Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Integrative Transcriptomics Identifies Ubiquitination-Related Genes *BIRC2*, *COPS5*, and *TBK1* as Novel Biomarkers of T-Cell Dysregulation in Amyotrophic Lateral Sclerosis

Ailing Jiang, Yanzhen Huang, Xianting Que, Chonglin Li, Ziqun Lin, Wen Huang

Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China

Correspondence: Wen Huang, Department of Neurology, The First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Nanning, Guangxi, 530021, People's Republic of China, Tel +86-771-5356504, Email hwen1229@163.com

Purpose: Amyotrophic lateral sclerosis (ALS) is marked by immune dysregulation; however, the role of T cell-ubiquitination-related genes (TURGs) in its pathogenesis remains unclear. This study aimed to investigate the contribution of TURGs to T-cell dysfunction and ubiquitination imbalance in ALS.

Patients and Methods: Differentially expressed genes were identified through analysis of bulk transcriptomes (GSE112680). CIBERSORT deconvolution and weighted gene co-expression network analysis were employed to define T-cell-associated modules. Integration with ubiquitination-related gene sets yielded T cell–ubiquitination-related differentially expressed genes (TURDEGs). Functional enrichment analysis and protein-protein interaction network construction, combined with multi-algorithm selection, facilitated the development of a risk-prediction model. Mechanistic insights were derived from Gene Set Enrichment Analysis, immune profiling, co-expression and regulatory network analyses, and drug-target prediction. Single-cell transcriptomic analysis provided insights into cellular-level pathogenic mechanisms in ALS. qPCR was used to validate core TURDEGs expression in peripheral blood samples from patients with ALS.

Results: Thirty-nine TURDEGs were identified and exhibited significant enrichment in pathways related to ubiquitination, immune activation, autophagy, and NOD-like receptor signaling. BIRC2, COPS5, and TBK1 were identified as core genes. The resulting risk-prediction model demonstrated significant potential for clinical application. Immune infiltration analysis revealed positive correlations between core genes and $CD4^+$ resting memory T cells, as well as negative correlations between *COPS5, TBK1*, and regulatory T cells. Adavosertib and MRS2211 were identified as potent modulators of *TBK1* and *BIRC2*, respectively. Single-cell transcriptomics highlighted enhanced T cell–neutrophil interactions, suggesting a remodeling of the immune communication network in ALS. qPCR validation confirmed significantly increased expression of these genes in patients with ALS ($p<0.05$).

Conclusion: TURDEGs-mediated T cell dysfunction and ubiquitination imbalance play critical roles in ALS pathogenesis, unveiling novel biomarkers and potential personalized therapeutic targets.

Keywords: amyotrophic lateral sclerosis, t cell ubiquitination-related genes, immune dysregulation, risk-prediction model, biomarkers

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare, universally fatal neurodegenerative disorder characterized by the progressive degeneration of motor neurons. Its incidence ranges from 0.26 to 2.46 cases per 100,000 individuals, with a median survival of 3–5 years post-diagnosis.[1] The pathogenesis of ALS is complex, involving excitotoxicity, oxidative stress, mitochondrial dysfunction, RNA metabolic disturbances, abnormal neuron–immune interactions, and proteostasis imbalance.[2,3] While riluzole and edaravone have been approved for use, effective treatments that significantly improve patient prognosis remain scarce, highlighting the urgent need to better understand the underlying pathogenic mechanisms and identify promising therapeutic targets.[4]

https://doi.org/10.2147/JIR.S585872
Received: 3 December 2025
Accepted: 11 April 2026
Published: 5 May 2026

Although the central nervous system (CNS) is traditionally considered immune-privileged, recent research has shown that peripheral immune cells can infiltrate the CNS through the cerebrospinal fluid or microcracks in the blood-brain barrier, thereby influencing ALS pathology.[5,6] Immune dysfunction is not only a hallmark of ALS but also a potential contributor to its progression.[7] Moreover, immune system activation in ALS establishes a chronic pro-inflammatory state, that fosters reciprocal interactions between the CNS and peripheral immune compartments.[8] Persistent inflammation undermines barrier integrity, accelerating motor neuron degeneration, axonal injury, and neuromuscular junction dysfunction.[9] Activated $CD4^+$ memory T cells secrete interferon-γ and interleukin-17, exacerbating neuroinflammation, while reduced frequencies of regulatory T (Treg) cells correlate with faster disease progression and decreased survival in ALS.[6,10] Treg-based therapies have been shown to prolong survival and reduce neuronal loss, highlighting their neuroprotective potential.[11] These findings suggest that T cell dysfunction plays a role in ALS progression; however, the involvement of T cell–related genes (TRGs) remains poorly understood.

The imbalance in the ubiquitin–proteasome system (UPS) is a hallmark of ALS pathogenesis, driving disease progression by disrupting proteostasis and impairing stress responses.[12] Ubiquitinated protein aggregates have been detected in neurons of patients with ALS, and UPS dysfunction exacerbates the accumulation of misfolded proteins, ultimately triggering motor neuron apoptosis.[13] Key regulators of ubiquitination, such as *OPTN* and *UBQLN2*, coordinate UPS activity with selective autophagy. Disruption of these regulators promotes intraneuronal aggregate formation and impairs clearance, thereby increasing ALS susceptibility.[14,15] In addition to its role in neuronal proteostasis, the UPS also influences immune cell function and differentiation. For example, E3 ligase Cbl-b lowers the activation threshold of T cells, leading to functional dysregulation.[16–18] However, the precise molecular mechanisms linking UPS imbalance to T cell dysfunction in ALS remain poor understood.

This study integrated bulk transcriptomic and single-cell RNA sequencing data through bioinformatics analyses and validated the finding via qPCR to identify T cell–ubiquitination-related differentially expressed genes (TURDEGs) in ALS. Additionally, the cell-type-specific expression patterns and functional roles of key TURDEGs within the ALS immune microenvironment were characterized. Our results identified *BIRC2, COPS5*, and *TBK1* as core TURDEGs. Using these genes, this study developed an ALS risk-prediction model and unveiled a synergistic pathogenic axis linking T cell dysfunction with UPS imbalance in ALS. These findings offer a framework for early diagnosis based on potential biomarkers and highlight personalized therapeutic targets for future intervention.

