

1 The Transcriptome Profile of a Turkey B-cell Line Upon
2 Infection With Turkey Hemorrhagic Enteritis Virus

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15 **ABSTRACT**

16 INTRODUCTION

17 Turkey hemorrhagic enteritis virus (THEV), belonging to the family *Adenoviridae*, genus *Siadenovirus*,
18 infects turkeys, chickens, and pheasants (1, 2). Infecting its hosts via the feco-oral route, THEV causes
19 hemorrhagic enteritis (HE) in turkeys, a debilitating disease affecting predominantly 6-12-week-old turkey
20 pouls characterized by immunosuppression (IMS), depression, splenomegaly, intestinal lesions leading to
21 bloody droppings, and up to 80% mortality (3–6). The clinical disease usually persists in affected flocks for
22 about 7-10 days. However, secondary bacterial infections may extend the duration of illness and mortality for
23 an additional 2-3 weeks due to the immunosuppressive nature of the virus, exacerbating the economic losses
24 (5, 7). Low pathogenic (avirulent) strains of THEV have been isolated, which show subclinical infections
25 but retain the immunosuppressive effects. Since its isolation from a pheasant spleen, the Virginia Avirulent
26 Strain (VAS) has been used effectively as a live vaccine despite the immunosuppressive side-effects, but
27 the vaccinated birds are rendered more susceptible to opportunistic infections and death than unvaccinated
28 cohorts leading to significant economic losses (4, 5, 8–10).

29 It is well-established that THEV primarily infects and replicates in turkey B-cells of the bursa and spleen and
30 somewhat in macrophages, inducing apoptosis and necrosis. Consequently, a significant drop in number of
31 B-cells (specifically, IgM+ B-cells) and macrophages ensue along with increased T-cell counts with abnormal
32 T-cell subpopulation (CD4+ and CD8+) ratios. The cell death seen in the B-cells and macrophages is
33 generally proposed as the major cause of THEV-induced IMS as both humoral and cell-mediated immunity
34 are impaired (5, 6, 8, 11). It is also thought that the virus replication in the spleen attracts T-cells and
35 peripheral blood macrophages to the spleen where the T-cells are activated by cytokines from activated
36 macrophages and vice versa. The activated T-cells undergo clonal expansion and secrete interferons: type I
37 (IFN- α and IFN- β) and type II (IFN- γ) as well as tumor necrosis factor (TNF) while activated macrophages
38 secrete interleukin 6 (IL-6), TNF, and nitric oxide (NO), an antiviral agent with immunosuppressive properties.
39 The inflammatory cytokines released by T-cells and macrophages (e.g., TNF and IL-6) may also induce
40 apoptosis in bystander splenocytes, exacerbating the already numerous apoptotic and necrotic splenocytes,
41 culminating in IMS (8, 11) (see **Figure 1**). However, the precise molecular mechanisms of THEV-induced
42 IMS or pathways involved are poorly understood (6). Elucidating the specific mechanisms and pathways of
43 THEV-induced IMS is the most crucial step in THEV research as it will present a means of mitigating the IMS.

44 Next generation sequencing (NGS) is a groundbreaking technology that has significantly enhanced our
45 understanding of DNA and RNA structure and function and facilitated exceptional advancements in all
46 domains of biology and the Life Sciences (12). mRNA sequencing (RNA-seq), an NGS approach to
47 transcriptomic studies, is a versatile, high throughput, and cost-effective technology that allows a broad scan

48 of the entire transcriptome, thereby uncovering the active genes and molecular pathways and processes.
49 This technology has been leveraged in an ever increasing number of studies to elucidate active cellular
50 processes under a wide range of treatment conditions, including the transcriptomics of viral infections (12–16).
51 In RNA-seq studies, differentially expressed genes (DEGs) identified under different experimental conditions
52 are key to unlocking the interesting biology or mechanism under study. Identified DEGs are typically used for
53 functional enrichment analysis in large curated knowledgebases which connect genes to specific biological
54 processes, functions, and pathways such as gene ontology (GO) and Kyoto Encyclopedia of Genes and
55 Genomes (KEGG) pathways, shedding light on the biological question under study (17, 18).
56 To the best of our knowledge, no study has leveraged the wealth of information offered by RNA-seq to
57 elucidate the molecular mechanisms and pathways leading to THEV-induced IMS. To effectively counteract
58 the immunosuppressive effect of the vaccine, it is essential to unravel the host mechanisms/pathways
59 influenced by the virus to bring about IMS. In this study, we present the first transcriptomic profile of a
60 THEV infection using paired-end RNA-seq in a turkey B-cell line (MDTC-RP19), highlighting key host genes,
61 cellular/molecular processes and pathways affected during a THEV infection. Our RNA-seq yielded 149 bp
62 long high quality (mean PHRED Score of 36) sequences from each end of cDNA fragments, which were
63 mapped to the genome of domestic turkey (*Meleagris gallopavo*).

64 **RESULTS**

65 **Sequencing Results**

66 To identify the host transcriptome profile during THEV infection, MDTC-RP19 cells were THEV-infected
67 or mock-infected in triplicates or duplicates, respectively, and collected in like manner at 4-, 12-, 24-, and
68 72-hours post infection (hpi). mRNAs extracted from mock- or THEV-infected cells were sequenced on the
69 Illumina platform, yielding a total of **776.1** million raw reads (149 bp in length) across all samples (statistics
70 for the sequencing reads obtained from each RNA library are presented in **Table 1**). After trimming off
71 low-quality reads, the remaining **742.8** million total paired-end trimmed reads (approximately, **34.7-47.9**
72 million reads per sample) were mapped to the genome of *Meleagris gallopavo* obtained from the National
73 Center for Biotechnology Information (NCBI). The percentage of reads mapping to the host genome across
74 all samples ranged from **32.4-89.2%**. Although our sequencing reads have excellent quality scores (see
75 **Table 1**) at all time points, the DEGs identified at 4- and 72-hpi did not yield any results in the functional
76 enrichment analyses (i.e, GO term and KEGG pathway analysis); hence, they were excluded from all
77 subsequent analyses. In the remaining samples from 12- and 24-hpi, there is a high correlation was seen
78 between biological replicates (**Figure 2A** and **B**)

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

80 Gene expression levels were estimated with the StringTie software (19) in Fragments per kilobase of
81 transcript per million (FPKM) units. The analysis of DEGs was performed with the DESeq2 R package (20)
82 which employs negative binomial distribution model for read count comparisons. Using a P_{adjusted} -value
83 cutoff ≤ 0.05 as the inclusion criteria, a total of **2,343** and **3,295** genes were identified as differentially
84 expressed at 12-hpi and 24-hpi, respectively. ~~DEG analyses results at 12 and 24 hpi are presented in~~
85 **Supplementary Tables 1 and 2**, respectively. At 12-hpi, **1,079** genes were upregulated and **1,264** genes
86 downregulated, whereas **1,512** genes were upregulated and **1,783** genes downregulated at 24-hpi (**Figure**
87 **2C**,~~and~~ **Figure 3A-C**). The log₂fold-change(FC) values at 12-hpi ranged between **-1.4** and **+1.7** for **TMEM156**
88 (**Transmembrane Protein 156**) and **LIPG** (**Lipase G**), respectively. At 24-hpi, the log₂FC values ranged
89 between **-2.0** and **+2.6** for **C1QTNF12** (**C1q And TNF Related 12**) and **KCNG1** (**Potassium Voltage-Gated**
90 **Channel Modifier Subfamily G Member 1**), respectively.

