Host Turkey B-cell Transcriptomics During THEV Infection Highlights Upregulated Cell Death and Breakdown Pathways and Other Biological Processes

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## ABSTRACT

## INTRODUCTION

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

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

Next generation sequencing (NGS) is a groundbreaking technology that has significantly enhanced our understanding of DNA and RNA structure and function and facilitated exceptional advancements in all domains of biology and the Life Sciences (12). mRNA sequencing (RNA-seq), an NGS approach to transcriptomic studies, is a versatile, high throughput, and cost-effective technology that allows a broad scan of the entire transcriptome, thereby uncovering the active genes and molecular pathways and processes. This technology has been leveraged in an ever increasing number of studies to elucidate active cellular processes under a wide range of treatment conditions, including the transcriptomics of viral infections (12–16). In RNA-seq studies, differentially expressed genes (DEGs) identified under different experimental conditions are key to unlocking the interesting biology or mechanism under study. Identified DEGs are typically used for functional enrichment analysis in large curated knowledgebases which connect genes to specific biological processes, functions, and pathways such as gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, shedding light on the biological question under study (17, 18).

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

## RESULTS

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

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

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

**Functional Enrichment Analyses (GO, KEGG pathway, and interaction network analyses)**  
Gene ontology (GO) enrichment analysis was performed for 12- and 24-hpi DEGs with the gprofiler2 (version **0.2.3**) R package (21), which outputs results in three GO categories – cellular components (CP), biological processes (BP), and molecular functions (MF). Results with Padjusted-value 0.05 were considered functionally enriched. The GO enrichment analyses results at 12-hpi and 24-hpi showed significant similarities among all three GO categories. At both time points, cellular breakdown processes were upregulated while cellular maintenance processes and structures were downregulated in all three GO categories (**Table 2A-B** and **Table 3A-B**).

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

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

KEGG analysis on the DEGs was performed using both the gprofiler2 R package (21) and the DAVID (Database for Annotation, Visualization and Integrated Discovery; version 2021) online resource (22). Both analysis resources gave similar results but the results from DAVID (**Table 4A**) includes more information than the gprofiler2 results (**Table 4B**). The KEGG pathway analysis was congruent with the GO results, revealing that generally, cell maintenance and upkeep pathways were downregulated while cell death and breakdown pathways were upregulated. Cell maintenance pathways such as DNA replication and repair, ribosome biogenesis, spliceosome, and oxidative phosphorylation were downregulated at 12-hpi. Similar pathways were downregulated at 24-hpi. Cell breakdown pathways such as: autophagy, response to virus (Influenza A), and steroid biosynthesis were upregulated at 12-hpi similar to 24-hpi, where pathways such as: autophagy, ubiquitin-mediated proteolysis, lysosome, protein processing in endoplasmic reticulum, and steroid biosynthesis were upregulated.

It is well-established that THEV induces cell death (apoptosis and necrosis) in infected B-cells, which is linked to the IMS associated with THEV (8, 11, 23). Hence, we are particularly interested in cellular processes and pathways associated with cell death and pathways that may affect the survivability of the host cells, thereby accounting for THEV-induced IMS. We highlight the upregulated cell death (apoptosis and autophagy) pathways, cellular metabolism, and responses to stimuli (especially ER-stress response) pathways identified by our GO and KEGG analyses as the likely key aspects of THEV-host cell interaction leading to IMS.

**Cell Death and Breakdown Pathways Upregulated by THEV**  
Many virus families, including adenoviruses, herpesviruses, poxviruses, baculoviruses, parvoviruses, retroviruses, rhabdoviruses, pararmyxoviruses, orthomyxoviruses, togaviruses, and picornaviruses are known to trigger apoptosis in infected host cells either by a direct action of a viral protein or due to the host antiviral response (24–26). Adenoviruses generally possess the protein, E1B 19K to inhibit host cell apoptosis long enough to complete their replication cycle (24, 26, 27). However, no such protein is known in THEV. A recent paper showed several novel transcripts and open reading frames (ORFs) in the genome of THEV which may offer a similar anti-apoptotic functions but the functions of these novel ORFs are yet to be shown (28). Our data shows that apoptotic and autophagic pathways are upregulated during THEV infection, supporting previous findings of apoptosis and necrosis of THEV-infected cells (8, 11, 23). For example, …, which are proapoptotic, were upregulated.

