistent with the GO results, revealing that generally, cell maintenance and upkeep pathways were  
225 downregulated while cell death and breakdown pathways were upregulated. We observed that cell main-  
226 tenance pathways such as DNA replication and repair, ribosome biogenesis, spliceosome, and oxidative  
227 phosphorylation were downregulated at both 12- and 24-hpi. Pathways such as: autophagy, response to  
228 virus (Influenza A), and steroid biosynthesis were upregulated at 12-hpi, which is similar to 24-hpi, where  
229 pathways such as: autophagy, ubiquitin-mediated proteolysis, lysosome, protein processing in endoplasmic  
230 reticulum, and steroid biosynthesis were upregulated.

231 It is well-established that THEV induces cell death (apoptosis and necrosis) in infected B-cells, which is  
232 linked to THEV-induced IMS (8, 11, 30). Hence, we were particularly interested in cellular processes and  
233 pathways associated with cell death and pathways that may affect the survival of the host B-cells, thereby  
234 accounting for THEV-induced IMS. We highlight the upregulated cell death (apoptosis and autophagy),  
235 ubiquitin-dependent endoplasmic reticulum [ER]-mediated protein degradation, and suppressed cell main-  
236 tenance pathways as well as cytokine deregulation identified by our GO and KEGG analyses as the likely  
237 key aspects of THEV-host cell interaction relevant to THEV-induced IMS.

### 238 **3.1.4 Cell Death and Breakdown Pathways Upregulated by THEV**

239 Many virus families, including adenoviruses, herpesviruses, poxviruses, baculoviruses, parvoviruses, retro-  
240 viruses, rhabdoviruses, paramyxoviruses, orthomyxoviruses, togaviruses, and picornaviruses are known  
241 to trigger apoptosis in infected host cells either through direct viral protein action or the host antiviral re-  
242 sponse (31–33). Our data show that apoptotic and autophagic pathways are upregulated during THEV  
243 infection, supporting previous findings of apoptosis and necrosis of THEV-infected cells (8, 11, 30). For  
244 example, several proapoptotic members of the BCL2 (B-cell lymphoma 2) protein family such as BCL2  
245 antagonist/killer 1 (*BAK1*), BCL2 interacting protein 3 like (*BNIP3L*), BCL2 interacting protein 3 (*BNIP3*),  
246 and Bcl2 modifying factor (*BMF*) were upregulated. Additionally, Fas cell surface death receptor (*FAS*),

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

### 260 **3.1.5 Downregulation of Cell Maintenance Pathways**

261 Forcibly transitioning the host cell cycle to the S phase during the early phase of infection is a prerequisite  
262 for a productive adenovirus infection (34). Interaction of the viral E1A early proteins with the host pRb  
263 (retinoblastoma) protein releases the host transcription factor E2F, which activates genes required for S  
264 phase cell cycle induction. Viral E1A also binds the host transcriptional co-activator p300/CBP (34, 35).  
265 Our GO and KEGG pathway results showed that at 12-hpi, several key genes involved with cell cycle  
266 transition were upregulated. Notably, E1A binding protein p300 (*EP300*), cyclin genes (*CCND3*, *CCNG1*,  
267 *CCNG2*, *CDK6*), anaphase promoting complex subunit 1 (*ANAPC1*), and cell division cycle 27 (*CDC27*)  
268 were upregulated. However, unlike the observation in Mastadenoviruses, the cell cycle regulation at 12- and  
269 24-hpi seem complicated as some key cell cycle-related genes and general cell maintenance processes  
270 were concurrently downregulated.

271 We found that several essential cell maintenance processes whose suppression can trigger apoptosis were  
272 downregulated. Severe DNA damage is a known mechanism of apoptosis induction, called DNA damage-  
273 dependent apoptosis (36). Repression of host RNA and protein synthesis is also strongly associated with  
274 apoptosis (37). Several processes related to DNA and RNA synthesis, maintenance, and repair such as nu-  
275 cleotide biosynthesis and metabolism, double strand break repair, DNA excision repair, RNA biosynthesis,  
276 RNA processing, DNA replication, mitotic cell cycle process, protein-RNA complex organization, and DNA  
277 damage response were downregulated at both timepoints. Notable genes identified include DNA ligase 1  
278 (*LIG1*), X-ray repair cross complementing 1 (*XRCC1*), cyclin dependent kinase 1 and 2 (*CDK1*, *CDK2*),

279 checkpoint kinase 1 (*CHEK1*), 8-oxoguanine DNA glycosylase (*OGG1*), BLM RecQ-like-helicase (*BLM*),  
280 BRCA1 DNA repair associated (*BRCA1*), and several RAD family proteins (*RAD21*, *RAD51*, *RAD51B*,  
281 *RAD51C*, *RAD54B*).

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

### 289 **3.1.6 Endoplasmic Reticulum (ER) Stress Response during THEV infection**

290 Our KEGG pathway analysis (**Table 4**) showed that protein processing in the ER, and ubiquitin-mediated  
291 proteolysis are significantly upregulated (**Figure 5**). The GO results (**Table 3A**) showed that specifically,  
292 ER stress and the ER-associated protein degradation (ERAD) pathway, a branch of the unfolded protein  
293 response (UPR) were upregulated during THEV infection, especially at 24-hpi. The ER is the major site for  
294 protein synthesis, folding and quality control, and sorting (38). Upon ER stress or continued accumulation  
295 of unfolded proteins in the ER lumen, the UPR pathways are activated to restore ER homeostasis. The  
296 ERAD pathway, a ubiquitin-proteasome-dependent pathway, is a protein quality control system activated for  
297 degradation of misfolded and unassembled proteins (38). In our results, the THEV-infected samples showed  
298 significant increase in ERAD pathway effector proteins, such as valosin containing protein (*VCP*), ubiquitin  
299 recognition factor in ER associated degradation 1 (*UFD1*), ER degradation enhancing alpha-mannosidase  
300 like proteins 1 and 3 (*EDEM1*, *EDEM3*), cullin 1 (*CUL1*), and ubiquilin 1 (*UBQLN1*). Other genes related to  
301 other UPR pathways such as *HSPA5* and *ATF4* were also upregulated. Our KEGG pathway (**Table S2**) and  
302 GO (**Figure 4B**) results indicated a significant upregulation of ubiquitin-mediated proteolysis with other ubiq-  
303 uitination pathway proteins such as ubiquitin conjugating enzymes (*UBE2J2*, *UBE2E3*, *UBE2Z*), ubiquitin  
304 protein ligases (*UBE3A*, *UBE3B*), NPL4 homolog ubiquitin recognition factor (*NPLOC4*), and ubiquitin-like  
305 modifier activating enzyme 6 (*UBA6*) showing significant upregulation. Additionally, the heat shock family of  
306 chaperone proteins such as the DnaJ heat shock protein family (*HSP40*) members (*DNAJB11*, *DNAJB12*,  
307 *DNAJB2*, *DNAJC10*), heat shock protein family A (*HSP70*) members (*HSPA4L*, *HSPA5*, *HSPA8*), and heat  
308 shock protein 90 alpha family class A member 1 (*HSP90AA1*) were upregulated at 24-hpi. Moreover, the  
309 KEGG pathway analysis (**Table 4**) shows a significant upregulation in lysosome formation, lumen acidifi-  
310 cation, and lysosomal degradation, likely an indication of ER-to-lysosome-associated degradation. Taken