Materials and Methods

Data Collection

The GSE112680 dataset,[19] which includes 164 ALS and 137 control samples, was retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) and used as the training set. The GSE112676 dataset, comprising 233 ALS and 508 control samples, was downloaded as the validation set.[19] Clinical details are provided in Supplementary Table S1. Notably, GSE112680 was selected for the training set due to its inclusion of patients early-stage ALS (mean disease duration: 12.5±4.1 months), while GSE112676 with its larger cohort and longitudinal design, was used as the validation set. A total of 1,295 ubiquitin-related genes (URGs) were curated from the IUUCD database (https://iuucd.biocuckoo.org/) (Supplementary Table S2). Additionally, the single-cell RNA-seq dataset GSE244263,[20] including 10 controls and 30 ALS samples, was retrieved from the GEO database.

Identification of TURDEGs in ALS

Differentially expressed genes (DEGs) in the GSE112680 dataset were identified using the "limma" R package.[21] Immune infiltration analysis was performed with the CIBERSORT algorithm, and a Wilcoxon rank-sum test ($p < 0.05$) was applied to identify significantly different T cell subtypes. Weighted gene co-expression network analysis (WGCNA) was then conducted using the infiltration levels of the distinct T cell subtypes as phenotypic traits to identify gene modules associated with these phenotypes. The resulting modules were intersected with DEGs and URGs to pinpoint TURDEGs.

Functional Enrichment Analysis of TURDEGs

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of TURDEGs were performed with the R package "clusterProfiler".[22] KEGG pathway visualization was conducted using the R package "Pathview".[23]

Identification of Core TURDEGs

A protein-protein interaction (PPI) network was constructed using the STRING database (https://cn.string-db.org/) with a confidence score threshold of >0.4. The CytoHubba plugin in Cytoscape software (version 3.9.1) was used to select the top 10 genes identified by each of the eight algorithms for further screening of key genes. Core TURDEGs were defined as those exhibiting consistent expression trends between the training and the validation sets, as determined by the Wilcoxon rank-sum test. Spearman correlation analysis was performed to assess correlations among the core TURDEGs, and binary logistic regression analysis was applied to evaluate their association with ALS risk.

Construction and Evaluation of the ALS Risk Prediction Model

A nomogram was generated using the "rms" package (version 6.5.0) to predict ALS risk, assigning weighted scores to each core TURDEG, with the sum reflecting overall disease probability. Model accuracy and discrimination were evaluated through calibration curves generated by the "calibrate" function in RMS. Decision curve analysis (DCA), performed with ggDCA (v1.1), quantified the net clinical benefit across thresholds,[24] while clinical impact curves plotted with the "clinicalrisk" package provided additional validation of predictive performance. Receiver operating characteristic (ROC) analysis was performed to calculate the area under the curve (AUC) and its 95% confidence interval (CI), with an AUC > 0.7 considered indicative of good diagnostic performance.

Gene Set Enrichment Analysis (GSEA) of Core TURDEGs

Spearman correlation coefficients between each core TURDEG and the transcriptome were calculated using the "psych" R package (version 2.4.3). GSEA of core TURDEGs was performed using the "clusterProfiler" package against the MSigDB KEGG collection (c2.cp.kegg.v7.5.1.symbols.gmt). Pathways meeting significance criteria ($p < 0.05$ and $|NES| > 1$) were ranked by p-value, and the top five enriched terms were reported.

Correlation Analysis Between Immune Cells and Core TURDEGs

Spearman correlations among differentially infiltrated immune cell subsets were calculated using the "psych" R package (version 2.4.3) and visualized as a heatmap with the "corrplot" package. To further explore relationships between core genes and immune cell populations, Spearman correlation analysis was repeated with the identified infiltration profiles, and results were visualized as lollipop plots generated in "ggplot2" (version 3.5.1).

Co-Expression and Regulatory Network Analysis of Core TURDEGs

A co-expression network of core TURDEGs was constructed using the GeneMANIA database. Functional similarity analysis (Friends analysis) among these genes was conducted with the "GOSemSim" package.[25] Subcellular localization predictions were retrieved from the mRNALocater database.[26] Candidate miRNAs and transcription factors targeting the core genes were predicted through NetworkAnalyst, and an integrated TF–mRNA–miRNA regulatory network was constructed in Cytoscape (version 3.9.1).

Drug Prediction and Protein Regulation Analysis of Core TURDEGs

Drug–gene interactions involving the core TURDEGs were systematically catalogued through the Drug–Gene Interaction Database (DGIdb) and visualized as a regulatory network in Cytoscape (version 3.9.1). To investigate the spatial conformation of the proteins encoded by the core genes and their functional associations, the crystal structure files of TURDEGs were downloaded from the RCSB Protein Data Bank (PDB, https://www.rcsb.org/) and the three-dimensional structures of the proteins

were visualized. Relevant supplementary information from the GeneCards database (https://www.genecards.org/) was integrated to provide functional annotations for these genes and their protein effectors, guiding subsequent analyses.

Single-Cell Transcriptomic Analysis

Single-cell transcriptomes from GSE244263 were encapsulated in a Seurat object[27] and subjected to quality control, normalization, and Principal Component Analysis (PCA) for dimensionality reduction. Cell identities were annotated using scMayoMap, and cluster-level functional enrichment was performed with Reactome GSA,[28] integrating Fisher's exact and rank-sum tests to identify key cell types associated with ALS. Pseudotemporal trajectories were inferred using Monocle2, and intercellular signaling networks were reconstructed with CellChat.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Verification

Blood samples were collected from five patients with ALS and five healthy controls at the First Affiliated Hospital of Guangxi Medical University. Clinical details are provided in Supplementary Table S3. Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, USA), and reverse transcribed into complementary DNA (cDNA). The relative expression levels of key genes were measured by RT-qPCR. The qPCR was conducted using cDNA as the template and the following primers: GAPDH forward, CGGAGTCAACGGATTTGGTCGTAT; GAPDH reverse, AGCCTTCTCCATGGTGGTGAAGAC; TBK1 forward, CCTCCCTAAAGTACATCCACG; TBK1 reverse, CAATCAGCCATCGTATCCCC; BIRC2 forward, CTGTGGTGGGAAGCTCAGTA; BIRC2 reverse, TCATTCGAGCTGCATGTGTC; COPS5 forward, AACTGGCCAACAACATGCAG; COPS5 reverse, CTGGCATGCATCACCATCTT. Gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method. Data visualization was performed with GraphPad Prism (version 10.1.0). Written informed consent was obtained from all participants, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Statistical Analysis

All statistical analyses were conducted using R (v4.2.2) or GraphPad Prism (v10.1.0). Differences in gene expression between groups were assessed using the two-sided Wilcoxon rank-sum test. Associations between core genes and ALS risk were evaluated through univariate and binary logistic regression within a generalized linear model framework. Spearman correlation was applied to quantify inter-molecular relationships, and Fisher's exact test was used to compare cell-cluster distributions. Statistical significance was defined as $p<0.05$.