91 **Functional Enrichment Analyses (GO, KEGG pathway, and interaction network analyses)**

92 Gene ontology (GO) enrichment analysis was performed for 12- and 24-hpi DEGs with the gprofiler2
93 (version **0.2.3**) R package (21), which outputs results in three GO categories – cellular components (CP),
94 biological processes (BP), and molecular functions (MF). Results with P_{adjusted} -value ≤ 0.05 were considered

95 functionally enriched. The GO enrichment analyses results at 12-hpi and 24-hpi showed significant similarities
96 among all three GO categories. At both time points, cellular breakdown processes were upregulated while
97 cellular maintenance processes and structures were downregulated in all three GO categories (**Table 2A-B**
98 and **Table 3A-B**).

99 For upregulated DEGs at 12-hpi, GO terms annotated under the biological processes (BP) category broadly
100 cluster into: apoptosis, catabolic processes, cellular metabolism, response to stimuli, and protein processing
101 (**Figure 4A**). Under the cellular components (CC) GO category, the GO terms relate with cytoplasmic
102 vacuolation while the GO terms under the molecular functions (MF) category broadly fit under protein
103 binding (**Table 2A**). For 12-hpi downregulated DEGs, the GO terms in BP category generally fall under:
104 translation, protein biosynthesis and folding; ribosome biogenesis; nitrogen compound metabolism; nucleic
105 acid synthesis, metabolism, processing, and replication; and energy metabolism (**Figure 4C**). As for the CC
106 category GO terms, they broadly group into: ribosome, mitochondria, respirosome, and nucleus while the
107 MF category GO terms generally belong to: translation regulator activity, protein folding chaperone, catalytic
108 activity (acting on a nucleic acids), and ATP hydrolysis activity (**Table 2B**).

109 At 24-hpi, the GO terms under the BP GO category for the upregulated DEGs are connected with: catabolic
110 process, protein ubiquitination and proteolysis, cell signalling, and cell metabolism (**Figure 4B**). The GO terms
111 of the CC category, similar to those identified at 12-hpi, are also related with cytoplasmic vacuolation. The
112 MF category GO terms group into: protein ubiquitination activity, acyltransferase activity, and macromolecule
113 binding activity (**Table 3A**). The GO terms for the downregulated DEGs are markedly similar to those at
114 12-hpi in all three GO categories. Lastly, the BP category GO terms broadly group into: translation, peptide
115 biosynthesis and folding, ribosome biogenesis, aerobic respiration and ATP synthesis, and cell cycle process
116 and DNA replication (**Figure 4D**). The GO terms of the CC category group under: ribosome, organelle,
117 mitochondrion, nucleus and chromosomes while the MF category GO terms group into: structural constituent
118 of ribosome and translation regulator activity, catalytic activity acting on a nucleic acid and nucleic acid
119 binding, aminoacyl-tRNA ligase activity, and NAD binding (**Table 3B**).

120 KEGG analysis on the DEGs was performed using both the gprofiler2 R package (21) and the DAVID
121 (Database for Annotation, Visualization and Integrated Discovery; version 2021) online resource (22). Both
122 analysis resources gave similar results but the results from DAVID (**Table 4A**) includes more information
123 than the gprofiler2 results (**Table 4B**)... revealed that ...

124 **DISCUSSION**

125 **CONCLUSIONS**

126 **MATERIALS AND METHODS**

127 **Cell culture and THEV Infection**

128 The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete
129 Leibovitz's L-15/McCoy's 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5%
130 tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100 μ g/mL Streptomycin),
131 at 41°C in a humidified atmosphere with 5% CO₂. Infected cells were maintained in 1:1 serum-reduced
132 Leibovitz's L15/McCoy's 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic solution. A
133 commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain). The stock
134 virus was titrated using an in-house qPCR assay with titer expressed as genome copy number (GCN)/mL,
135 similar to Mahshoub *et al* (23). Cells were THEV-infected or mock-infected in triplicates or duplicates,
136 respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41°C for 1 hour, and washed
137 three times with phosphate buffered saline (PBS) to get rid of free virus particles. At each time point (4-, 12-,
138 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were harvested for total
139 RNA extraction.

140 **RNA extraction and Sequencing**

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

151 **Quality Control and Mapping Process**

152 Sequencing reads were processed following a well-established protocol described by Pertea *et al* (19),
153 using Snakemake - version 7.32.4 (24), a popular workflow management system to drive the pipeline.
154 Briefly, raw sequencing reads were trimmed with Cutadapt - version 1.10 (25) and the quality of trimmed
155 reads evaluated using the FastQC software, version 0.12.1 (Bioinformatics Group at the Babraham Institute,
156 Cambridge, United Kingdom; www.bioinformatics.babraham.ac.uk), achieving an overall Mean Sequence
157 Quality (PHRED Score) of 36. Trimmed reads were mapped the reference *Meleagris gallopavo* genome

158 (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/GCF_000146
159 605.3_Turkey_5.1_genomic.fna.gz) with Hisat2 - version 2.2.1 (19) using the accompanying gene transfer
160 format (GTF) annotation file (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3
161 _Turkey_5.1/GCF_000146605.3_Turkey_5.1_genomic.gtf.gz) to build a genomic index. Samtools - version
162 1.19.2 was used to convert the output Sequence Alignment Map (SAM) file to the more manageable Binary
163 Alignment Map (BAM) format. The StringTie (v2.2.1) software (19), set to expression estimation mode was
164 used to generate normalized gene expression estimates from the BAM files for genes in the reference GTF
165 file after which the prepDE.py3 script was used to extract read count information from the StringTie gene
166 expression files, providing an expression-count matrix for downstream DEG analysis.

167 **DEG Analysis and Functional Enrichment Analysis**

168 DEG analysis between mock- and THEV-infected samples was performed using the very popular DESeq2
169 (20), which employs a Negative Binomial distribution model for read count comparisons. Genes with P_{adjusted} -
170 value ≤ 0.05 were considered as differentially expressed. The read count data are deposited at Gene
171 Expression Omnibus (GEO) under accession number ###. The functional profiling of DEGs (GO and KEGG
172 analyses) were performed based on GO databases and KEGG databases using the R package gprofiler2
173 (21) with *Meleagris gallopavo* as the reference organism. Results with P_{adjusted} -value ≤ 0.05 were included
174 as functionally enriched. Additionally, the DAVID (Database for Annotation, Visualization and Integrated
175 Discovery; version 2021) online analysis tool was used for KEGG pathway analysis. All visualization plots
176 were made using ggplot2, pheatmap, and ggvenn R packages (26–28).