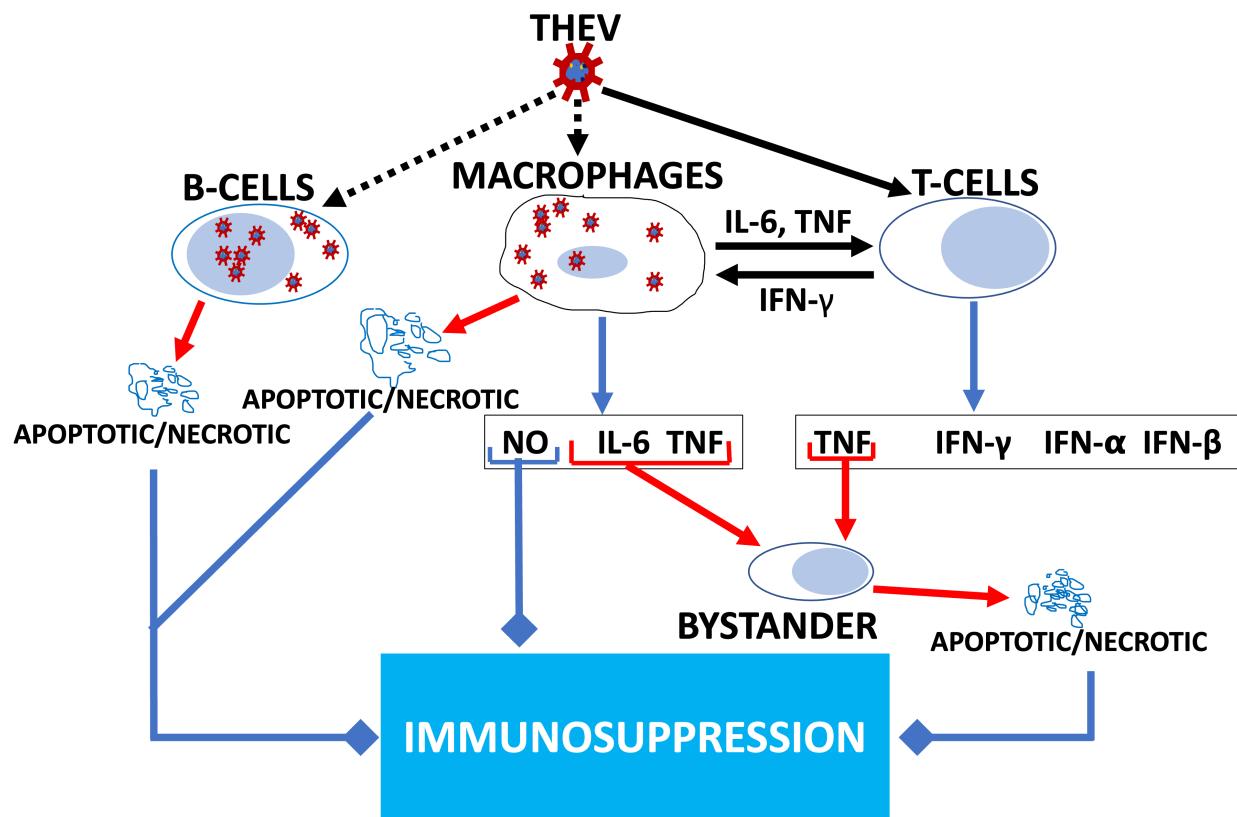
311 together, these results suggest that THEV infection triggers significant ER-associated protein degradation,  
312 which may contribute to cell death and IMS.

313 **3.1.7 Differential Expression of Cytokine and Cytokine Receptor-encoding Genes**

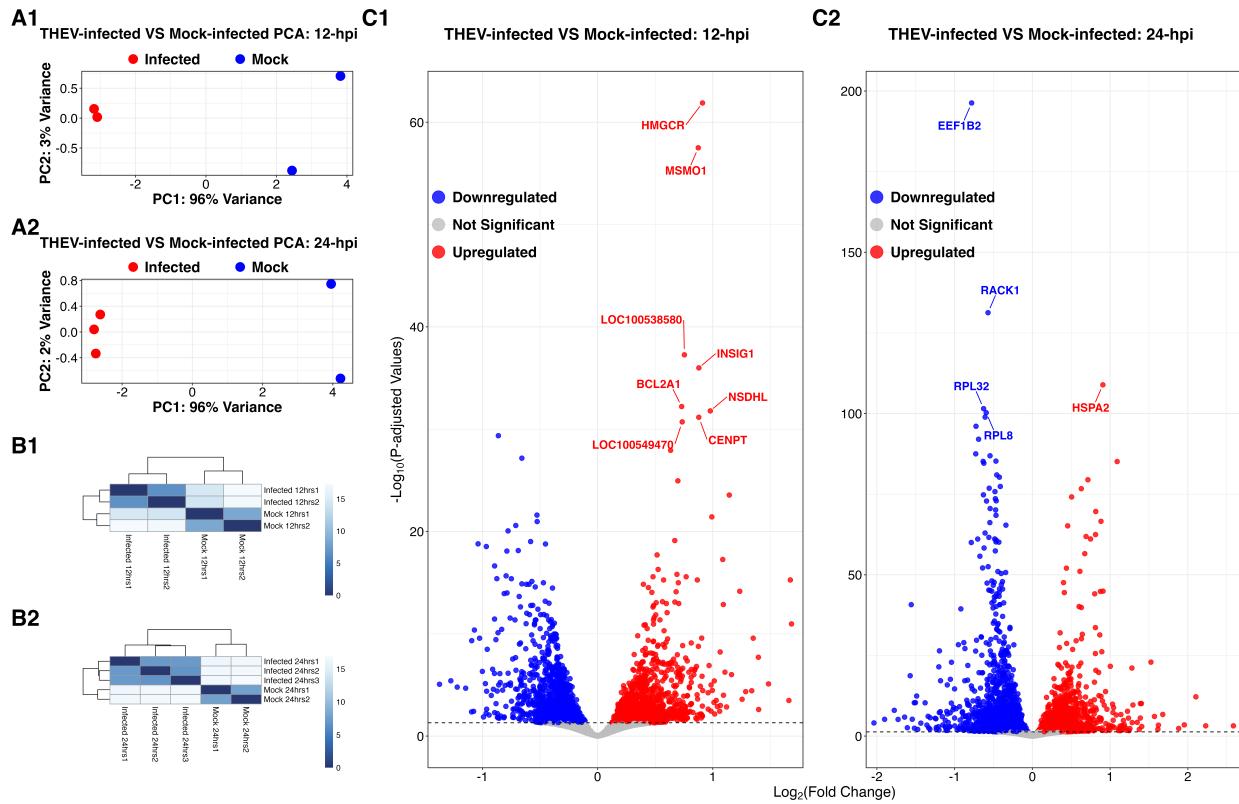
314 Our KEGG pathway results showed that a pathway similar to immune response to influenza A infection  
315 was upregulated at 12-hpi. Our GO analysis also identified terms such as regulation of lymphocyte activa-  
316 tion and regulation of cytokine production as upregulated at both 12- and 24-hpi. Genes involved include  
317 *IL18*, *IL2RB*, *IL4R*, *IL5RA*, TNF receptor associated factors (*TRAF2*, *TRAF3*, *TRAF6*, *TRAF7*, *TRAFD1*),  
318 TNF receptor superfamily members (*TNFRSF1B*, *TNFRSF8*, *TNFSF4*), interferon-induced with helicase  
319 C domain 1 (*IFIH1*), interferon-induced double-stranded RNA-activated protein kinase (*PKR*), and *CD80*.  
320 In contrast, cytokine inhibitors such as suppressor of cytokine signaling (*SOCS3* and *SOCS5*) were also  
321 upregulated at both 12 and 24-hpi and immunoglobulin production and isotype switching GO terms were  
322 downregulated at 12-hpi. This inconsistency is likely an indicator of the struggle between the virus and its  
323 host. While several cytokines were regulated by THEV as in the proposed model of THEV immunopatho-  
324 genesis (**Figure 1**), the cytokines in the model (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  TNF, and IL-6) were not significantly  
325 differentially expressed in our data. However, some of the differentially expressed cytokines and cytokine  
326 receptors (*TNFRSF8*, *TRAF7*) identified in this study are positive regulators of apoptosis; therefore, they  
327 may play a role in THEV-induced IMS.