**Cellular Metabolism Changes During THEV Infection**  
Many viruses, such as hepatitis C virus, human cytomegalovirus, influenza virus, and rhinovirus, have been documented to manipulate the cellular metabolism process for their use

**Cellular Responses to Stimuli during THEV infection**

## DISCUSSION

We may not have seen a measurable immune response/pathway enrichment in the infected host cells because the these B-cells may likely require other immune cells such as macrophages and T-cells for activation/mount an immune response. Secondly, the curated data in the GO, and KEGG databases are most complete for human and other model organisms; hence, there may not be enough information curated for turkeys to highlight the anything that is not very strong immune response.

## CONCLUSIONS

## MATERIALS AND METHODS

**Cell culture and THEV Infection**  
The Turkey B-cell line (MDTC-RP19, ATCC CRL-8135) was grown as a suspension culture in 1:1 complete Leibovitz’s L-15/McCoy’s 5A medium with 10% fetal bovine serum (FBS), 20% chicken serum (ChS), 5% tryptose phosphate broth (TPB), and 1% antibiotic solution (100 U/mL Penicillin and 100/mL Streptomycin), at 41oC in a humidified atmosphere with 5% CO2. Infected cells were maintained in 1:1 serum-reduced Leibovitz’s L15/McCoy’s 5A media (SRLM) with 2.5% FBS, 5% ChS, 1.2% TPB, and 1% antibiotic solution. A commercially available THEV vaccine was purchased from Hygieia Biological Labs (VAS strain). The stock virus was titrated using an in-house qPCR assay with titer expressed as genome copy number (GCN)/mL, similar to Mahshoub *et al* (29). Cells were THEV-infected or mock-infected in triplicates or duplicates, respectively at a multiplicity of infection (MOI) of 100 GCN/cell, incubated at 41oC for 1 hour, and washed three times with phosphate buffered saline (PBS) to get rid of free virus particles. At each time point (4-, 12-, 24-, and 72-hpi), triplicate (THEV-infected) and duplicate (mock-infected) samples were harvested for total RNA extraction.

**RNA extraction and Sequencing**  
Total RNA was extracted from infected cells using the Thermofisher RNAqueous™-4PCR Total RNA Isolation Kit (which includes a DNase I digestion step) per manufacturer’s instructions. An agarose gel electrophoresis was performed to check RNA integrity. The RNA quantity and purity was initially assessed using nanodrop, 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. Extracted total RNA samples were sent to LC Sciences, Houston TX for poly-A-tailed mRNA sequencing. RNA integrity was checked with Agilent Technologies 2100 Bioanalyzer High Sensitivity DNA Chip and poly(A) RNA-seq library was prepared following Illumina’s TruSeq-stranded-mRNA sample preparation protocol. Paired-end sequencing, generating 150 bp reads was performed on the Illumina NovaSeq 6000 sequencing system. The paired-end 150bp sequences obtained during this study and all expression data have been submitted to the Gene Expression Omnibus database, under accession no #######

**Quality Control and Mapping Process**  
Sequencing reads were processed following a well-established protocol described by Pertea *et al* (19), using Snakemake - version 7.32.4 (30), a popular workflow management system to drive the pipeline. Briefly, raw sequencing reads were trimmed with Cutadapt - version 1.10 (31) and the quality of trimmed reads evaluated using the FastQC software, version 0.12.1 (Bioinformatics Group at the Babraham Institute, Cambridge, United Kingdom; www.bioinformatics.babraham.ac.uk), achieving an overall Mean Sequence Quality (PHRED Score) of 36. Trimmed reads were mapped the reference *Meleagris gallopavo* genome (<https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/GCF_000146605.3_Turkey_5.1_genomic.fna.gz>) with Hisat2 - version 2.2.1 (19) using the accompanying gene transfer format (GTF) annotation file (<https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/146/605/GCF_000146605.3_Turkey_5.1/GCF_000146605.3_Turkey_5.1_genomic.gtf.gz>) to build a genomic index. Samtools - version 1.19.2 was used to convert the output Sequence Alignment Map (SAM) file to the more manageable Binary Alignment Map (BAM) format. The StringTie (v2.2.1) software (19), set to expression estimation mode was used to generate normalized gene expression estimates from the BAM files for genes in the reference GTF file after which the prepDE.py3 script was used to extract read count information from the StringTie gene expression files, providing an expression-count matrix for downstream DEG analysis.