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

178 **Statistical Analysis**

¹⁷⁹ **DATA AVAILABILITY**

¹⁸⁰ **CODE AVAILABILITY**

¹⁸¹ **ACKNOWLEDGMENTS**

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211 TABLES AND FIGURES

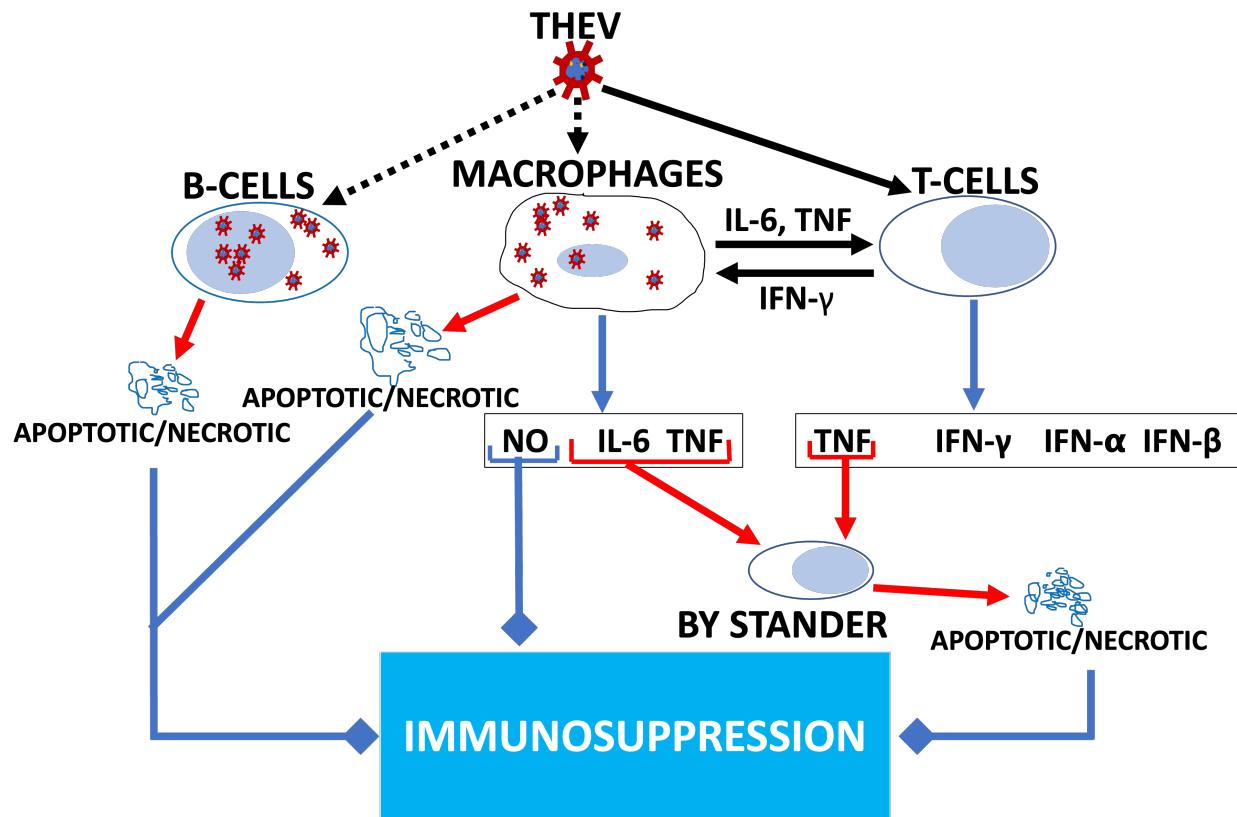


Figure 1: Model of THEV-induced immunosuppression in turkeys. THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicate signals leading to apoptosis. Blue arrows indicate all cytokines released by the cell. Blue arrows with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

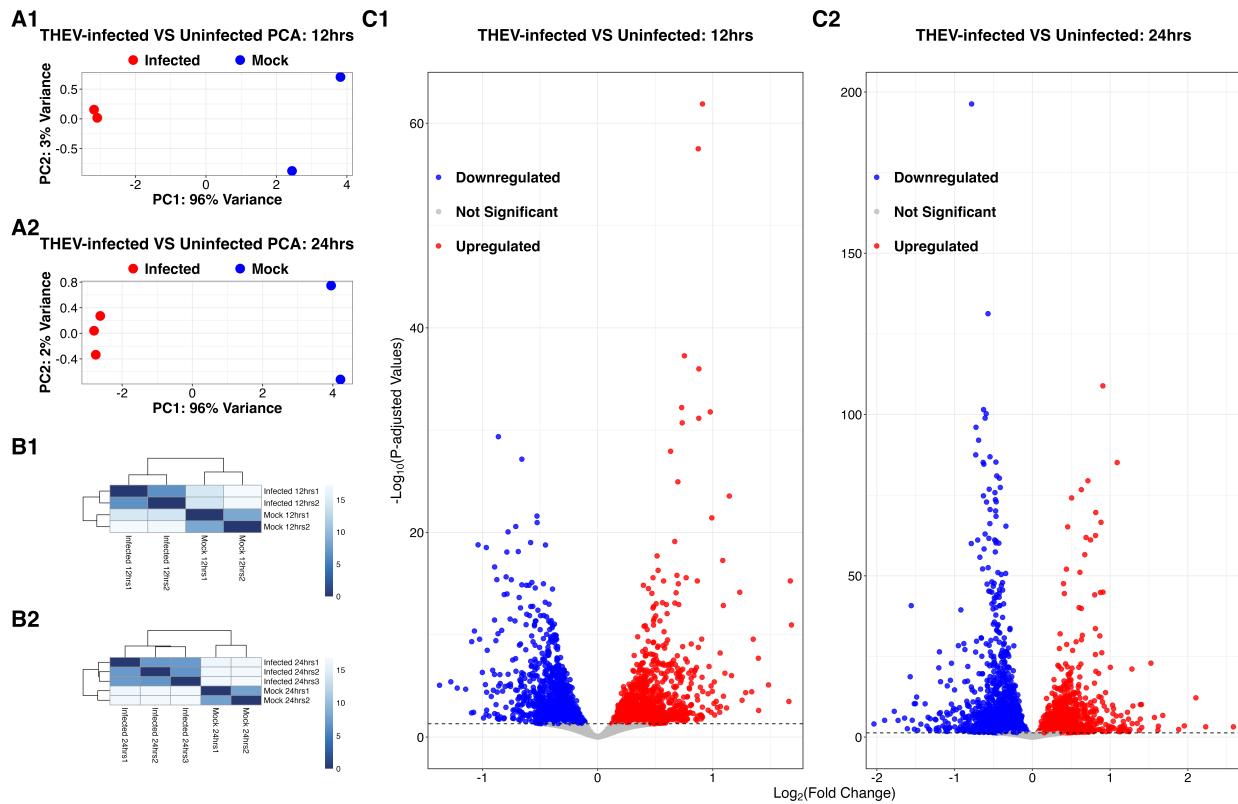


Figure 2. (A) Principal component analysis (PCA) of turkey B-cells during THEV infection. At 12-hpi (**A1**), the results indicate that the first (PC1) and second (PC2) principal components account for 96% and 3% of the variation in the samples, respectively. Whereas PC1 and PC2 account for 96% and 2%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