328 **3.1.8 Validation of DEGs by Reverse Transcriptase Quantitative PCR (RT-qPCR)**

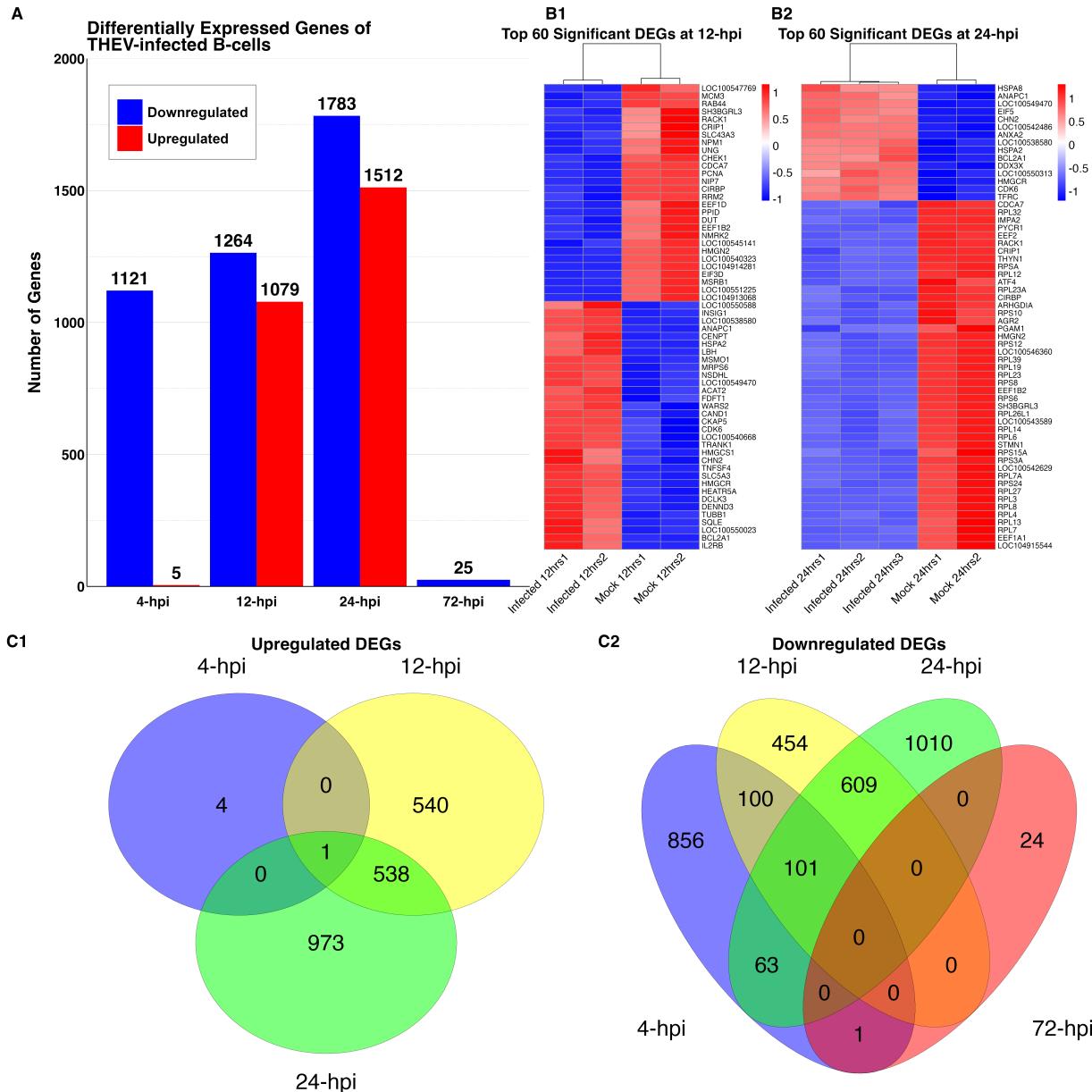
329 To validate the RNA-seq results, 12 DEGs (8 upregulated and 4 downregulated) were selected for RT-  
330 qPCR. The DEGs were representative of apoptosis (*APAF1*, *BMF*, *FADD*, *MADD*, and *PDCD4*), ERAD and  
331 ubiquitination (*VCP*, *UFD1*, *EDEM1*), and ribosome biosynthetic (*EIF3D*, *EIF3M*, *RPL8*, *RPL10A*) pathways.  
332 As shown in **Figure 6**, the RT-qPCR results corroborate the RNA-seq results, further reinforcing the validity  
333 of the RNA-seq transcriptomic profile results. Although there was no inconsistency between the RNA-  
334 seq and RT-qPCR results in terms of gene regulations, the fold changes in the RT-qPCR results were  
335 consistently higher than observed in the RNA-seq results. Our RT-qPCR primers showed excellent target  
336 specificity; only one amplicon of the expected size was amplified as shown by the melt curves (data not  
337 shown) and gel electrophoresis (**Figure S1**). According to our Student's T-test and Mann-Whitney U test,  
338 the difference in gene expression levels in all the selected genes were statistically significant.



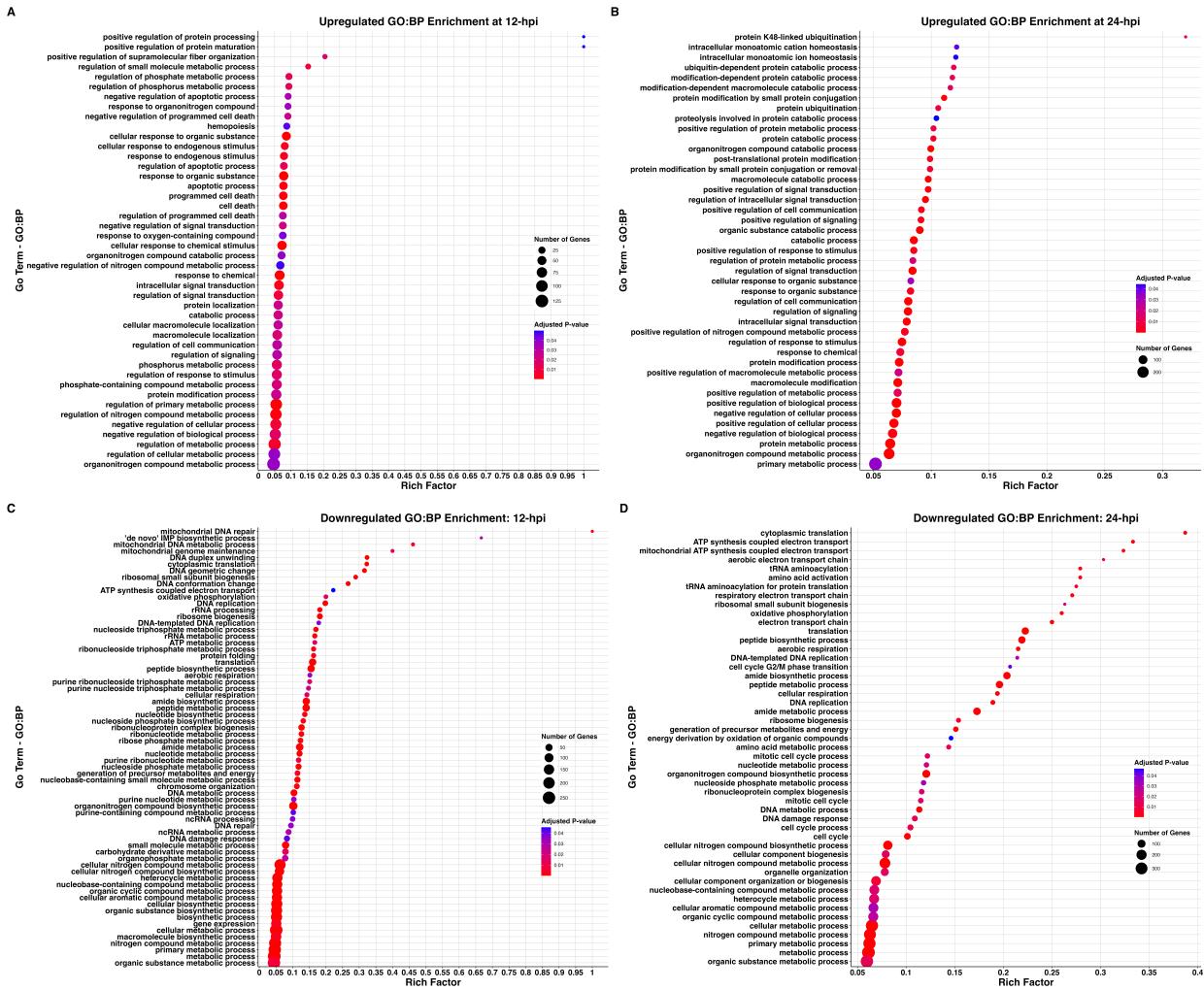
**Figure 1.** Model of THEV-induced immunosuppression in turkeys. THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicate signals leading to cell death (apoptosis/necrosis). Blue arrows indicate all cytokines released by the cell. Blue arrows with square heads indicated 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**).