Results

Identification of TURDEGs in ALS

A total of 987 DEGs were identified in the GSE112680 dataset, comprising 539 upregulated and 448 downregulated genes (Figure 1A). Immune cell populations were quantified per sample using the CIBERSORT algorithm, which revealed six differentially infiltrated immune cell subsets between ALS and control groups, including three T cell subtypes: activated $CD4^+$ memory T cells, resting $CD4^+$ memory T cells, and Tregs (Figure 1B). WGCNA was conducted with the infiltration levels of these T cell subsets as traits, identifying two key modules: the turquoise module (2,120 genes) positively correlated with resting $CD4^+$ memory T cells ($r = 0.37$) and negatively correlated with Tregs ($r=–0.30$), while the brown module (955 genes) showed a negative correlation with activated $CD4^+$ memory T cells ($r=–0.35$) (Figure 1C and D). By intersecting the 987 DEGs, 3,075 module genes, and 1,295 URGs, 39 TURDEGs were identified (Figure 1E and Supplementary Table S4).

Functional Enrichment Analysis of TURDEGs

Functional analysis of the 39 TURDEGs through GO and KEGG enrichment revealed significant associations with protein polyubiquitination, proteasome-mediated ubiquitin-dependent protein catabolism, and immune-response activating signaling pathways in the GO Biological Process category. Cellular Component terms highlighted the ubiquitin ligase complex, cullin–RING ubiquitin ligase complex, and nuclear speckles. Molecular Function terms were predominantly

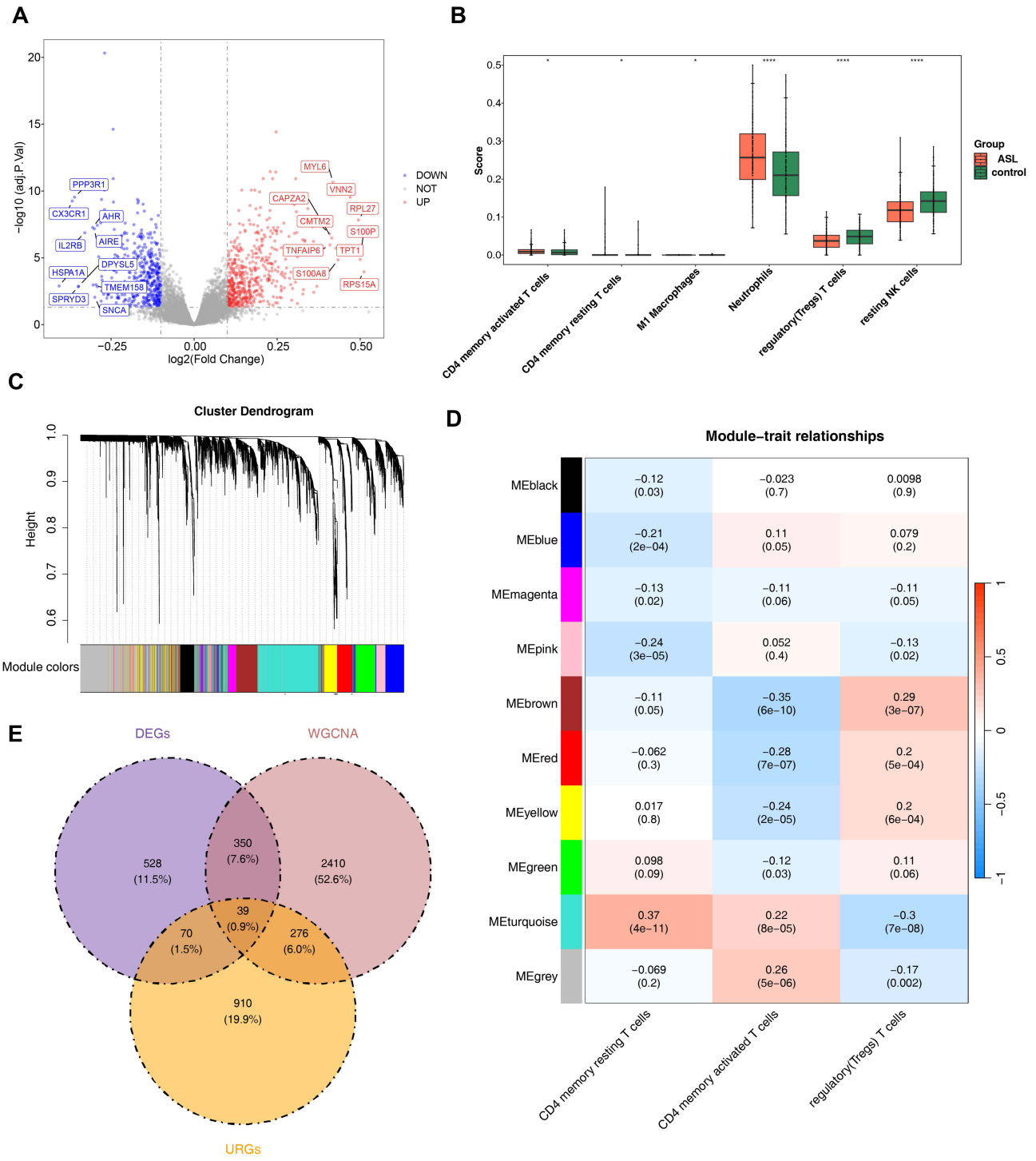

Figure 1 T cell–ubiquitination-related differentially expressed genes (TURDEGs) in amyotrophic lateral sclerosis (ALS). (**A**) Volcano plot showing differentially expressed genes between ALS and control samples; red indicates significantly upregulated genes, blue indicates significantly downregulated genes, and gray represents non-significant genes. (**B**) Differential infiltration analysis of immune cell subsets, *p < 0.05, **** p < 0.0001. (**C**) Identification of co-expression modules via Weighted Gene Co-expression Network Analysis (WGCNA). (**D**) Heatmap of module–trait correlations obtain through WGCNA. (**E**) Venn diagram for identifying TURDEGs.