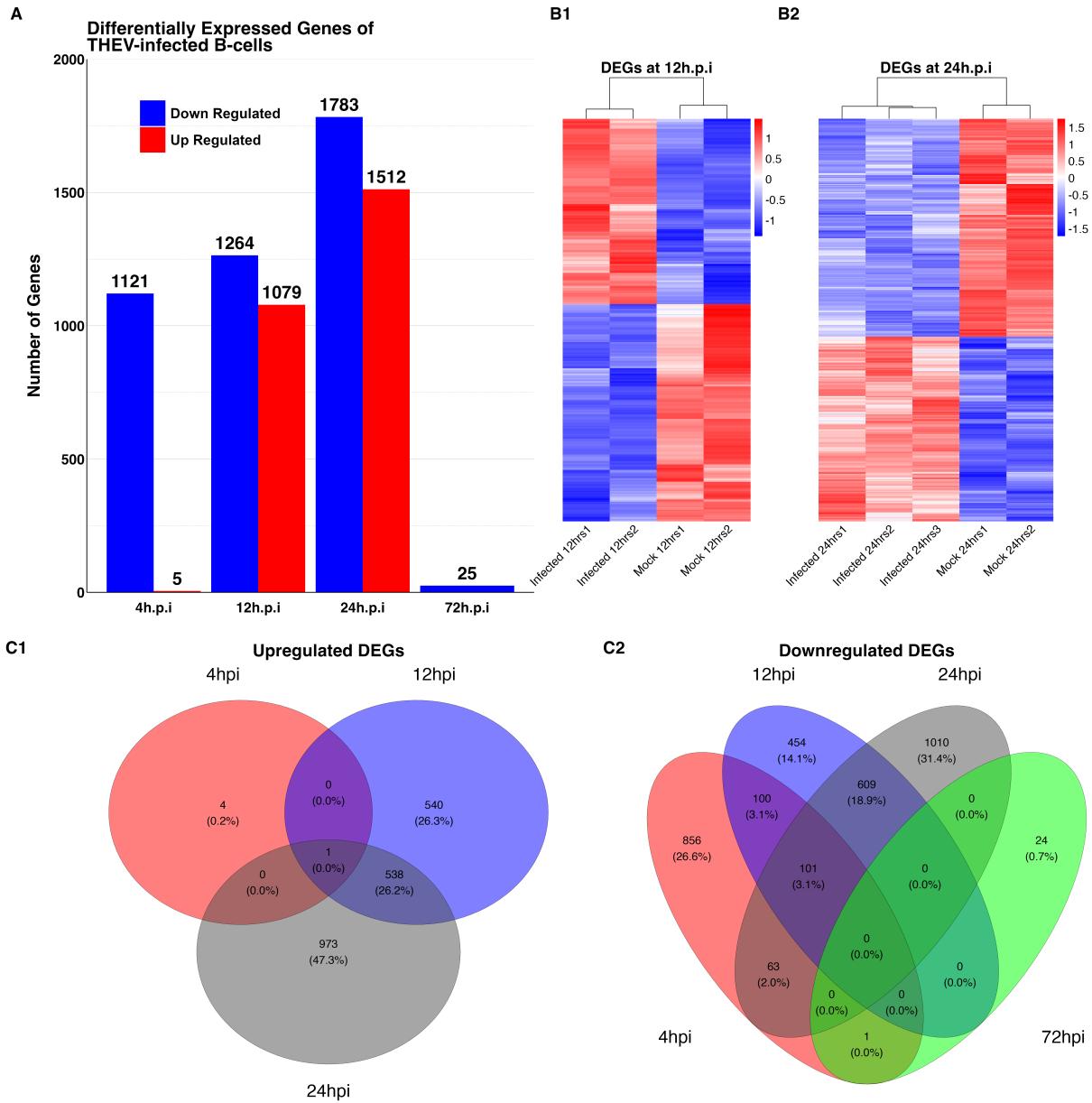


Figure 3: Differentially expressed genes (DEGs) of THEV-infected versus mock-infected samples at different time points. (A) Bar plot of number DEGs identified. Red represents upregulated genes and blue represents downregulated genes. **(B) Heatmaps of scaled expression data (Z-scores) of DEGs.** DEGs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

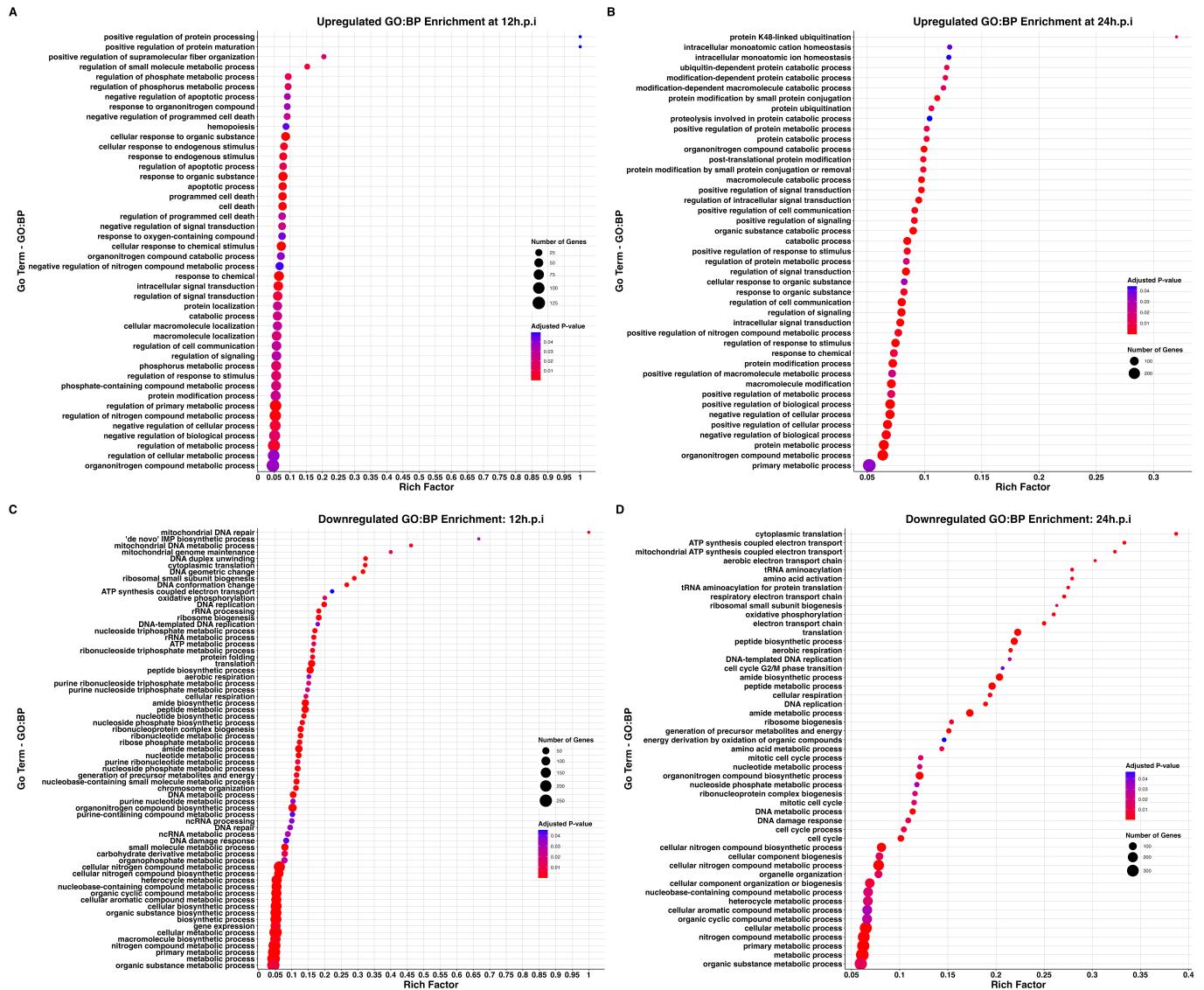


Figure 4: Dotplot of Enriched Gene Ontology Biological Processes (BP). Significant BP GO terms identified for upregulated DEGs at 12-hpi and 24-hpi are shown in (A) and (B), respectively. Significant BP GO terms for downregulated DEGs at 12-hpi and 24-hpi are shown in (C) and (D), respectively. The y-axis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

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

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

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

^MAll values for number of reads are in millions;

^{Inf}These are infected samples indicated by the letter 'I' and 'S' in sample names

^{Mk}These are mock-infected samples indicated by the letters 'U' and 'N' in sample names