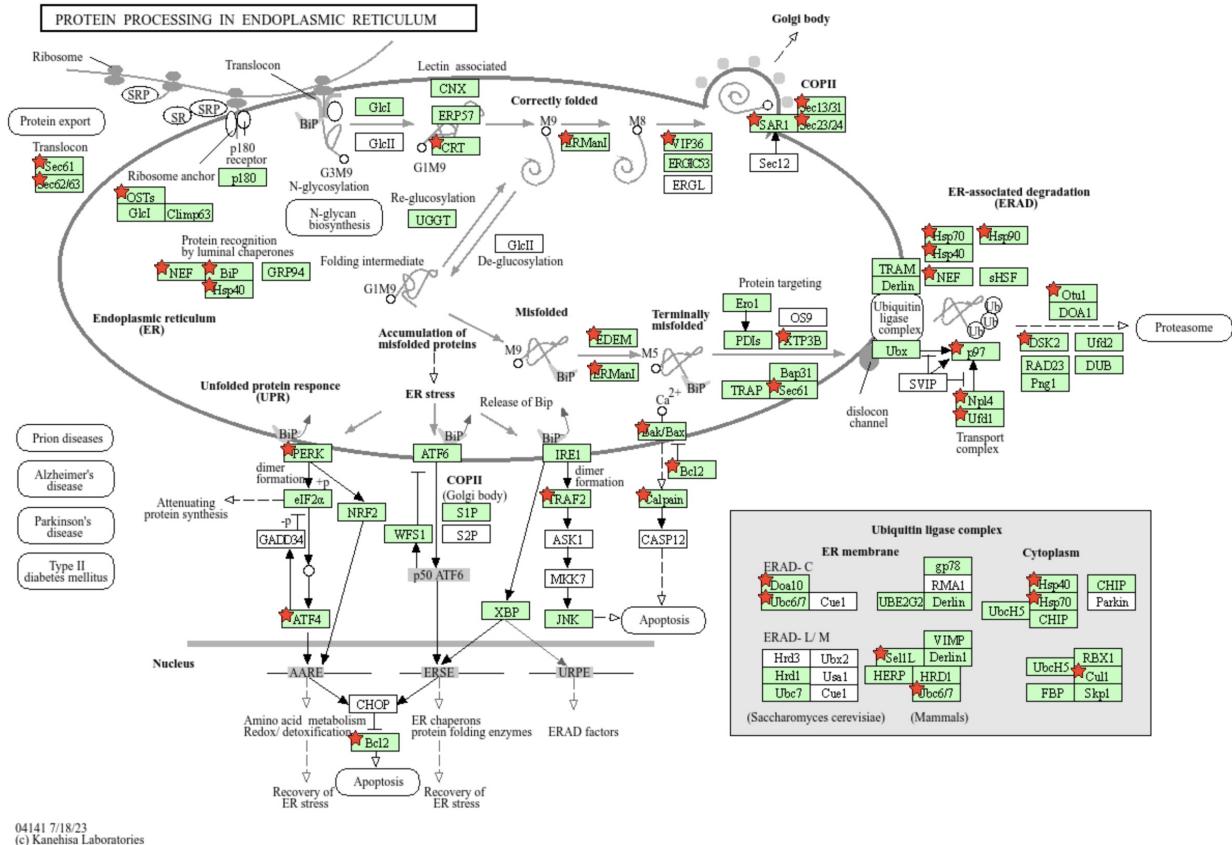


**Figure 3.** DEGs of THEV-infected versus mock-infected samples at different time points. **(A)** Bar plot of number DEGs identified. Red represents upregulated genes and blue represents downregulated genes. **(B)** Heatmaps of scaled expression data (Z-scores) of DEGs. DEGs identified at 12-hpi are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



Results from gprofiler2 R package

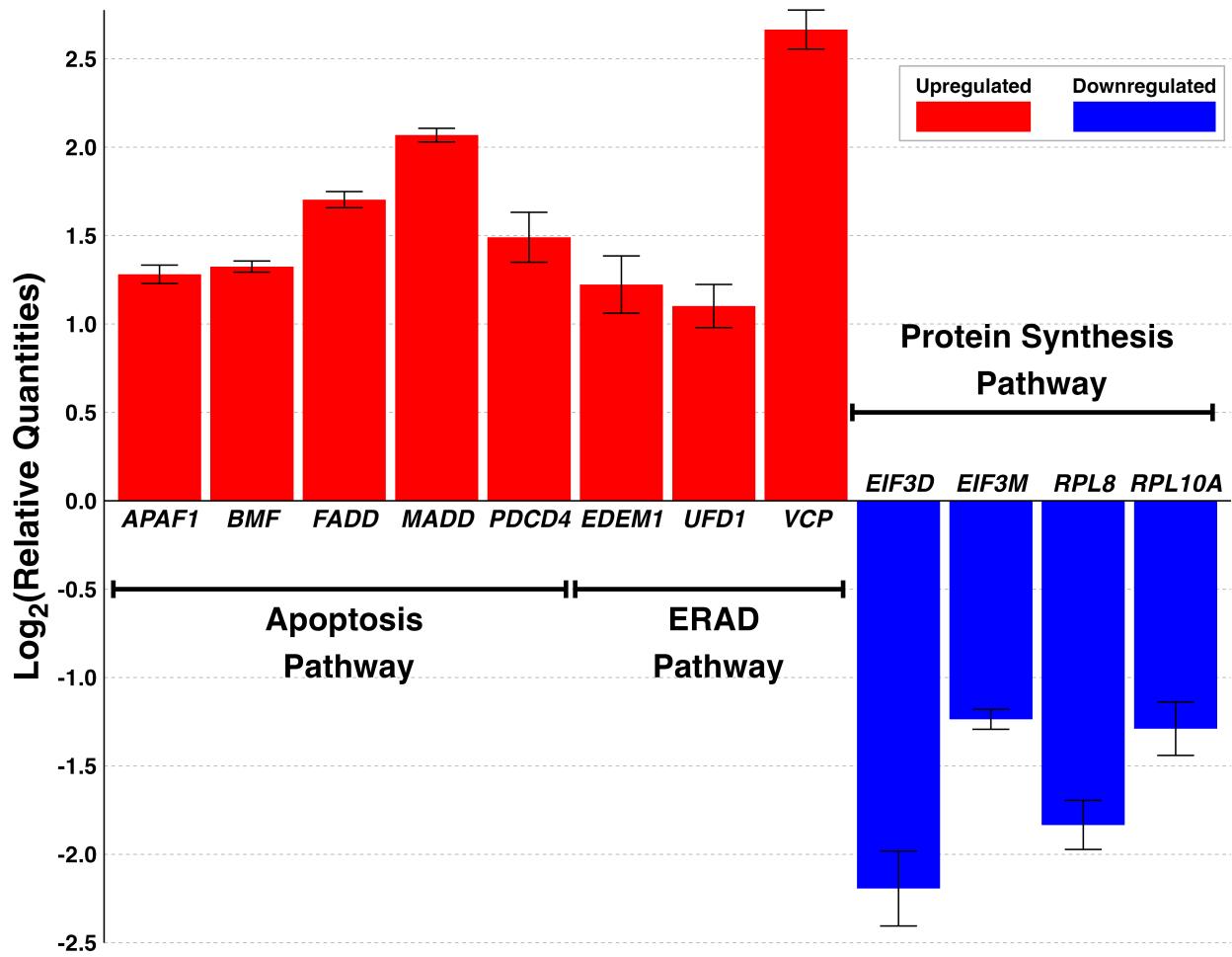
**Figure 4.** Dotplot of Enriched Gene Ontology Biological Processes (BP). Significant BP GO terms identified for upregulated DEGs at 12-hpi and 24-hpi are shown in (A) and (B), respectively. Significant BP GO terms for downregulated DEGs at 12-hpi and 24-hpi are shown in (C) and (D), respectively. The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