**DEG Analysis and Functional Enrichment Analysis**  
DEG analysis between mock- and THEV-infected samples was performed using the very popular DESeq2 (20), which employs a Negative Binomial distribution model for read count comparisons. Genes with Padjusted-value 0.05 were considered as differentially expressed. The read count data are deposited at Gene Expression Omnibus (GEO) under accession number ###. The functional profiling of DEGs (GO and KEGG analyses) were performed based on GO databases and KEGG databases using the R package gprofiler2 (21) with *Meleagris gallopavo* as the reference organism. Results with Padjusted-value 0.05 were included as functionally enriched. Additionally, the DAVID (Database for Annotation, Visualization and Integrated Discovery; version 2021) online analysis tool was used for KEGG pathway analysis. All visualization plots were made using ggplot2, pheatmap, and ggvenn R packages (32–34).

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

**Statistical Analysis**

## DATA AVAILABILITY

## CODE AVAILABILITY

## ACKNOWLEDGMENTS

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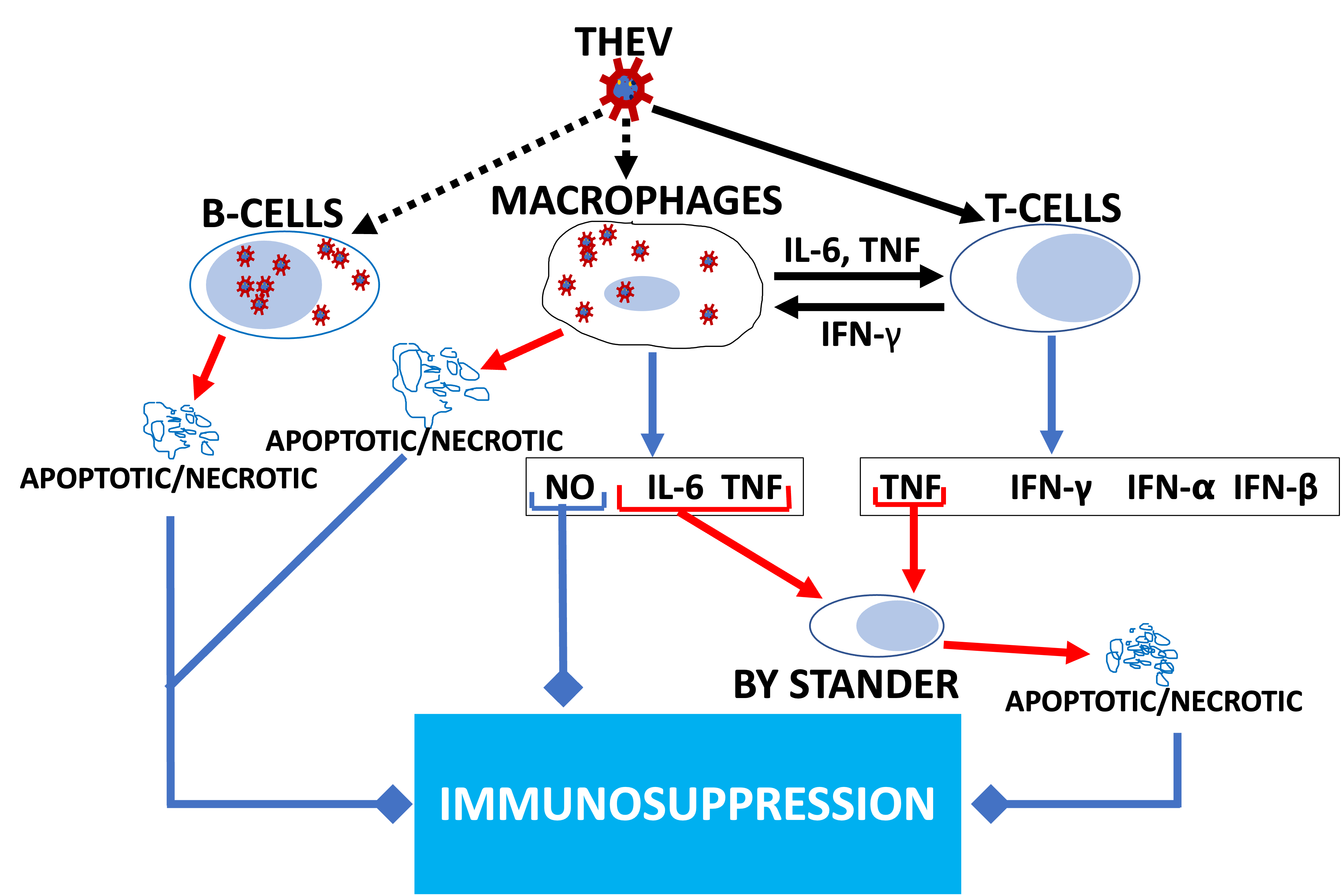
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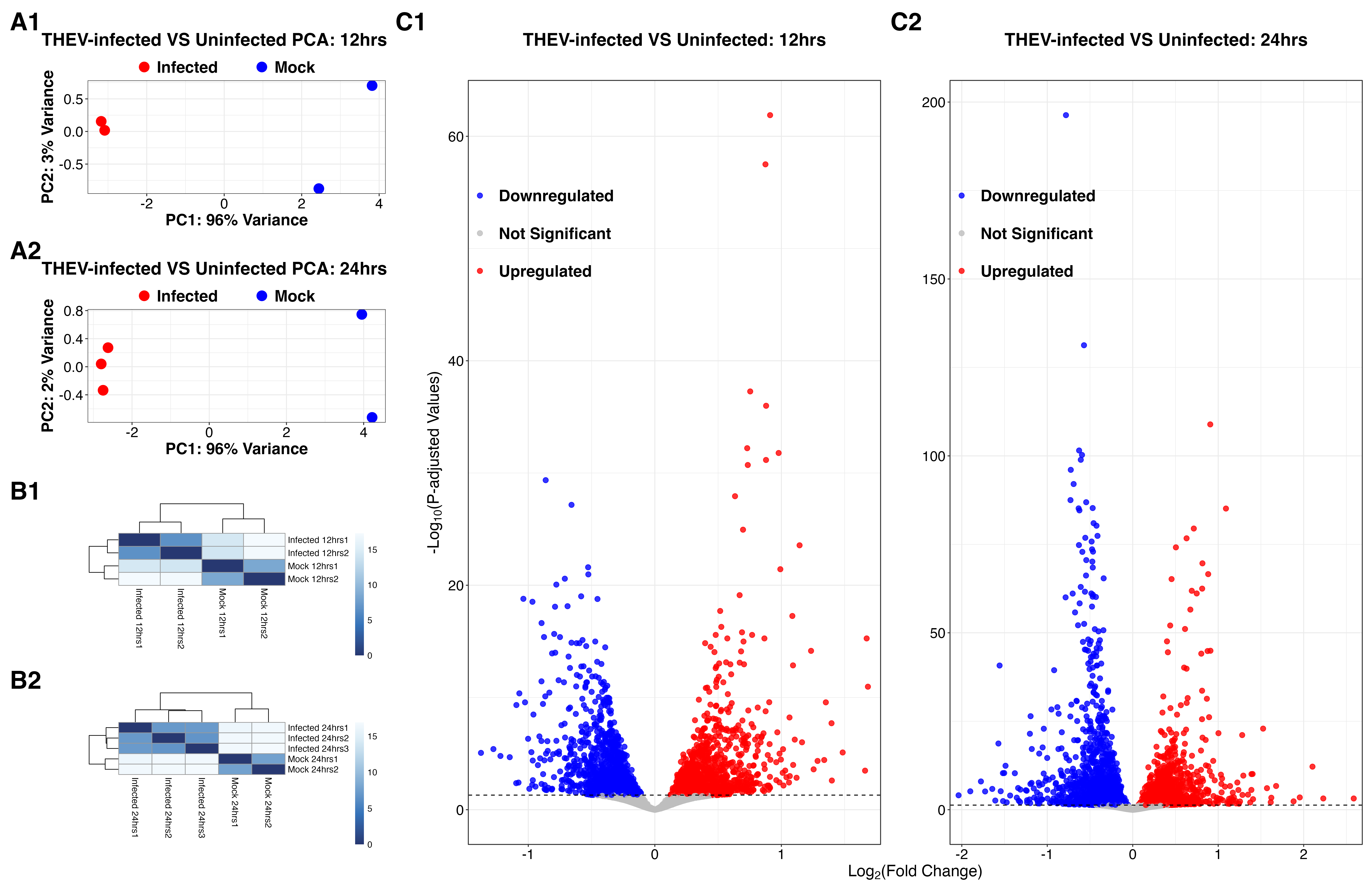
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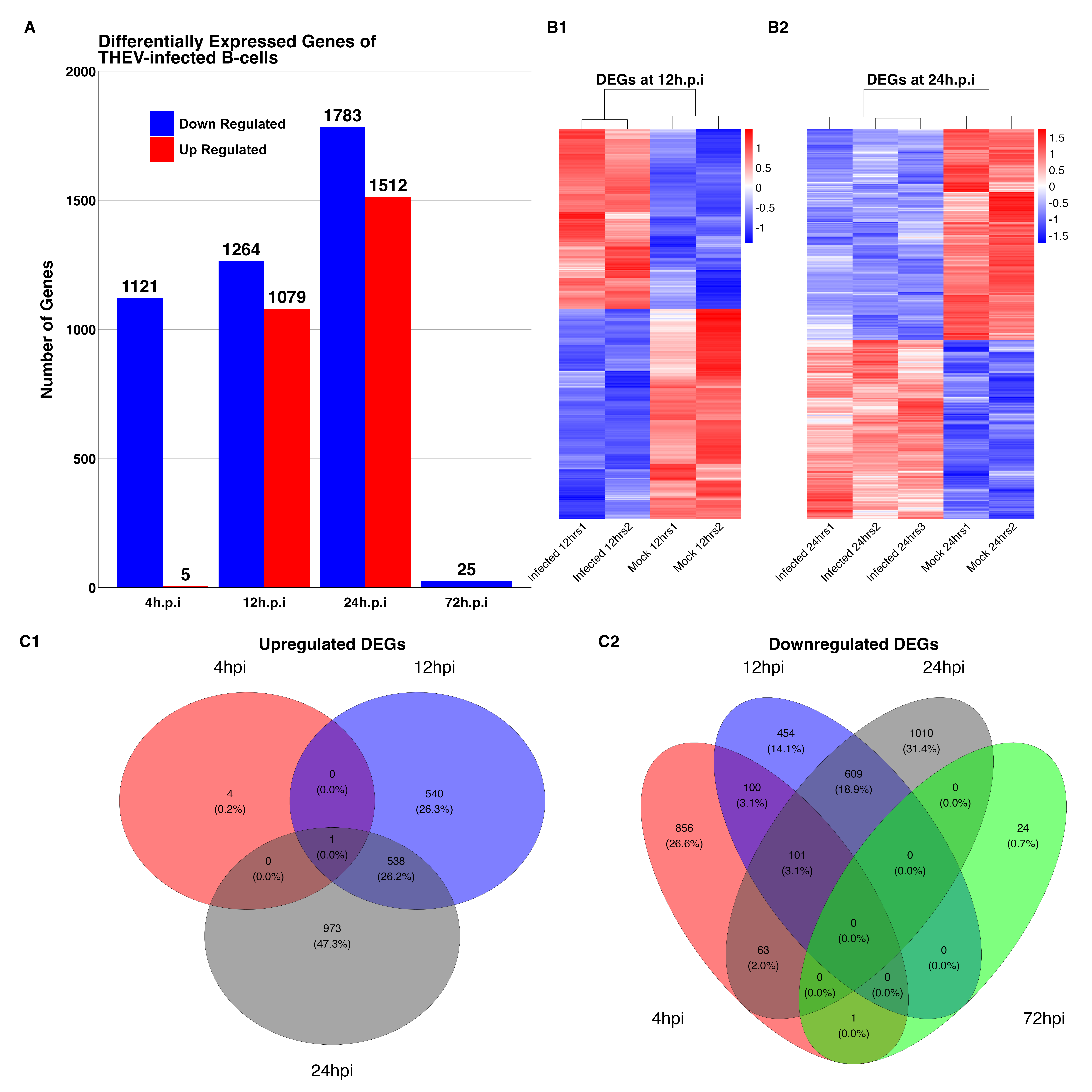
## TABLES AND FIGURES