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

Time Point	Regulation	GO Term	DEG Count	Fold Enrichment	P-value	Benjamini
12-hpi	down	Ribosome	80	6.59	4.87e-51	7.50e-49
12-hpi	down	Oxidative phosphorylation	37	3.18	1.94e-10	1.39e-08
12-hpi	down	DNA replication	18	5.94	3.15e-10	1.39e-08
12-hpi	down	Ribosome biogenesis in eukaryotes	27	3.99	3.62e-10	1.39e-08
12-hpi	down	Spliceosome	30	2.47	5.09e-06	1.57e-04
12-hpi	down	Nucleocytoplasmic transport	22	2.27	4.53e-04	1.16e-02
12-hpi	down	Mismatch repair	9	4.24	6.31e-04	1.23e-02
12-hpi	down	Base excision repair	13	3.06	6.39e-04	1.23e-02
12-hpi	down	Nucleotide excision repair	14	2.83	8.36e-04	1.43e-02
12-hpi	up	Steroid biosynthesis	10	6.14	1.07e-05	1.65e-03
12-hpi	up	Autophagy - animal	29	2.34	2.74e-05	2.12e-03

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

Time Point	Regulation	GO Term	DEG Count	Fold Enrichment	P-value	Benjamini
12-hpi	up	Cell cycle	27	2.30	7.55e-05	3.90e-03
12-hpi	up	Influenza A	22	2.13	1.22e-03	4.74e-02
24-hpi	down	Ribosome	88	5.54	1.71e-51	2.81e-49
24-hpi	down	Oxidative phosphorylation	50	3.28	3.30e-15	2.71e-13
24-hpi	down	Carbon metabolism	39	2.98	1.97e-10	1.08e-08
24-hpi	down	Aminoacyl-tRNA biosynthesis	22	3.78	2.69e-08	1.10e-06
24-hpi	down	Biosynthesis of amino acids	24	3.02	8.22e-07	2.50e-05
24-hpi	down	Citrate cycle (TCA cycle)	15	4.36	9.16e-07	2.50e-05
24-hpi	down	DNA replication	15	3.78	8.25e-06	1.93e-04
24-hpi	down	Spliceosome	33	2.08	5.30e-05	1.09e-03
24-hpi	down	Metabolic pathways	225	1.22	1.77e-04	3.04e-03
24-hpi	down	Cell cycle	36	1.89	1.86e-04	3.04e-03
24-hpi	down	Propanoate metabolism	12	3.24	5.05e-04	7.53e-03
24-hpi	down	Fatty acid degradation	14	2.86	5.68e-04	7.77e-03
24-hpi	down	Glycolysis / Gluconeogenesis	17	2.42	9.40e-04	1.19e-02
24-hpi	down	One carbon pool by folate	9	3.78	1.16e-03	1.35e-02
24-hpi	down	Nucleotide excision repair	15	2.31	3.41e-03	3.73e-02
24-hpi	down	Pyruvate metabolism	12	2.59	4.10e-03	4.20e-02

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

Time Point	Regulation	GO Term	DEG Count	Fold Enrichment	P-value	Benjamini
24-hpi	up	Steroid biosynthesis	11	5.15	1.21e-05	1.92e-03
24-hpi	up	Lysosome	29	2.24	4.95e-05	3.94e-03
24-hpi	up	Terpenoid backbone biosynthesis	9	4.43	4.13e-04	1.73e-02
24-hpi	up	Glycosaminoglycan biosynthesis - heparan sulfate / heparin	10	3.90	5.02e-04	1.73e-02
24-hpi	up	Protein processing in endoplasmic reticulum	30	1.94	5.45e-04	1.73e-02
24-hpi	up	Autophagy - animal	30	1.85	1.20e-03	3.19e-02

Table 4B: Significantly Enriched KEGG Pathways from DEGs identified at 12 and 24-hpi (Results from the gprofiler2 R package)

Time Point	Regulation	GO 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

Table 4B: Significantly Enriched KEGG Pathways from DEGs identified at 12 and 24-hpi (Results from the gprofiler2 R package)

Time Point	Regulation	GO Term	DEG Count	P-value (Adjusted)
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

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

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
Biological Process			
GO:BP	cellular response to organic substance	3.38e-06	48
GO:BP	response to organic substance	7.40e-06	55
GO:BP	cellular response to chemical stimulus	6.25e-05	56
GO:BP	response to chemical	1.92e-04	66

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	regulation of primary metabolic process	3.18e-04	102
GO:BP	cell death	6.58e-04	43
GO:BP	programmed cell death	6.58e-04	43
GO:BP	apoptotic process	1.08e-03	41
GO:BP	regulation of nitrogen compound metabolic process	1.29e-03	98
GO:BP	intracellular signal transduction	1.89e-03	61
GO:BP	response to endogenous stimulus	3.24e-03	36
GO:BP	cellular response to endogenous stimulus	3.27e-03	34
GO:BP	regulation of metabolic process	4.54e-03	116
GO:BP	negative regulation of cellular process	6.47e-03	89
GO:BP	regulation of small molecule metabolic process	7.16e-03	13
GO:BP	regulation of signal transduction	8.44e-03	58
GO:BP	regulation of apoptotic process	1.33e-02	32
GO:BP	regulation of phosphate metabolic process	1.40e-02	23
GO:BP	regulation of phosphorus metabolic process	1.49e-02	23
GO:BP	negative regulation of biological process	1.62e-02	92
GO:BP	phosphorus metabolic process	1.63e-02	69
GO:BP	regulation of response to stimulus	1.73e-02	69
GO:BP	positive regulation of supramolecular fiber organization	1.81e-02	9
GO:BP	macromolecule localization	2.02e-02	63

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	phosphate-containing compound metabolic process	2.12e-02	68
GO:BP	catabolic process	2.30e-02	55
GO:BP	negative regulation of signal transduction	2.31e-02	33
GO:BP	negative regulation of programmed cell death	2.51e-02	23
GO:BP	protein localization	2.56e-02	54
GO:BP	protein modification process	2.60e-02	73
GO:BP	cellular macromolecule localization	2.81e-02	54
GO:BP	regulation of cell communication	2.87e-02	62
GO:BP	regulation of programmed cell death	3.01e-02	32
GO:BP	regulation of signaling	3.12e-02	62
GO:BP	negative regulation of apoptotic process	3.36e-02	22
GO:BP	response to organonitrogen compound	3.59e-02	22
GO:BP	organonitrogen compound metabolic process	3.75e-02	129
GO:BP	regulation of cellular metabolic process	3.81e-02	106
GO:BP	organonitrogen compound catabolic process	3.92e-02	35
GO:BP	response to oxygen-containing compound	4.31e-02	31
GO:BP	hemopoiesis	4.65e-02	23
GO:BP	negative regulation of nitrogen compound metabolic process	4.83e-02	39
GO:BP	positive regulation of protein processing	5.00e-02	3
GO:BP	positive regulation of protein maturation	5.00e-02	3

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
Cellular Component			
GO:CC	cytoplasm	5.42e-15	201
GO:CC	intracellular anatomical structure	3.33e-09	253
GO:CC	cytosol	5.72e-09	78
GO:CC	intracellular membrane-bounded organelle	2.13e-06	197
GO:CC	membrane-bounded organelle	5.72e-06	201
GO:CC	intracellular organelle	1.18e-04	218
GO:CC	nucleoplasm	4.02e-04	66
GO:CC	organelle	4.91e-04	219
GO:CC	nucleus	9.73e-04	130
GO:CC	endomembrane system	1.15e-03	75
GO:CC	bounding membrane of organelle	2.72e-03	37
GO:CC	perinuclear region of cytoplasm	4.96e-03	17
GO:CC	organelle membrane	7.16e-03	59
GO:CC	vesicle	7.68e-03	37
GO:CC	cytoplasmic vesicle	2.58e-02	34
GO:CC	intracellular vesicle	2.96e-02	34
Molecular Function			
GO:MF	enzyme binding	7.66e-07	50
GO:MF	identical protein binding	1.40e-04	47