related to ubiquitin-like protein transferase activity, amino acyltransferase activity, and ubiquitin-protein transferase activity (Figure 2A). KEGG pathway analysis further indicated involvement in ubiquitin-mediated proteolysis, autophagy and mitophagy, NOD-like receptor signaling, and pathogen-related pathways, including Yersinia infection, Kaposi sarcoma-associated herpesvirus infection, small cell lung cancer, and shigellosis (Figure 2B).

https://doi.org/10.2147/JIR.S585872

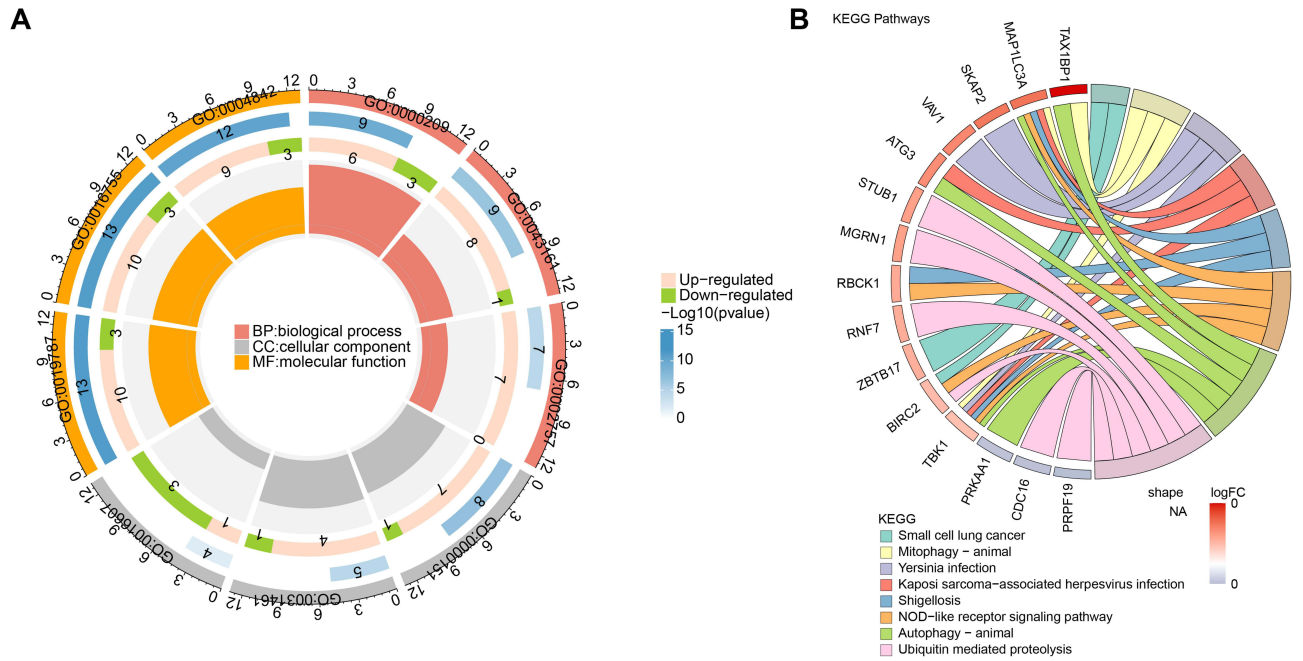

Figure 2 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for T cell–ubiquitination-related differentially expressed genes (TURDEGs). (**A**) GO analysis diagram for TURDEGs: The first layer displays GO annotation function IDs, with red indicating biological processes, grey indicating cellular components, and yellow representing molecular functions. The second layer visualizes the significance level, with color intensity representing the number of genes enriched in each function, while bar length and width correspond to gene count. The third layer shows the counts of up-regulated or down-regulated genes for each function, with colors differentiating between them. The innermost color blocks represent distinct functions, with block size reflecting the Rich Factor of the corresponding pathway. (**B**) KEGG pathway enrichment analysis diagram: The left half of the circular diagram lists enriched gene names, with color intensity representing the magnitude of logFC differences; darker shades correspond to greater fold changes. The right half illustrates the enriched functional pathways.

Identification and Validation of Core TURDEGs

PPI analysis of the 39 candidate genes, conducted via STRING, generated a network of 27 nodes and 35 edges (Figure 3A). Core TURDEGs were defined by intersecting the top 10 nodes ranked by eight centrality algorithms, resulting in *COPS5, RBCK1, BIRC2*, and *TBK1* (Figure 3B). To validate the robustness and reproducibility of these biomarkers, expression trends of these four genes were evaluated in both the training dataset (GSE112680) and the validation dataset (GSE112676). *BIRC2, COPS5*, and *TBK1* consistently exhibited significantly upregulated expression in both datasets, establishing them as core TURDEGs (Figure 3D–E). In contrast, although *RBCK1* showed significant upregulation in the training set, it did not reach statistical significance in the validation set and was excluded from further analysis. Spearman correlation analysis revealed strong positive correlations between *BIRC2* and both *COPS5* (r = 0.65) and *TBK1* (r = 0.70) (Figure 3C). Finally, binary logistic regression analysis confirmed that *COPS5, BIRC2*, and *TBK1* were strongly positively associated with ALS risk (Figure 3F–H).