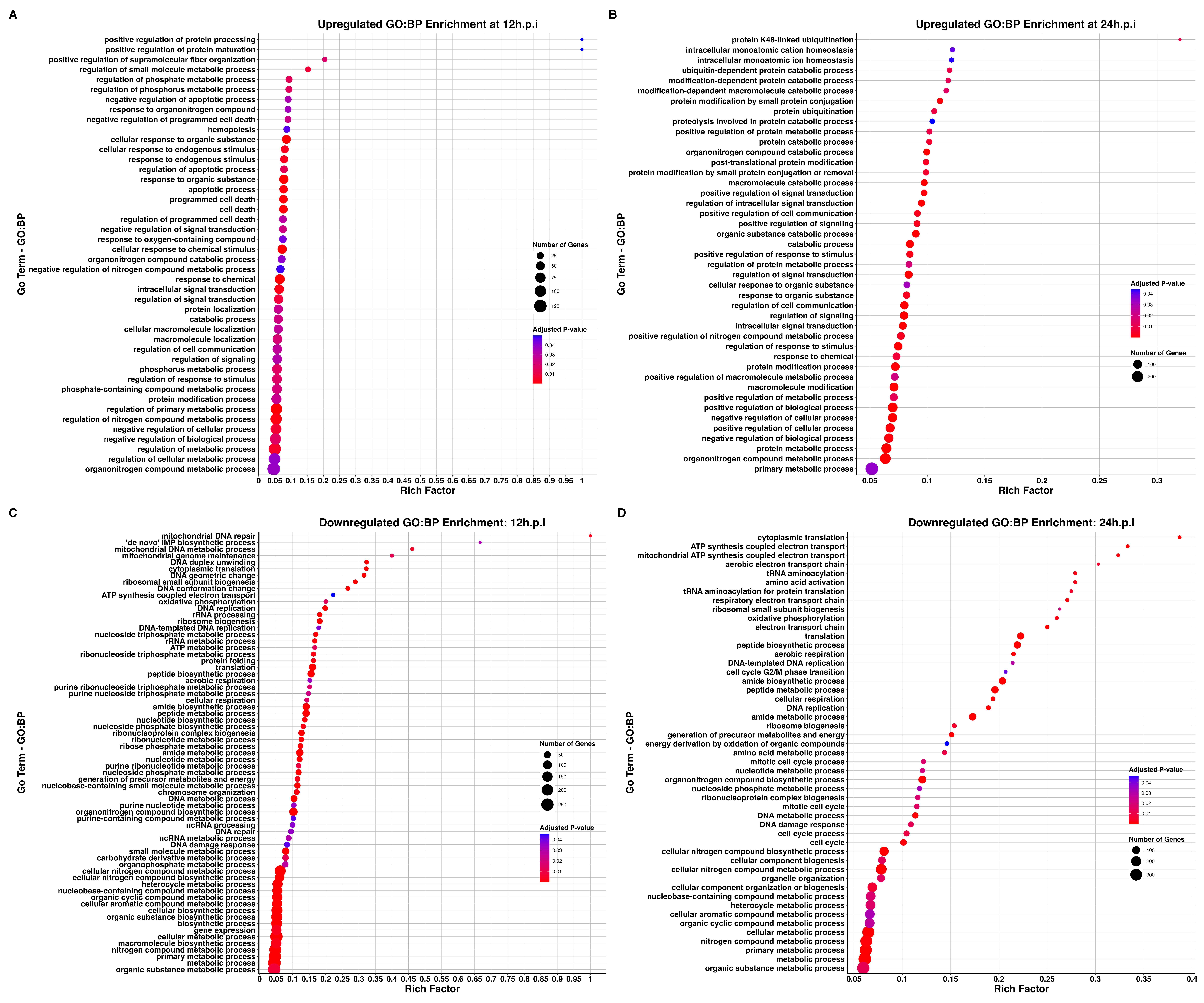
**Figure 1: Model of THEV-induced immunosuppression in turkeys**. THEV infection of target cells is indicated with black dotted arrows. Black unbroken arrows indicate cell activation. Red arrows indicated signals leading to apoptosis. Blue arrows indicate all cytokines released by the cell. Blue arrows 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: 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 ReadsM** | **Trimmed ReadsM** | **Mapped ReadsM** | **Uniquely Mapped  ReadsM** | **Non-uniquely  Mapped ReadsM** | **Q20%** | **Q30%** | **GC Content (%)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| I\_12hrsS1Inf | 40.6 | 39.0 | 34.7 (88.92%) | 33.1 (84.78%) | 1.6 (4.14%) | 99.95 | 97.23 | 47.5 |
| I\_12hrsS3Inf | 38.8 | 37.3 | 33.1 (88.78%) | 31.7 (84.95%) | 1.4 (3.83%) | 99.95 | 97.53 | 47.5 |
| I\_24hrsS1Inf | 42.7 | 41.0 | 36.2 (88.13%) | 34.5 (84.2%) | 1.6 (3.93%) | 99.95 | 96.95 | 46.5 |
| I\_24hrsS2Inf | 42.0 | 40.4 | 35.6 (88.1%) | 33.9 (83.83%) | 1.7 (4.27%) | 99.94 | 97.05 | 46.5 |
| I\_24hrsS3Inf | 40.5 | 38.9 | 34.2 (88.01%) | 32.7 (84.12%) | 1.5 (3.89%) | 99.95 | 97.08 | 47.0 |
| I\_4hrsS1Inf | 39.1 | 37.4 | 33 (88.16%) | 31.2 (83.43%) | 1.8 (4.73%) | 99.93 | 97.04 | 48.5 |
| I\_4hrsS2Inf | 41.3 | 39.6 | 35.3 (89.24%) | 33.6 (84.92%) | 1.7 (4.33%) | 99.95 | 97.15 | 47.0 |
| I\_4hrsS3Inf | 41.5 | 39.8 | 35.5 (89.2%) | 33.2 (83.29%) | 2.4 (5.91%) | 99.95 | 97.11 | 47.5 |
| I\_72hrsS1Inf | 41.2 | 39.8 | 28.3 (71.09%) | 26.9 (67.7%) | 1.3 (3.38%) | 99.96 | 97.23 | 44.5 |
| I\_72hrsS2Inf | 39.3 | 38.0 | 27 (71.11%) | 25.8 (67.86%) | 1.2 (3.25%) | 99.96 | 97.34 | 44.5 |
| I\_72hrsS3Inf | 39.9 | 37.1 | 28.3 (76.36%) | 26.1 (70.3%) | 2.2 (6.05%) | 99.87 | 96.14 | 52.5 |
| U\_12hrsN1Mk | 42.1 | 40.4 | 35.9 (88.72%) | 34.1 (84.39%) | 1.7 (4.33%) | 99.95 | 97.04 | 47.5 |
| U\_12hrsN2Mk | 41.0 | 39.3 | 34.7 (88.4%) | 33.2 (84.53%) | 1.5 (3.86%) | 99.94 | 97.08 | 47.5 |
| U\_24hrsN1Mk | 38.4 | 37.0 | 32.7 (88.46%) | 31.4 (84.74%) | 1.4 (3.72%) | 99.96 | 97.48 | 47.5 |
| U\_24hrsN2Mk | 39.9 | 38.4 | 34 (88.58%) | 32.6 (84.96%) | 1.4 (3.61%) | 99.95 | 96.95 | 47.0 |
| U\_4hrsN1Mk | 39.4 | 37.9 | 33.7 (88.9%) | 32 (84.41%) | 1.7 (4.49%) | 99.96 | 97.36 | 47.0 |
| U\_4hrsN2Mk | 37.6 | 34.7 | 22 (63.43%) | 18.5 (53.18%) | 3.6 (10.25%) | 99.80 | 94.96 | 61.0 |
| U\_72hrsN1Mk | 50.3 | 47.9 | 15.5 (32.4%) | 11.7 (24.5%) | 3.8 (7.9%) | 99.88 | 96.54 | 56.0 |
| U\_72hrsN2Mk | 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; | | | | | | | | |
| InfThese are infected samples indicated by the letter 'I' and 'S' in sample names | | | | | | | | |
| MkThese 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** | **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: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: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 |
| **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: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: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: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: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: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: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: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: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: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: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: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: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: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: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: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: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: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: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: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 |

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

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

Table 4B: 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 |

## SUPPLEMENTARY INFORMATION/MATERIALS