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:MF	protein binding	2.24e-04	192
GO:MF	binding	1.36e-03	302
GO:MF	enzyme regulator activity	2.94e-02	36
GO:MF	small molecule binding	2.96e-02	147
GO:MF	transcription regulator activator activity	4.99e-02	3

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

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
Biological Process			
GO:BP	translation	1.67e-17	54
GO:BP	peptide biosynthetic process	6.71e-17	54
GO:BP	peptide metabolic process	1.64e-15	56
GO:BP	organonitrogen compound biosynthetic process	2.83e-15	83
GO:BP	amide biosynthetic process	6.63e-15	54
GO:BP	cellular nitrogen compound metabolic process	1.77e-14	188
GO:BP	amide metabolic process	2.25e-13	59
GO:BP	cellular metabolic process	5.28e-11	254
GO:BP	ribosome biogenesis	1.26e-08	26
GO:BP	DNA replication	8.81e-08	22

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	ribonucleoprotein complex biogenesis	1.45e-07	35
GO:BP	DNA metabolic process	4.04e-07	44
GO:BP	cellular biosynthetic process	1.11e-06	188
GO:BP	organic substance biosynthetic process	2.66e-06	189
GO:BP	biosynthetic process	3.20e-06	190
GO:BP	cellular nitrogen compound biosynthetic process	6.31e-06	116
GO:BP	DNA geometric change	7.10e-06	12
GO:BP	nucleotide metabolic process	1.84e-05	29
GO:BP	nitrogen compound metabolic process	2.14e-05	232
GO:BP	nucleobase-containing small molecule metabolic process	2.14e-05	31
GO:BP	DNA duplex unwinding	2.32e-05	11
GO:BP	heterocycle metabolic process	2.81e-05	150
GO:BP	nucleoside phosphate metabolic process	3.22e-05	29
GO:BP	primary metabolic process	5.30e-05	244
GO:BP	small molecule metabolic process	5.59e-05	55
GO:BP	DNA conformation change	5.96e-05	12
GO:BP	organic cyclic compound metabolic process	7.26e-05	153
GO:BP	ribosomal small subunit biogenesis	8.54e-05	11
GO:BP	metabolic process	1.02e-04	267
GO:BP	nucleobase-containing compound metabolic process	1.06e-04	145

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	cytoplasmic translation	1.07e-04	10
GO:BP	rRNA processing	1.28e-04	16
GO:BP	nucleoside triphosphate metabolic process	1.43e-04	17
GO:BP	cellular aromatic compound metabolic process	1.90e-04	148
GO:BP	rRNA metabolic process	4.46e-04	16
GO:BP	ribonucleotide metabolic process	4.77e-04	22
GO:BP	ribose phosphate metabolic process	7.10e-04	22
GO:BP	nucleotide biosynthetic process	8.95e-04	19
GO:BP	chromosome organization	1.21e-03	24
GO:BP	protein folding	1.38e-03	15
GO:BP	ribonucleoside triphosphate metabolic process	1.38e-03	15
GO:BP	nucleoside phosphate biosynthetic process	1.55e-03	19
GO:BP	macromolecule biosynthetic process	1.69e-03	159
GO:BP	mitochondrial DNA repair	2.13e-03	4
GO:BP	gene expression	2.39e-03	149
GO:BP	generation of precursor metabolites and energy	2.59e-03	22
GO:BP	mitochondrial DNA metabolic process	3.90e-03	6
GO:BP	organic substance metabolic process	6.79e-03	250
GO:BP	purine ribonucleotide metabolic process	8.33e-03	19
GO:BP	mitochondrial genome maintenance	1.07e-02	6

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	carbohydrate derivative metabolic process	1.23e-02	37
GO:BP	ATP metabolic process	1.29e-02	12
GO:BP	oxidative phosphorylation	1.33e-02	10
GO:BP	cellular respiration	1.54e-02	14
GO:BP	purine ribonucleoside triphosphate metabolic process	1.68e-02	13
GO:BP	purine nucleoside triphosphate metabolic process	2.17e-02	13
GO:BP	organophosphate metabolic process	2.67e-02	35
GO:BP	ncRNA metabolic process	2.90e-02	27
GO:BP	'de novo' IMP biosynthetic process	3.01e-02	4
GO:BP	purine nucleotide metabolic process	3.37e-02	20
GO:BP	aerobic respiration	3.42e-02	12
GO:BP	DNA repair	3.48e-02	23
GO:BP	ncRNA processing	3.64e-02	21
GO:BP	DNA-templated DNA replication	3.78e-02	10
GO:BP	DNA damage response	4.19e-02	29
GO:BP	purine-containing compound metabolic process	4.21e-02	20
GO:BP	ATP synthesis coupled electron transport	4.54e-02	8
Cellular Component			
GO:CC	intracellular anatomical structure	2.34e-20	315
GO:CC	protein-containing complex	2.35e-19	177

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	ribosomal subunit	6.90e-19	28
GO:CC	cytosolic ribosome	2.02e-18	21
GO:CC	ribosome	4.82e-16	39
GO:CC	intracellular organelle	4.96e-16	284
GO:CC	cytosolic large ribosomal subunit	1.59e-15	15
GO:CC	ribonucleoprotein complex	4.59e-15	62
GO:CC	organelle	2.14e-14	284
GO:CC	intracellular membrane-bounded organelle	1.66e-12	245
GO:CC	large ribosomal subunit	9.19e-12	18
GO:CC	membrane-bounded organelle	1.37e-10	246
GO:CC	organelle lumen	1.92e-10	118
GO:CC	intracellular organelle lumen	1.92e-10	118
GO:CC	membrane-enclosed lumen	1.92e-10	118
GO:CC	envelope	8.50e-10	47
GO:CC	organelle envelope	8.50e-10	47
GO:CC	nucleus	2.90e-09	169
GO:CC	cytoplasm	4.68e-09	212
GO:CC	intracellular non-membrane-bounded organelle	5.50e-09	135
GO:CC	non-membrane-bounded organelle	5.50e-09	135
GO:CC	mitochondrion	6.70e-09	58

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	mitochondrial inner membrane	7.34e-08	26
GO:CC	organelle inner membrane	1.42e-07	27
GO:CC	nuclear lumen	1.69e-07	103
GO:CC	mitochondrial envelope	6.30e-07	32
GO:CC	mitochondrial membrane	1.15e-06	30
GO:CC	small ribosomal subunit	2.19e-06	10
GO:CC	mitochondrial protein-containing complex	1.13e-05	20
GO:CC	respirasome	3.77e-05	12
GO:CC	inner mitochondrial membrane protein complex	5.48e-05	14
GO:CC	respiratory chain complex	8.15e-05	11
GO:CC	catalytic complex	8.42e-05	55
GO:CC	cytosol	1.29e-04	75
GO:CC	preribosome	1.72e-04	10
GO:CC	cytosolic small ribosomal subunit	1.76e-04	6
GO:CC	respiratory chain complex I	2.46e-04	8
GO:CC	NADH dehydrogenase complex	2.46e-04	8
GO:CC	mitochondrial respirasome	2.85e-04	10
GO:CC	protein folding chaperone complex	3.55e-04	7
GO:CC	oxidoreductase complex	3.58e-04	12
GO:CC	nucleoplasm	5.96e-04	74