Construction and Evaluation of the ALS Risk Prediction Model

A nomogram integrating the expression levels of *BIRC2, COPS5*, and *TBK1* was developed to quantify ALS risk (Figure 4A). Calibration curve demonstrated alignment between predicted probabilities and actual disease prevalence, closely approximating the ideal prediction curve (dashed line) (Figure 4B and C). CIC analysis revealed that high risk predictions were strongly correlated with actual case counts (Figure 4D). The multi-gene model outperformed single-gene models in identifying high-risk individuals, highlighting its enhanced clinical applicability. The area under the receiver operating characteristic curve (AUC) was 0.60, indicating moderate discriminatory power, although the model's overall predictive performance remained limited (Figure 4E).

https://doi.org/10.2147/JIR.S585872

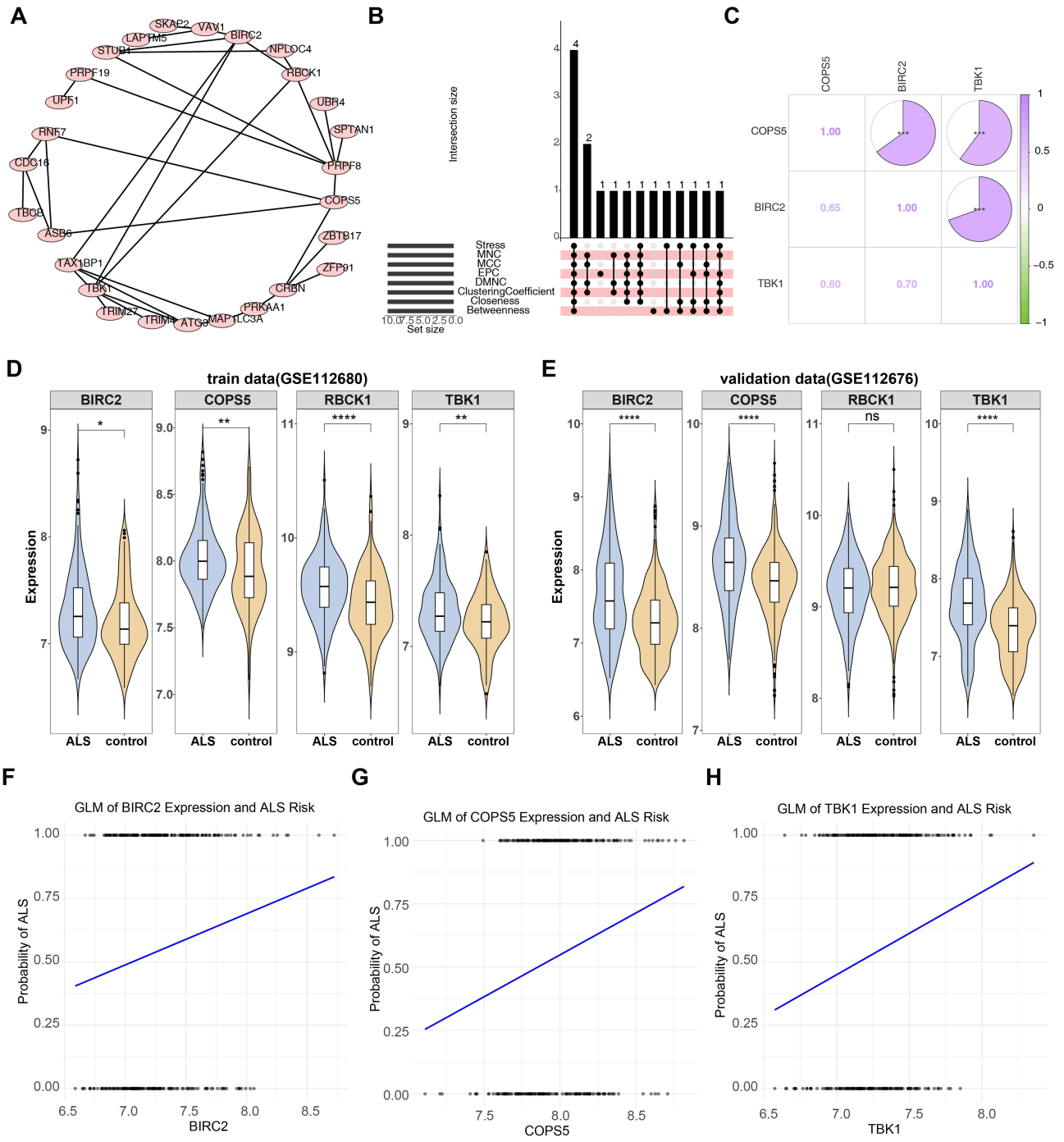

Figure 3 Identification and validation of core T cell–ubiquitination-related differentially expressed genes (TURDEGs). (**A**) Protein–protein interaction network of 39 candidate genes as rendered by STRING; nodes represent proteins, and edges indicate documented interactions. (**B**) Intersection of the top 10 hub genes selected by eight centrality algorithms, visualized as an UpSet plot: left bars represent the size of each algorithm's top 10 set, and the right matrix shows the size of each intersection. (**C**) Spearman correlation analysis among the four core TURDEGs, *** $p < 0.001$. (**D**) Differential expression of core TURDEGs in GSE112680 training set, *$p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. (**E**) Differential expression of core TURDEGs in the GSE112676 validation set, **** $p < 0.0001$, ns, no significance. (**F–H**) Binary logistic regression analyses illustrating the association between the expression of each core gene and amyotrophic lateral sclerosis.

GSEA and Immune Cell Correlation

To explore the signaling pathways and biological functions associated with *COPS5, BIRC2*, and *TBK1*, GSEA was performed. *COPS5* was significantly enriched in processes relate to ribosome function, regulation of the actin cytoskeleton, oxidative phosphorylation, lysosomal function, and pathways linked to Parkinson's disease. *BIRC2* showed