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(c) Kanehisa Laboratories

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

## RT-qPCR Validation of Select DEGs



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

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

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

Sample	Raw Reads <sup>M</sup>	Trimmed Reads <sup>M</sup>	Mapped Reads <sup>M</sup>	Uniquely Mapped Reads <sup>M</sup>	Non-uniquely Mapped Reads <sup>M</sup>	Q20%	Q30%	GC Content (%)
U_72hrsN2 <sup>Mk</sup>	40.5	38.9	34.5 (88.82%)	32.7 (84.14%)	1.8 (4.68%)	99.95	97.04	46.5

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

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

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

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	cellular component disassembly	2.46	21	1.31e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	embryo development	1.93	29	3.43e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	limb development	4.20	9	3.40e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	negative regulation of cellular biosynthetic process	1.74	68	8.52e-04
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	phosphorus metabolic process	1.63	146	1.11e-06
GO:BP	phosphorylation	1.80	74	1.96e-04
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	protein localization	1.58	104	4.21e-04
GO:BP	protein localization to organelle	1.90	52	1.15e-03
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	transport	1.24	183	3.18e-02
GO:BP	vesicle-mediated transport	1.51	80	9.41e-03
<b>Cellular Component</b>				
GO:CC	Golgi apparatus	1.52	69	1.40e-02
GO:CC	bounding membrane of organelle	1.70	92	3.49e-05
GO:CC	chromatin	1.84	40	9.58e-03
GO:CC	chromosome	1.58	63	1.10e-02
GO:CC	cytoplasm	1.28	590	7.06e-17
GO:CC	cytoplasmic vesicle	1.57	88	1.12e-03
GO:CC	cytoplasmic vesicle membrane	1.69	41	2.85e-02
GO:CC	cytosol	1.69	166	6.96e-10
GO:CC	early endosome	2.11	22	3.70e-02
GO:CC	endomembrane system	1.48	200	3.53e-07
GO:CC	endoplasmic reticulum	1.54	86	2.07e-03
GO:CC	endosome	1.69	48	1.37e-02
GO:CC	endosome membrane	2.02	25	3.21e-02
GO:CC	intracellular anatomical structure	1.19	774	8.23e-20
GO:CC	intracellular membrane-bounded organelle	1.29	578	1.08e-16
GO:CC	intracellular organelle	1.23	655	7.52e-16
GO:CC	intracellular organelle lumen	1.51	135	4.91e-05

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:CC	intracellular vesicle	1.55	88	1.62e-03
GO:CC	membrane-bounded organelle	1.26	595	7.52e-16
GO:CC	membrane-enclosed lumen	1.51	135	4.91e-05
GO:CC	nuclear lumen	1.55	119	6.06e-05
GO:CC	nucleoplasm	1.75	104	1.57e-06
GO:CC	nucleus	1.42	371	1.33e-13
GO:CC	organelle	1.21	666	2.49e-14
GO:CC	organelle lumen	1.51	135	4.91e-05
GO:CC	organelle membrane	1.59	154	3.53e-07
GO:CC	organelle subcompartment	1.56	65	1.21e-02
GO:CC	perinuclear region of cytoplasm	2.51	25	2.11e-03
GO:CC	phagophore assembly site	4.73	8	2.83e-02
GO:CC	protein-DNA complex	1.76	42	1.40e-02
GO:CC	spindle	2.04	25	2.85e-02
GO:CC	transcription regulator complex	1.99	30	1.40e-02
GO:CC	vacuole	1.82	34	2.73e-02
GO:CC	vesicle	1.51	95	1.94e-03
GO:CC	vesicle membrane	1.74	43	1.40e-02
<b>Molecular Function</b>				
GO:MF	ATP binding	1.33	128	2.71e-02

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:MF	protein kinase activity	1.59	67	1.20e-02
GO:MF	protein kinase binding	2.10	31	1.18e-02
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)
<b>Biological Process</b>				
GO:BP	'de novo' AMP biosynthetic process	10.16	5	2.26e-02
GO:BP	'de novo' IMP biosynthetic process	11.43	5	1.51e-02
GO:BP	'de novo' XMP biosynthetic process	18.29	4	1.71e-02
GO:BP	'de novo' post-translational protein folding	8.31	10	6.05e-05
GO:BP	'de novo' protein folding	7.95	10	8.96e-05
GO:BP	AMP biosynthetic process	7.84	6	1.71e-02
GO:BP	ATP biosynthetic process	6.58	9	1.26e-03
GO:BP	ATP metabolic process	4.29	19	1.86e-05
GO:BP	ATP synthesis coupled electron transport	6.10	18	1.95e-07
GO:BP	DNA damage response	2.35	69	7.26e-09
GO:BP	DNA geometric change	4.33	9	2.22e-02
GO:BP	DNA integrity checkpoint signaling	3.75	16	6.72e-04
GO:BP	DNA metabolic process	2.74	82	3.18e-14
GO:BP	DNA recombination	2.07	23	3.64e-02
GO:BP	DNA repair	2.39	51	1.44e-06
GO:BP	DNA replication	4.95	39	2.28e-14
GO:BP	DNA replication checkpoint signaling	6.36	8	5.00e-03
GO:BP	DNA replication initiation	5.23	8	1.71e-02

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	aerobic electron transport chain	5.97	16	2.15e-06
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	chromosome organization	2.15	38	6.51e-04
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	macromolecule biosynthetic process	2.69	313	8.24e-63
GO:BP	macromolecule metabolic process	1.79	466	6.20e-45
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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
<b>Cellular Component</b>				
GO:CC	90S preribosome	8.05	20	1.61e-11
GO:CC	Arp2/3 protein complex	7.89	5	2.33e-02
GO:CC	Ctf18 RFC-like complex	15.77	5	1.30e-03
GO:CC	DNA replication preinitiation complex	9.46	5	1.18e-02
GO:CC	INO80-type complex	5.68	6	2.68e-02
GO:CC	Ino80 complex	7.89	5	2.33e-02
GO:CC	MCM complex	7.28	5	3.08e-02
GO:CC	Sm-like protein family complex	3.57	13	2.69e-03
GO:CC	U2-type prespliceosome	6.31	6	1.77e-02
GO:CC	U2-type spliceosomal complex	4.32	13	4.48e-04
GO:CC	catalytic complex	1.63	136	2.38e-07
GO:CC	catalytic step 2 spliceosome	3.40	14	2.49e-03
GO:CC	chaperonin-containing T-complex	7.36	7	2.67e-03
GO:CC	chromatin	1.64	41	2.10e-02