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	chaperonin-containing T-complex	1.70e-03	4
GO:CC	small-subunit processome	2.12e-03	8
GO:CC	nucleolus	2.15e-03	41
GO:CC	mitochondrial respiratory chain complex I	2.34e-03	7
GO:CC	chromosome	8.26e-03	37
GO:CC	organelle membrane	1.24e-02	66
GO:CC	nuclear protein-containing complex	1.93e-02	40
GO:CC	Ctf18 RFC-like complex	3.81e-02	3
GO:CC	eukaryotic translation initiation factor 3 complex, eIF3m	3.81e-02	3
GO:CC	eukaryotic 48S preinitiation complex	3.82e-02	4
GO:CC	rough endoplasmic reticulum	3.92e-02	5

Molecular Function

GO:MF	structural constituent of ribosome	1.10e-15	35
GO:MF	organic cyclic compound binding	1.33e-09	171
GO:MF	nucleic acid binding	1.13e-06	101
GO:MF	RNA binding	1.46e-06	52
GO:MF	structural molecule activity	3.18e-06	41
GO:MF	DNA helicase activity	2.69e-05	9
GO:MF	unfolded protein binding	3.20e-05	11
GO:MF	ATP hydrolysis activity	3.51e-05	24

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:MF	catalytic activity, acting on a nucleic acid	5.60e-05	34
GO:MF	translation regulator activity	1.25e-04	13
GO:MF	catalytic activity, acting on DNA	2.97e-04	18
GO:MF	heterocyclic compound binding	4.39e-04	88
GO:MF	nucleoside phosphate binding	7.58e-04	84
GO:MF	nucleotide binding	7.58e-04	84
GO:MF	protein folding chaperone	9.92e-04	9
GO:MF	adenyl nucleotide binding	1.16e-03	68
GO:MF	ATP-dependent protein folding chaperone	1.38e-03	8
GO:MF	purine nucleotide binding	3.23e-03	78
GO:MF	ATP-dependent activity, acting on DNA	3.67e-03	12
GO:MF	hydrolase activity, acting on acid anhydrides	7.09e-03	33
GO:MF	ribonucleoprotein complex binding	7.21e-03	11
GO:MF	ATP-dependent activity	7.43e-03	31
GO:MF	translation regulator activity, nucleic acid binding	7.88e-03	10
GO:MF	anion binding	1.10e-02	83
GO:MF	helicase activity	1.39e-02	12
GO:MF	pyrophosphatase activity	1.47e-02	32
GO:MF	NAD binding	1.64e-02	8

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:MF	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	1.67e-02	32
GO:MF	translation factor activity, RNA binding	2.37e-02	9
GO:MF	ribonucleoside triphosphate phosphatase activity	2.53e-02	30
GO:MF	hydroxymethyl-, formyl- and related transferase activity	2.61e-02	4
GO:MF	mRNA binding	4.75e-02	12

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

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
Biological Process			
GO:BP	positive regulation of biological process	4.76e-06	133
GO:BP	organonitrogen compound metabolic process	7.66e-06	176
GO:BP	organic substance catabolic process	8.02e-06	68
GO:BP	catabolic process	9.68e-06	77
GO:BP	regulation of signal transduction	1.08e-05	79
GO:BP	regulation of cell communication	1.78e-05	86
GO:BP	regulation of signaling	2.03e-05	86
GO:BP	organonitrogen compound catabolic process	6.25e-05	49
GO:BP	negative regulation of cellular process	8.39e-05	116

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	protein metabolic process	1.17e-04	148
GO:BP	regulation of intracellular signal transduction	1.51e-04	51
GO:BP	macromolecule catabolic process	1.74e-04	48
GO:BP	regulation of response to stimulus	1.79e-04	91
GO:BP	macromolecule modification	2.23e-04	104
GO:BP	intracellular signal transduction	2.96e-04	76
GO:BP	protein modification process	3.57e-04	96
GO:BP	positive regulation of cellular process	5.36e-04	115
GO:BP	protein modification by small protein conjugation	6.56e-04	34
GO:BP	positive regulation of signal transduction	8.37e-04	43
GO:BP	negative regulation of biological process	8.76e-04	118
GO:BP	positive regulation of response to stimulus	1.83e-03	55
GO:BP	positive regulation of cell communication	1.92e-03	46
GO:BP	positive regulation of signaling	2.15e-03	46
GO:BP	response to organic substance	2.75e-03	58
GO:BP	positive regulation of nitrogen compound metabolic process	3.07e-03	68
GO:BP	post-translational protein modification	3.53e-03	37
GO:BP	protein catabolic process	4.85e-03	34
GO:BP	protein modification by small protein conjugation or removal	4.88e-03	36
GO:BP	response to chemical	7.06e-03	74

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	protein ubiquitination	8.16e-03	30
GO:BP	protein K48-linked ubiquitination	9.05e-03	8
GO:BP	positive regulation of protein metabolic process	9.19e-03	32
GO:BP	ubiquitin-dependent protein catabolic process	9.45e-03	24
GO:BP	modification-dependent protein catabolic process	1.12e-02	24
GO:BP	positive regulation of metabolic process	1.23e-02	78
GO:BP	modification-dependent macromolecule catabolic process	1.44e-02	24
GO:BP	regulation of protein metabolic process	1.81e-02	46
GO:BP	positive regulation of macromolecule metabolic process	2.04e-02	72
GO:BP	cellular response to organic substance	3.15e-02	46
GO:BP	primary metabolic process	3.44e-02	272
GO:BP	intracellular monoatomic cation homeostasis	3.99e-02	20
GO:BP	intracellular monoatomic ion homeostasis	4.37e-02	20
GO:BP	proteolysis involved in protein catabolic process	4.40e-02	26
Cellular Component			
GO:CC	cytoplasm	6.93e-15	268
GO:CC	intracellular anatomical structure	8.47e-08	343
GO:CC	intracellular membrane-bounded organelle	7.95e-07	270
GO:CC	cytosol	3.71e-06	92
GO:CC	membrane-bounded organelle	7.07e-06	274

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	organelle membrane	1.00e-05	88
GO:CC	endomembrane system	1.94e-05	106
GO:CC	intracellular organelle	3.19e-04	298
GO:CC	organelle	1.28e-03	300
GO:CC	perinuclear region of cytoplasm	1.51e-03	22
GO:CC	bounding membrane of organelle	4.31e-03	47
GO:CC	nucleoplasm	6.28e-03	82
GO:CC	Golgi apparatus	2.20e-02	44
GO:CC	vacuole	2.85e-02	21
GO:CC	vacuolar membrane	4.14e-02	15

Molecular Function

GO:MF	acyltransferase activity	7.50e-04	34
GO:MF	aminoacyltransferase activity	9.34e-04	24
GO:MF	ubiquitin-like protein ligase activity	1.68e-03	17
GO:MF	transferase activity	2.20e-03	101
GO:MF	small molecule binding	3.46e-03	187
GO:MF	ubiquitin-like protein transferase activity	4.36e-03	22
GO:MF	ubiquitin protein ligase activity	4.44e-03	16
GO:MF	adenyl nucleotide binding	4.80e-03	80
GO:MF	adenyl ribonucleotide binding	5.52e-03	76