https://doi.org/10.2147/JIR.S585872

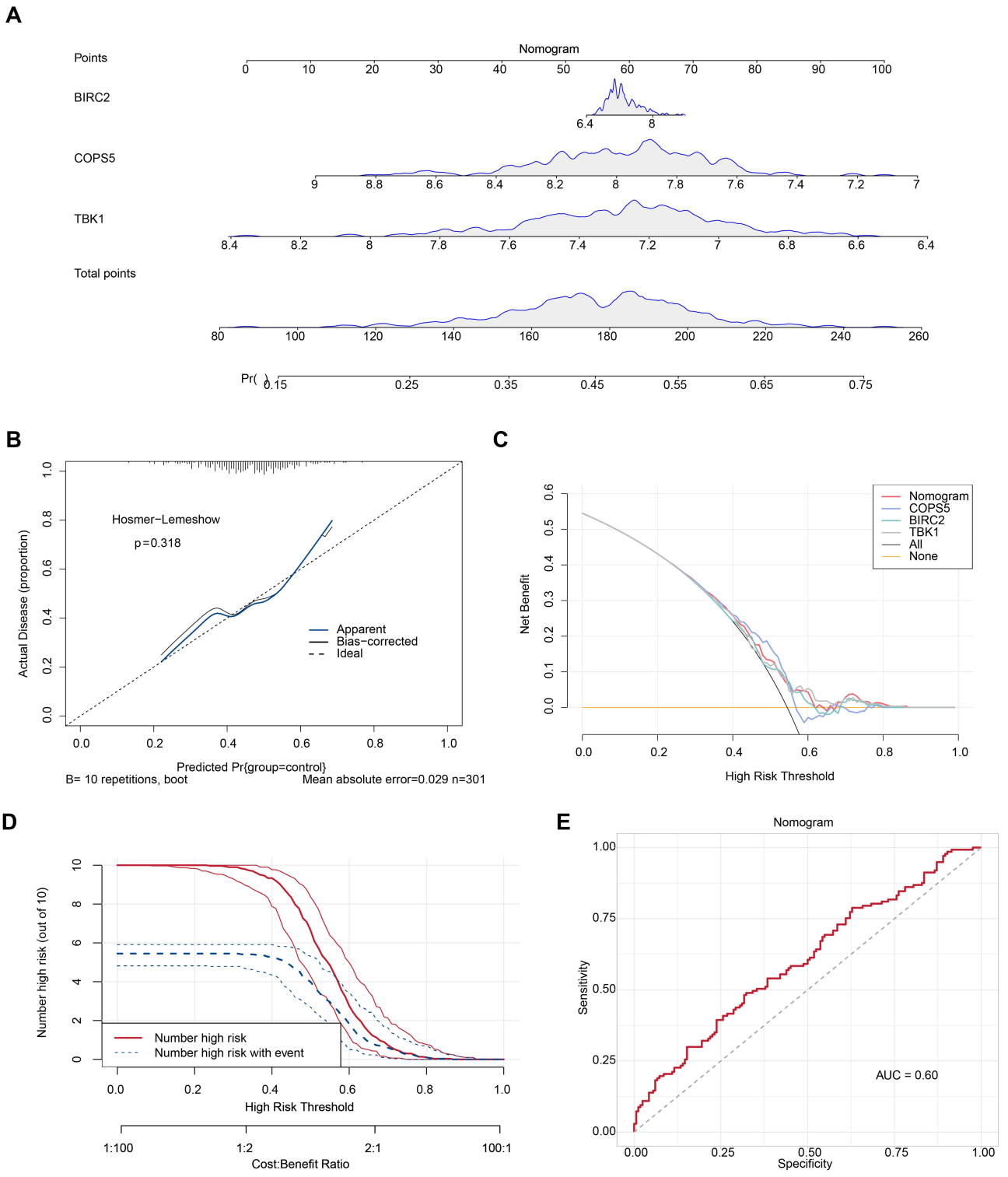

Figure 4 Evaluation of the multigene nomogram for amyotrophic lateral sclerosis (ALS). (**A**) Nomogram integrating *BIRC2*, *COPS5*, and *TBK1* expression levels to estimate individual ALS risk. (**B**) Calibration curve of the nomogram, with the x-axis representing predicted probability and the y-axis representing observed disease incidence; the dashed diagonal denotes perfect concordance. (**C**) Decision curve analysis, plotting net benefit against risk threshold; colored lines compare the nomogram with single-gene models. (**D**) Clinical impact curve, showing the number of individuals classified as high-risk by the nomogram (solid red line) versus the actual case count (dashed blue line) across varying risk thresholds. (**E**) Receiver operating characteristic (ROC) curves for the nomogram.

https://doi.org/10.2147/JIR.S585872

enrichment in ribosome activity, lysosomal function, ubiquitin-mediated proteolysis, regulation of the actin cytoskeleton, and p53 signaling. *TBK1* was involved in ribosome-related processes, olfactory transduction, ubiquitin-mediated proteolysis, lysosomal function, and p53 signaling (Figure 5A–C).

Spearman correlation analysis assessed the relationships between core genes and differentially infiltrated immune cell subsets. *COPS5* and *TBK1* demonstrated strong negative correlations with Treg cells, while *BIRC2, COPS5*, and *TBK1* showed robust positive correlations with $CD4^+$ memory resting T cells (Figure 5D). Furthermore, $CD4^+$ memory-activated and Treg cells exhibited an inverse correlation (r=–0.61) (Figure 5E).