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:CC	spliceosomal snRNP complex	3.86	11	4.98e-03
GO:CC	spliceosomal tri-snRNP complex	4.88	8	9.58e-03
GO:CC	translation preinitiation complex	13.10	9	1.03e-06
<b>Molecular Function</b>				
GO:MF	ATP hydrolysis activity	1.81	38	1.92e-02
GO:MF	ATP-dependent activity, acting on DNA	2.75	20	4.94e-03
GO: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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)
<b>Biological Process</b>				
GO:BP	ERAD pathway	6.28	14	1.77e-05
GO:BP	alcohol biosynthetic process	3.22	22	3.23e-04
GO:BP	autophagy	2.33	28	3.55e-03
GO:BP	biosynthetic process	1.21	252	1.72e-02
GO:BP	carbohydrate derivative metabolic process	1.47	84	1.58e-02
GO:BP	catabolic process	1.71	165	3.58e-09
GO:BP	cell death	1.73	42	2.50e-02
GO:BP	cellular biosynthetic process	1.24	220	1.58e-02
GO:BP	cellular catabolic process	1.76	64	1.02e-03
GO:BP	cellular homeostasis	1.64	45	3.99e-02
GO:BP	cellular lipid biosynthetic process	7.89	8	1.81e-03
GO:BP	cellular lipid metabolic process	1.60	87	1.20e-03
GO:BP	cellular localization	1.44	196	1.35e-05
GO:BP	cellular macromolecule localization	1.51	135	1.34e-04
GO:BP	cellular metabolic process	1.25	422	7.24e-06
GO:BP	cellular response to stress	1.69	105	1.87e-05
GO:BP	cellular response to topologically incorrect protein	2.87	13	4.82e-02
GO:BP	chaperone-mediated protein folding	3.70	10	3.73e-02

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (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
<b>Molecular Function</b>				
GO:MF	acyltransferase activity	1.63	72	7.45e-03
GO:MF	binding	1.07	924	1.02e-02
GO:MF	catalytic activity	1.16	522	1.47e-03
GO:MF	catalytic activity, acting on a protein	1.28	230	4.88e-03
GO:MF	enzyme binding	1.95	120	2.10e-09
GO:MF	identical protein binding	2.17	75	2.02e-07
GO:MF	kinase binding	2.08	45	1.47e-03
GO:MF	lipid binding	1.57	62	4.54e-02
GO:MF	manganese ion binding	4.71	10	2.12e-02
GO:MF	misfolded protein binding	7.40	10	9.76e-04

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

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	catabolic process	1.30	138	2.31e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	chromatin remodeling	2.07	37	1.70e-03
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	import into the mitochondrion	3.14	13	1.89e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	mitochondrial translation	3.64	14	2.78e-03
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	nucleic acid metabolic process	2.02	241	3.92e-25
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	oxidative phosphorylation	6.34	30	2.57e-14
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	purine nucleoside triphosphate biosynthetic process	4.29	13	1.08e-03
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	regulation of G2/M transition of mitotic cell cycle	3.44	15	2.78e-03
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	ribonucleoside diphosphate catabolic process	3.38	11	2.75e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:BP	tRNA metabolic process	2.28	34	5.83e-04
GO:BP	tetrahydrofolate metabolic process	6.24	6	3.75e-02
GO:BP	translation	5.77	136	3.59e-65
GO:BP	translational elongation	4.77	12	8.57e-04
GO:BP	translational initiation	5.34	15	1.59e-05
GO:BP	tricarboxylic acid cycle	5.34	15	1.59e-05
<b>Cellular Component</b>				
GO:CC	Arp2/3 protein complex	6.92	6	1.02e-02
GO:CC	Sm-like protein family complex	3.21	16	1.16e-03
GO:CC	U1 snRNP	5.10	7	1.67e-02
GO:CC	U12-type spliceosomal complex	4.62	7	2.88e-02
GO:CC	U2 snRNP	5.54	8	3.72e-03
GO:CC	U2-type spliceosomal complex	3.64	15	5.33e-04
GO:CC	U4 snRNP	10.39	6	1.12e-03
GO:CC	U5 snRNP	7.55	6	6.68e-03
GO:CC	aminoacyl-tRNA synthetase multienzyme complex	8.08	7	1.12e-03
GO:CC	catalytic complex	1.64	188	1.31e-10
GO:CC	catalytic step 2 spliceosome	3.02	17	1.37e-03
GO:CC	centrosome	1.78	55	5.33e-04
GO:CC	chromatin	1.69	58	1.18e-03

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:CC	chromosomal region	2.85	41	6.00e-08
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:CC	intracellular organelle	1.29	1,087	1.05e-42
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:CC	mitochondrion	2.39	235	6.18e-36
GO:CC	mitotic spindle	2.16	17	4.58e-02
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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
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: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

GO Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
<b>Molecular Function</b>				
GO:MF	ATP-dependent activity, acting on DNA	2.32	23	1.60e-02
GO:MF	NAD binding	3.78	17	5.51e-04
GO:MF	NAD <sup>+</sup> 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 Category	GO:Term	Fold Enrichment	Number of DEGs	P-value (Adjusted)
GO:MF	nucleoside phosphate binding	1.25	236	9.04e-03
GO:MF	nucleotide binding	1.25	236	9.04e-03
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 4: 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 4: 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

340 **4. Discussion**

341 THEV has a worldwide distribution, wreaking economic havoc on affected poultry farms, particularly due  
342 to its immunosuppressive trait allowing secondary opportunistic infections to devastate turkey populations  
343 (4, 6). HE in turkeys causes more economic losses than any disease caused in other birds like chicken  
344 or pheasants (4). While the current vaccine strain (VAS) has proven effective at preventing clinical HE in  
345 turkey poult, the retention of its immunosuppressive properties leaves some of the issues associated with  
346 economic losses unresolved. Elucidating the virus-host interactions leading to IMS is most pressing for not  
347 only the understanding of the viral infection and pathogenesis but also future antiviral therapy targets. Since  
348 both virulent and avirulent THEV cause IMS but the avirulent are used as vaccine, we believe that studying  
349 VAS would be more expedient for understanding THEV vaccine-induced IMS.

350 Only one cell line (MDTC-RP19 also known as RP19) has been found to be capable of supporting THEV  
351 replication (39). Thus, in this work, we establish the first transcriptomic profile of THEV infection in RP19  
352 cells using paired-end RNA-seq. We attempted a multi-time point experimental design but this being the  
353 first transcriptomic study of THEV infection, we faced some difficulties, including selecting our sampling time  
354 points based on the only study of THEV gene expression kinetics (40), leading to only 12- and 24-hpi provid-  
355 ing useful data. In total **2,343** and **3,295** DEGs were identified at 12-hpi and 24-hpi, respectively. At 12-hpi,  
356 **1,079** genes were upregulated and **1,264** genes downregulated, whereas **1,512** genes were upregulated  
357 and **1,783** genes downregulated at 24-hpi. Being a non-model organism, a significant proportion of the host  
358 (*M. gallopavo*) genes are not annotated and not recognized by the databases used for functional enrichment  
359 analysis. Thus, the obtained results are likely sub-optimal in amount of detail relative to results from well an-  
360 notated and curated genomes of model organisms. The DEGs were related to multiple biological processes  
361 all potentially playing a role in THEV infection but the most relevant to our study are apoptosis (upregulated),  
362 ER stress-induced unfolded protein response (upregulated), cell maintenance processes (downregulated),  
363 and cytokine functions (deregulated). Furthermore, the RT-qPCR results validated the RNA-seq results.  
364 Collectively, this study may shed light on some significant aspects of THEV-host interactions, which may  
365 benefit further mechanistic delineation of the viral infection and induction of IMS and inform future develop-  
366 ment of anti-THEV strategies. The biological processes most relevant to THEV-induced IMS highlighted by  
367 this study are further discussed below.