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:MF	ATP binding	5.94e-03	75
GO:MF	ubiquitin-protein transferase activity	7.85e-03	21
GO:MF	catalytic activity, acting on a protein	9.55e-03	95
GO:MF	active monoatomic ion transmembrane transporter activity	1.37e-02	17
GO:MF	protein phosphorylated amino acid binding	1.61e-02	7
GO:MF	phosphotyrosine residue binding	4.95e-02	6
GO:MF	ion binding	4.98e-02	176

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

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
Biological Process			
GO:BP	translation	1.81e-25	75
GO:BP	peptide biosynthetic process	2.30e-25	76
GO:BP	amide biosynthetic process	6.94e-24	78
GO:BP	peptide metabolic process	9.87e-23	78
GO:BP	amide metabolic process	8.33e-21	84
GO:BP	organonitrogen compound biosynthetic process	3.71e-13	98
GO:BP	cellular nitrogen compound metabolic process	1.11e-11	236
GO:BP	cellular nitrogen compound biosynthetic process	5.35e-07	155

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	cellular metabolic process	6.87e-07	324
GO:BP	cytoplasmic translation	2.39e-05	12
GO:BP	metabolic process	2.89e-05	364
GO:BP	electron transport chain	1.28e-04	16
GO:BP	primary metabolic process	1.46e-04	327
GO:BP	ATP synthesis coupled electron transport	1.67e-04	12
GO:BP	generation of precursor metabolites and energy	2.04e-04	29
GO:BP	DNA metabolic process	2.19e-04	48
GO:BP	DNA replication	2.40e-04	21
GO:BP	nitrogen compound metabolic process	3.91e-04	306
GO:BP	aerobic respiration	5.32e-04	17
GO:BP	cell cycle	5.70e-04	58
GO:BP	cellular respiration	6.24e-04	19
GO:BP	respiratory electron transport chain	7.64e-04	13
GO:BP	mitochondrial ATP synthesis coupled electron transport	8.01e-04	11
GO:BP	oxidative phosphorylation	1.28e-03	13
GO:BP	amino acid activation	1.47e-03	12
GO:BP	tRNA aminoacylation	1.47e-03	12
GO:BP	ribosome biogenesis	4.83e-03	22
GO:BP	tRNA aminoacylation for protein translation	4.88e-03	11

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	aerobic electron transport chain	5.18e-03	10
GO:BP	cellular component organization or biogenesis	6.34e-03	173
GO:BP	cell cycle process	9.13e-03	43
GO:BP	amino acid metabolic process	9.43e-03	23
GO:BP	DNA damage response	1.21e-02	38
GO:BP	organic substance metabolic process	1.27e-02	338
GO:BP	cellular component biogenesis	1.37e-02	94
GO:BP	organelle organization	1.43e-02	98
GO:BP	mitotic cell cycle	1.54e-02	33
GO:BP	heterocycle metabolic process	1.65e-02	185
GO:BP	mitotic cell cycle process	1.75e-02	29
GO:BP	ribonucleoprotein complex biogenesis	1.77e-02	32
GO:BP	nucleobase-containing compound metabolic process	1.78e-02	181
GO:BP	nucleotide metabolic process	2.06e-02	29
GO:BP	ribosomal small subunit biogenesis	2.10e-02	10
GO:BP	organic cyclic compound metabolic process	2.68e-02	190
GO:BP	DNA-templated DNA replication	2.91e-02	12
GO:BP	cellular aromatic compound metabolic process	3.19e-02	185
GO:BP	nucleoside phosphate metabolic process	3.30e-02	29
GO:BP	cell cycle G2/M phase transition	4.23e-02	12

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:BP	energy derivation by oxidation of organic compounds	4.68e-02	19
Cellular Component			
GO:CC	intracellular anatomical structure	1.68e-21	430
GO:CC	cytosolic ribosome	1.59e-19	24
GO:CC	ribosome	1.12e-17	48
GO:CC	protein-containing complex	1.59e-17	225
GO:CC	intracellular organelle	6.94e-17	386
GO:CC	cytosolic large ribosomal subunit	9.52e-17	17
GO:CC	ribosomal subunit	7.01e-16	29
GO:CC	organelle	1.29e-15	388
GO:CC	mitochondrion	3.64e-13	82
GO:CC	non-membrane-bounded organelle	2.70e-12	188
GO:CC	intracellular non-membrane-bounded organelle	2.70e-12	188
GO:CC	ribonucleoprotein complex	2.14e-11	69
GO:CC	large ribosomal subunit	2.57e-11	20
GO:CC	cytoplasm	9.78e-11	291
GO:CC	membrane-bounded organelle	8.65e-08	322
GO:CC	intracellular membrane-bounded organelle	1.46e-07	312
GO:CC	organelle lumen	2.56e-07	143
GO:CC	intracellular organelle lumen	2.56e-07	143

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	membrane-enclosed lumen	2.56e-07	143
GO:CC	respirasome	5.31e-07	16
GO:CC	catalytic complex	5.79e-07	77
GO:CC	mitochondrial respirasome	1.57e-06	14
GO:CC	mitochondrial inner membrane	1.78e-06	29
GO:CC	nucleoplasm	1.94e-06	106
GO:CC	respiratory chain complex	6.15e-06	14
GO:CC	oxidoreductase complex	1.20e-05	16
GO:CC	organelle inner membrane	1.80e-05	29
GO:CC	cytosol	1.90e-05	101
GO:CC	nuclear lumen	1.95e-05	127
GO:CC	mitochondrial membrane	2.93e-05	34
GO:CC	nucleus	3.43e-05	208
GO:CC	mitochondrial envelope	8.32e-05	35
GO:CC	chromosome	1.20e-04	53
GO:CC	inner mitochondrial membrane protein complex	1.38e-04	16
GO:CC	mitochondrial protein-containing complex	2.05e-04	22
GO:CC	envelope	5.02e-04	45
GO:CC	organelle envelope	5.02e-04	45
GO:CC	small ribosomal subunit	7.43e-04	9

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:CC	cytosolic small ribosomal subunit	1.52e-03	6
GO:CC	respiratory chain complex I	3.69e-03	8
GO:CC	NADH dehydrogenase complex	3.69e-03	8
GO:CC	chromosomal region	5.59e-03	20
GO:CC	mitochondrial respiratory chain complex I	2.48e-02	7
GO:CC	chromosome, centromeric region	2.55e-02	15
GO:CC	preribosome	2.79e-02	9
GO:CC	condensed chromosome	3.62e-02	15
GO:CC	chromatin	4.43e-02	29
GO:CC	protein-DNA complex	4.56e-02	31

Molecular Function

GO:MF	structural constituent of ribosome	5.85e-16	42
GO:MF	structural molecule activity	2.04e-06	53
GO:MF	nucleic acid binding	4.11e-06	134
GO:MF	translation regulator activity	9.31e-06	17
GO:MF	catalytic activity, acting on a nucleic acid	9.93e-05	43
GO:MF	organic cyclic compound binding	1.05e-04	214
GO:MF	RNA binding	3.34e-04	61
GO:MF	catalytic activity, acting on DNA	4.35e-04	22
GO:MF	ligase activity	8.09e-04	20

GO Category	GO:Term	P value (Adjusted)	Number of DEGs
GO:MF	aminoacyl-tRNA ligase activity	1.05e-03	11
GO:MF	ligase activity, forming carbon-oxygen bonds	1.05e-03	11
GO:MF	translation regulator activity, nucleic acid binding	1.75e-03	13
GO:MF	translation factor activity, RNA binding	3.82e-03	12
GO:MF	NAD binding	4.94e-02	9

²¹² **SUPPLEMENTARY INFORMATION/MATERIALS**