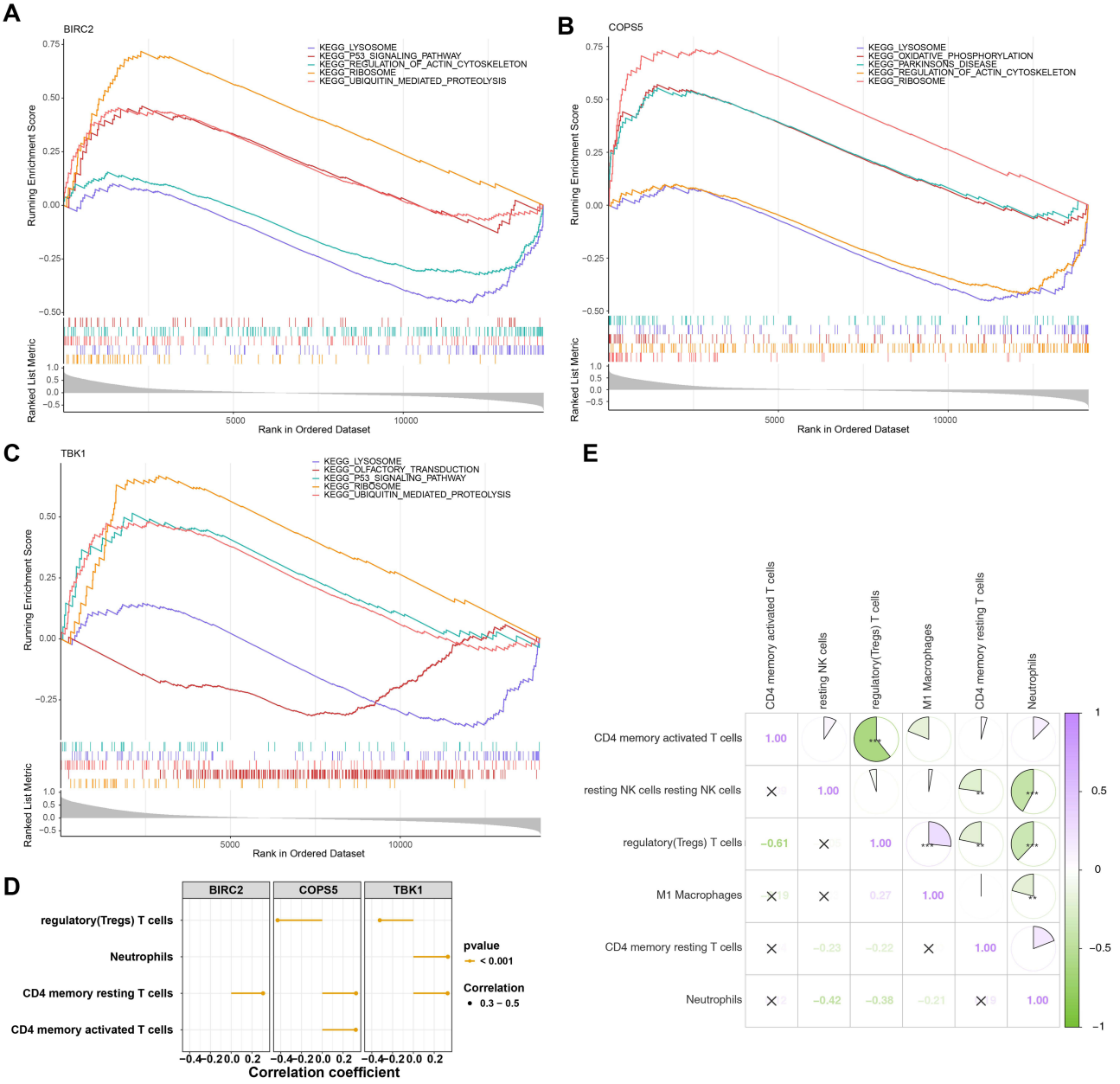

Figure 5 Gene set enrichment and immune correlation analyses. (**A–C**) GSEA plots for *BIRC2* (**A**), *COPS5* (**B**), and *TBK1* (**C**), illustrating the top enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. (**D**) Spearman correlation between core genes and differentially infiltrated immune cell subsets; the x-axis represents correlation coefficients, and the y-axis denotes immune cell types. (**E**) Spearman correlation matrix depicting interrelationships among the differentially infiltrated immune cell populations, **p < 0.01, ***p < 0.001, ×, no significance.

https://doi.org/10.2147/JIR.S585872

Co-Expression and Regulatory Network Analysis of Core TURDEGs

To further investigate genes and signaling pathways associated with *COPS5, BIRC2*, and *TBK1*, a co-expression network was generated using GeneMANIA. This analysis revealed significant enrichment in MyD88-independent and canonical Toll-like receptor signaling pathways, the IκB kinase/NF-κB signaling axis, and pattern recognition receptor pathways (Figure 6A). "Friends" analysis highlighted a robust functional association between *BIRC2, COPS5*, and *TBK1* (Figure 6B). Subcellular localization analysis via mRNALocator indicated that all three proteins exhibit a dual nuclear-cytoplasmic distribution. BIRC2 was primarily localized in the nucleus, COPS5 evenly distributed between the nucleus and cytoplasm, with a slight extracellular tendency, and TBK1 was predominantly localized in the cytoplasm with partial nuclear presence (Figure 6C). Three-dimensional structural analysis of their target proteins revealed distinct