368 Apoptosis is a key defense mechanism activated by cells in response to irreversible injury and virus infection  
369 to abrogate virus propagation. It is a formidable cellular defense network, non-specific to any virus family  
370 and therefore an important problem for any infecting virus to overcome (31–33). The adenovirus E1A pro-  
371 teins are strong inducers of apoptosis. They bind host pRb and p300/CBP protein, inducing p53-mediated

372 apoptosis, and can also sensitize infected cells to TNF $\alpha$  and TRAIL-induced apoptosis (34, 35). However,  
373 adenoviruses have developed multiple distinct anti-apoptotic mechanisms to counter almost all cellular pro-  
374 apoptotic programs. For example, E1A blocks its own induction of p53-dependent apoptosis and E1B  
375 proteins (E1B-19K and E1B-55K) counteract several types of apoptosis including TNF-induced apoptosis  
376 (34, 35). Despite the rich arsenal of countermeasures, transcriptomic studies of human adenovirus infec-  
377 tions suggest a complex set of virus-host interactions where both pro- and anti-apoptotic genes are turned  
378 on contemporaneously. For example, in human adenovirus 2 infection, both pro- and anti-apoptotic BCL2  
379 family genes were stimulated (34). Siadenoviruses including THEV are the smallest adenoviruses and  
380 therefore encode the fewest genes (10, 41). THEV encodes a mere 34 ORFs with no anti-apoptotic genes  
381 characterized (41). In agreement with these findings, in our results a strong signal indicative of apoptotic  
382 induction was observed. However, like Mastadenovirus infections, a complex relationship between pro and  
383 anti-apoptotic genes were observed. Pro-apoptotic genes such as *APAF1*, *BNIP3L*, *BMF*, *BAK1*, *RIPK1*,  
384 *FAS*, *FADD* and *ATF* were upregulated in concert with the anti-apoptotic genes *BCL2*, *BNIP2*, *BCL2A1*  
385 and *API5*. We speculate that this complex regulation is predictive of THEV possessing some anti-apoptotic  
386 genes but not sufficiently potent to thwart the cellular apoptotic response. It is also possible that using a  
387 naturally attenuated strain may account for the apparent balance in pro- and anti-apoptotic signals. Inter-  
388 estingly, pro-apoptotic genes in both intrinsic and extrinsic pathways were upregulated, possibly due to a  
389 concurrent stimulation of multiple apoptotic pathways or a positive feedback mechanism of one system acti-  
390 vating the other. The specific mechanism of apoptosis induction remains elusive. Further studies designed  
391 to elucidate these fine details are warranted and would benefit future THEV therapeutics.

392 The ER serves many specialized functions including biosynthesis and assembly of membrane and secre-  
393 tory proteins, calcium storage, and biosynthesis of lipids and sterols. It is also the site of protein folding  
394 and post-translational modifications and maintains stringent quality control systems, ensuring exported pro-  
395 teins are correctly folded and degradation of unfolded or misfolded proteins (16, 38, 42). Disruption of ER  
396 homeostasis or ER stress leads to accumulation of incorrect proteins in the ER lumen, triggering the UPR.  
397 The UPR restores ER normality by transiently attenuating general protein synthesis, increasing the luminal  
398 folding capacity, and the degradation of misfolded proteins through the ERAD pathway or autophagy (16,  
399 38, 42, 43). However, if incorrect luminal protein overload persists, the prolonged UPR will induce apop-  
400 tosis known as ER stress-associated programmed cell death (42, 43). Many DNA and RNA viruses are  
401 reported to induce ER stress and UPR pathways during infection (16). In our results, *ATF4* and PKR-like  
402 ER protein kinase (*PERK*), key proteins in the *PERK* branch of the UPR pathway were upregulated. A myr-  
403 iad of ERAD pathway proteins (e.g., *VCP*, *UFD1*, *EDEM1*, *EDEM3*, *CUL1*, *UBQLN1*), ubiquitination system

404 proteins (e.g., *UBE2J2*, *UBE2E3*, *UBE2Z*, *UBE3A*, *UBE3B*), and heat shock family of chaperone proteins  
405 (e.g., *HSPA5*, *HSP4L*, *HSPA8*, *HSP90AA1*) all showed increased expression according to our RNA-seq  
406 data with some validated with RT-qPCR. These data strongly suggest that THEV infection triggers the ER  
407 UPR pathways leading to a massive decrease of protein synthesis and deregulation of sterol biosynthesis,  
408 and ubiquitin-mediated proteolysis, all supported by our results. As noted above, a prolonged UPR activa-  
409 tion leads to ER stress-associated programmed cell death via genes such *ATF4* (42, 43). Thus, we suggest  
410 that the ER stress response likely plays a crucial role in THEV-induced IMS. Nonetheless, the mechanisms  
411 underlying the regulation of the UPR pathways by THEV remain to be clearly unraveled. Also, whether  
412 and how the ER stress response affects THEV infection and pathogenicity should be studied. Unsurpris-  
413 ingly, protein degradation was more evident at 24-hpi than at 12-hpi, reflecting the suggested two phases of  
414 UPR – Phase 1 allows the unfolded proteins time to refold without degradation and Phase 2 degrades any  
415 proteins which have failed to fold (43).

416 In the proposed model of THEV immunopathogenesis by Rautenschlein *et al* (**Figure 1**), while THEV di-  
417 rectly induced cell death in infected cells, cytokines are responsible for extending cell death to bystander  
418 splenocytes (8). However, the primary cytokines (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$  TNF, IL-6, and NO) highlighted in  
419 the model were not significantly differentially expressed in our data. This may be explained by the fact  
420 that the model was proposed based on data from splenocytes of THEV-infected turkeys, which have the  
421 full complement of immune cells (T-cells, B-cells, macrophages) shown in the model and not solely from  
422 B-cell culture data as in this study. From the model, T-cells and macrophages are the principal producers  
423 of the effector cytokines; thus, there is agreement with our data that B-cells alone would poorly recapitulate  
424 the cytokine communication network. This may also explain the very few immune-associated biological  
425 processes in our data as the B-cells may require cytokines from other immune cells such as macrophages  
426 and T-cells for optimal activation. Further transcriptomic studies with splenocytes would offer a wealth of  
427 insights regarding these ideas. It also likely that cytokines may only play a dominant role in some aspects of  
428 THEV-infection such as the clinical hemorrhage of the intestines but not the associated IMS since a study  
429 using the TNF-blocking drug (thalidomide) only prevented intestinal disease, not IMS (8). While some of the  
430 upregulated cytokines and receptors in our results are positive apoptosis regulators (*TNFRSF8*, *TRAF7*),  
431 most of the cytokines are either anti-apoptotic (*TNFRSF1B*, *TRAF2*), boost host antiviral defense (*IL18*,  
432 *TNFSF4*, *PKR*, *TRAFFD1*, *IFIH1*), or suppress cytokine signaling (*SOCS3*, *SOCS5*). Therefore, we specu-  
433 late that a non-cytokine-mediated apoptotic process such as ER stress-associated programmed cell death  
434 is more likely to mediate direct killing of infected cells. However, whether bystander cell death occurs and if  
435 it is cytokine-mediated as suggested by Rautenschlein *et al* are important questions that can be addressed

436 with future transcriptomic studies in splenocytes.

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

438 separated by the onset of viral DNA replication (34, 35). Based on DNA microarray analysis, adenovirus

439 type 2 (Ad2) infection has been divided into four stages. The first period is from 0 to 12-hpi during which

440 changes in cellular gene expression are likely to be triggered by the viral entry process. Most of the dereg-

441 ulated genes have functions linked to inhibition of cell growth. Therefore, growth suppression is most likely

442 the first response of the host cell to the incoming virus (34). The second period covers the time from

443 12 to 24-hpi and follows activation of the immediate early E1A gene, which forcibly transition cell cycle to

444 S phase (34). While the temporal changes of host gene expression for a THEV infection have not been

445 characterized in prior studies, our data suggest that during the first 24-hpi, cell growth was suppressed.

446 Cell maintenance processes involving nucleic acid and proteins were downregulated according to our data.

447 Protein synthesis-related processes including ribosome biogenesis, rRNA processing, ribosome assem-

448 bly, protein folding, translational initiation, protein maturation, and others were heavily affected. Additionally,

449 DNA and RNA synthesis, maintenance, and repair such as nucleotide biosynthesis and metabolism, double

450 strand break repair, and DNA excision repair were also repressed. As severe DNA damage leads to DNA

451 damage-dependent apoptosis (36) and repression of RNA and protein synthesis is also strongly associated

452 with apoptosis (37), these inhibitions may also play a role in THEV-induced IMS. Moreover, we speculate

453 that the ER UPR may contribute partly to the severe repression of protein synthesis as discussed above.

454 An in-depth study of temporal changes of host gene expression during THEV infection would be invaluable

455 in establishing if THEV follows the same pattern as Ad2.

## 456 5. Conclusions

457 THEV-induced IMS is a pressing concern for turkey farmers worldwide, causing substantial economic losses

458 annually. In this study, we establish the cellular transcriptomic profile of THEV infection in turkey RP19 B-

459 cells using paired-end RNA-seq, identifying **1,079** upregulated genes and **1,264** downregulated genes at

460 12-hpi and **1,512** upregulated genes and **1,783** downregulated genes at 24-hpi. Our data suggest that

461 several biological processes and pathways including apoptosis, immune response, ER response to stress,

462 ubiquitin-dependent proteolysis, and repression of essential cellular maintenance are significant aspects of

463 host cell response to THEV infection. All these processes are established apoptosis inducing mechanisms;

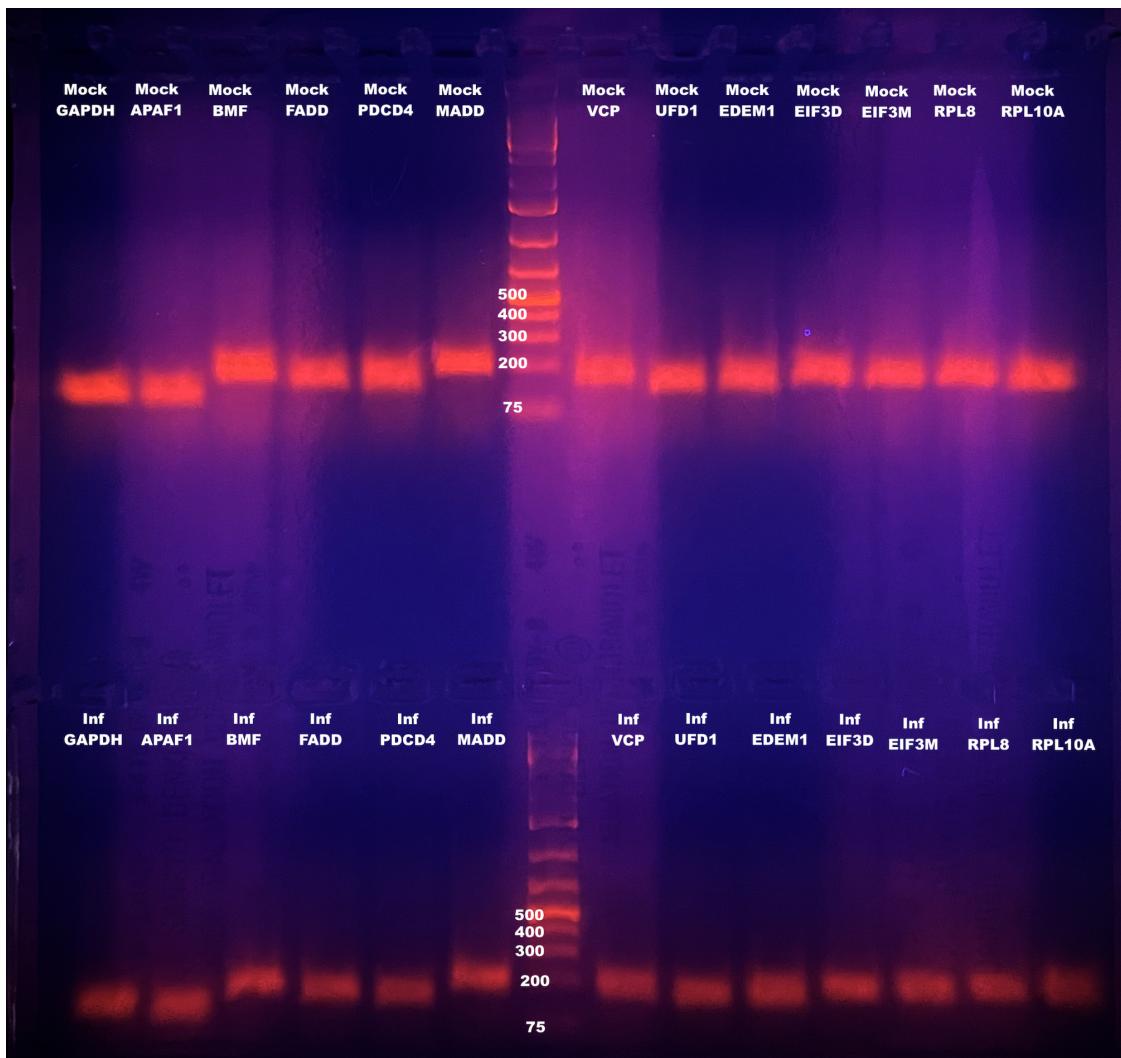
464 therefore, we believe that either one or synergistic interplay between multiple ones may mediate cell death

465 of infected B-cells, leading to IMS. These findings provide the first insights into THEV-host interactions

466 and may help advance the understanding of non-human adenoviral infection and pathogenesis, which may

<sup>467</sup> eventually inform the development of medical countermeasures for disease prevention and treatment.

468 **Supplementary Materials**



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

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

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

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

469 **Gene symbols:** glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); apoptotic peptidase activating  
 470 factor 1 (*APAF1*); Bcl2 modifying factor (*BMF*); FAS-associated protein with death domain (*FADD*); pro-  
 471 grammed cell death 4 (*PDCD4*); MAP kinase activating death domain (*MADD*); valosin containing protein  
 472 (*VCP/p97*); Ubiquitin Recognition Factor in ER Associated Degradation 1 (*UFD1*); ER degradation enhanc-  
 473 ing alpha-mannosidase like protein 1 (*EDEM1*); eukaryotic translation initiation factor 3 subunit D and M  
 474 (*EIF3D* and, *EIF3M*); ribosomal protein L8 and L10a (*RPL8*, and *RPL10A*).

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

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

475 **Author Contributions**

476 Conceptualization, Abraham Quaye, Brian D. Poole, Brett E. Pickett, Bradford K. Berges, and Joel S. Griffits; methodology, Abraham Quaye; software, Abraham Quaye; validation, Abraham Quaye; formal analysis, Abraham Quaye, Brett E. Pickett; investigation, Abraham Quaye; resources, Brian D. Poole; data curation, Abraham Quaye; writing—original draft preparation, Abraham Quaye; writing—review and editing, Abraham Quaye, Brian D. Poole, Brett E. Pickett, Bradford K. Berges, and Joel S. Griffits; visualization, Abraham Quaye; supervision, Brian D. Poole; project administration, Brian D. Poole; funding acquisition, Brian D. Poole. All authors have read and agreed to the published version of the manuscript.

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485 **Data Availability**

486 The raw sequencing read data (FastQ), gene expression counts, and total DEGs identified at 12- and 24-  
487 hpi have been deposited at the NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under  
488 accession number GSE286211.  
489 All the code/scripts in the entire analysis pipeline are available on GitHub (<https://github.com/Abraham->  
490 Quaye/host\_rna\_seq)

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494 work.

495 **Conflicts of Interest**

496 The authors declare no conflicts of interest.

497 **Abbreviations**

498 The following are abbreviations used in this manuscript:

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
ORF	Open Reading Frame
RNA-seq	RNA sequencing
RT-qPCR	Reverse Transcriptase Quantitative Polymerase Chain Reaction

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

499 **REFERENCES**

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