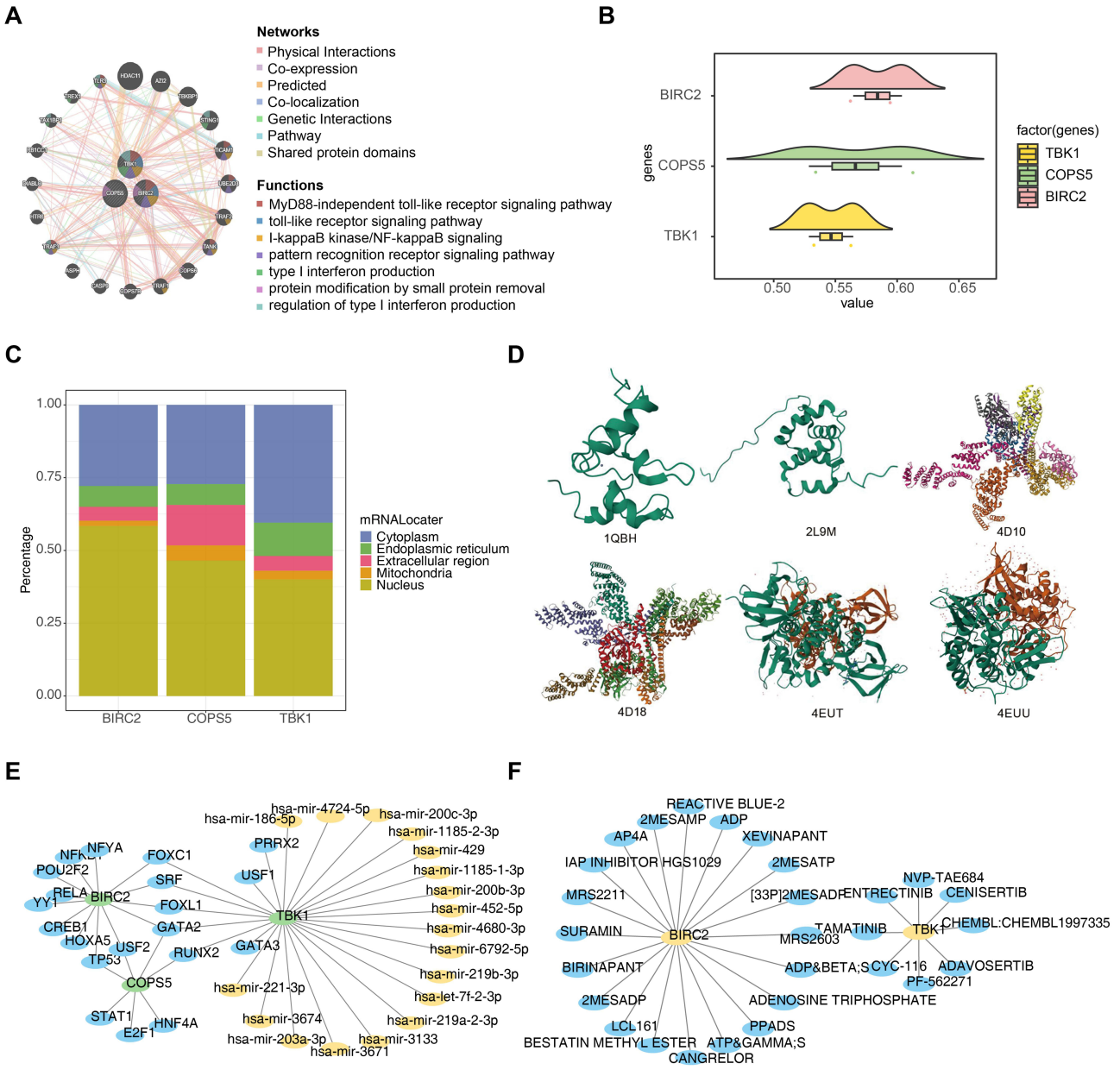

Figure 6 Functional and regulatory characterization of core genes. (**A**) GeneMANIA-derived co-expression network of *COPS5, BIRC2*, and *TBK1*. (**B**) "Friends" analysis depicting functional similarity scores between each core gene and its co-expressed partners. (**C**) Predicted subcellular localizations of the core gene products. (**D**) Three-dimensional structures of proteins regulated by the core genes. (**E**) Integrated transcription factor–mRNA–miRNA regulatory network (miRNAs in yellow, transcription factors in blue, mRNAs in green). (**F**) Drug–Gene Interaction Database-based drug–gene interaction network identifying candidate therapeutics (core genes in yellow, predicted drugs in blue).

https://doi.org/10.2147/JIR.S585872

characteristics: BIRC2-associated proteins (1QBH and 2L9M) displayed compact domain folds; COPS5 targets (4D10 and 4D18) formed multimeric complexes; and TBK1 targets (4EUT and 4EUU) exhibited the canonical kinase fold (Figure 6D). To explore upstream regulatory mechanisms, an integrated transcription factor–miRNA regulatory network was constructed, showing that *TBK1* is regulated by eight transcription factors and eighteen miRNAs (Figure 6E). Drug-gene interaction profiling using the DGIdb identified eight candidate compounds targeting *TBK1*, with adavosertib being the most notable, and twenty compounds targeting *BIRC2*, with MRS2211 ranked highest. No viable drug candidates were found for *COPS5* (Figure 6F).

Analyses of the Immune Microenvironment in ALS

To investigate the potential cellular-level pathogenic mechanisms underlying ALS, the single-cell RNA sequencing dataset GSE244263 was analyzed. After quality control and normalization using Seurat, the data quality was confirmed to be high (Figure 7A and B). Dimensionality reduction based on PCA, followed by UMAP visualization, revealed seven major immune cell populations: T cells, natural killer (NK) cells, neutrophils, B cells, monocytes, megakaryocytes, and erythroid progenitors (Figure 7C). Comparative abundance analysis showed a significant increase in T cells in patients with ALS compared to controls (Figure 7D). Functional enrichment analysis revealed significant positive associations between T cell abundance and several biological pathways, including TNFR1-mediated ceramide biosynthesis, biosynthesis of docosapentaenoic acid-derived specialized pro-resolving mediators (DPAn-6 SPMs), and regulation of FZD by ubiquitination (7E).

Expression profiling of core TURDEGs further demonstrated their predominant localization in T cells, B cells, and neutrophils, with the most significant differential expression observed in T cells (Figure 8A), identifying them as the central cell population. Pseudotime trajectory analysis revealed seven developmental states within the T cell compartment: BIRC2 expression decreased progressively, COPS5 levels modestly increased, and TBK1 remained low until a marked upregulation in the later stages (Figure 8B–E). CellChat analysis indicated that, in comparison to controls, patients with ALS exhibited significantly enhanced interactions between T cells and neutrophils, B lymphocytes, and NK cells, while interactions between T cells and monocytes were reduced (Figure 8F and G). These results suggest a remodeling of the immune communication network in ALS, characterized by intensified signaling among inflammation-related cell types and a shift towards a T cell–dominated pro-inflammatory immune architecture in the peripheral immune system.

RT-PCR Verification

To validate these bioinformatic results, RT-qPCR analysis of peripheral blood samples from patients with ALS and healthy controls was performed. Compared to controls, the mRNA expression levels of the three core genes were significantly upregulated in patients with ALS. Specifically, BIRC2 expression increased from 1.21 ± 0.82 to 2.60 ± 0.58 ($P = 0.015$), COPS5 from 1.13 ± 0.58 to 3.01 ± 0.74 ($P = 0.002$), and TBK1 from 1.07 ± 0.41 to 4.74 ± 1.40 ($P < 0.001$) (Figure 9A–C). These results were strongly corroborate the findings from our bioinformatic analyses.

Discussion

ALS is a progressive neurodegenerative disorder characterized by motor neuron loss, yet its etiology remains incompletely understood. This study presents the first systematic identification of TURDEGs in ALS, which were enriched in pathways related to ubiquitin–proteasome-mediated proteolysis, mitochondrial dysfunction, autophagy, NOD-like receptor signaling, and immune activation. A prognostic nomogram based on core TURDEGs demonstrated potential predictive value and clinical applicability. Immune infiltration analysis revealed significant positive correlations between core TURDEGs and $CD4^+$ resting memory T cells, as well as negative correlations between *COPS5, TBK1*, and Treg cells. Single-cell transcriptomics further established T cells as key players in ALS pathogenesis. These findings highlight a synergistic pathogenic axis between T cell and UPS dysregulation, providing novel biomarkers and promising personalized therapeutic targets for ALS.

Initially, three core TURDEGs were identified: *BIRC2, COPS5*, and *TBK1*. *BIRC2* encodes the IAP-family E3 ubiquitin ligase cIAP1, which regulates NF-κB signaling and inhibits caspase-mediated apoptosis.[29,30] Aberrant *BIRC2* expression may

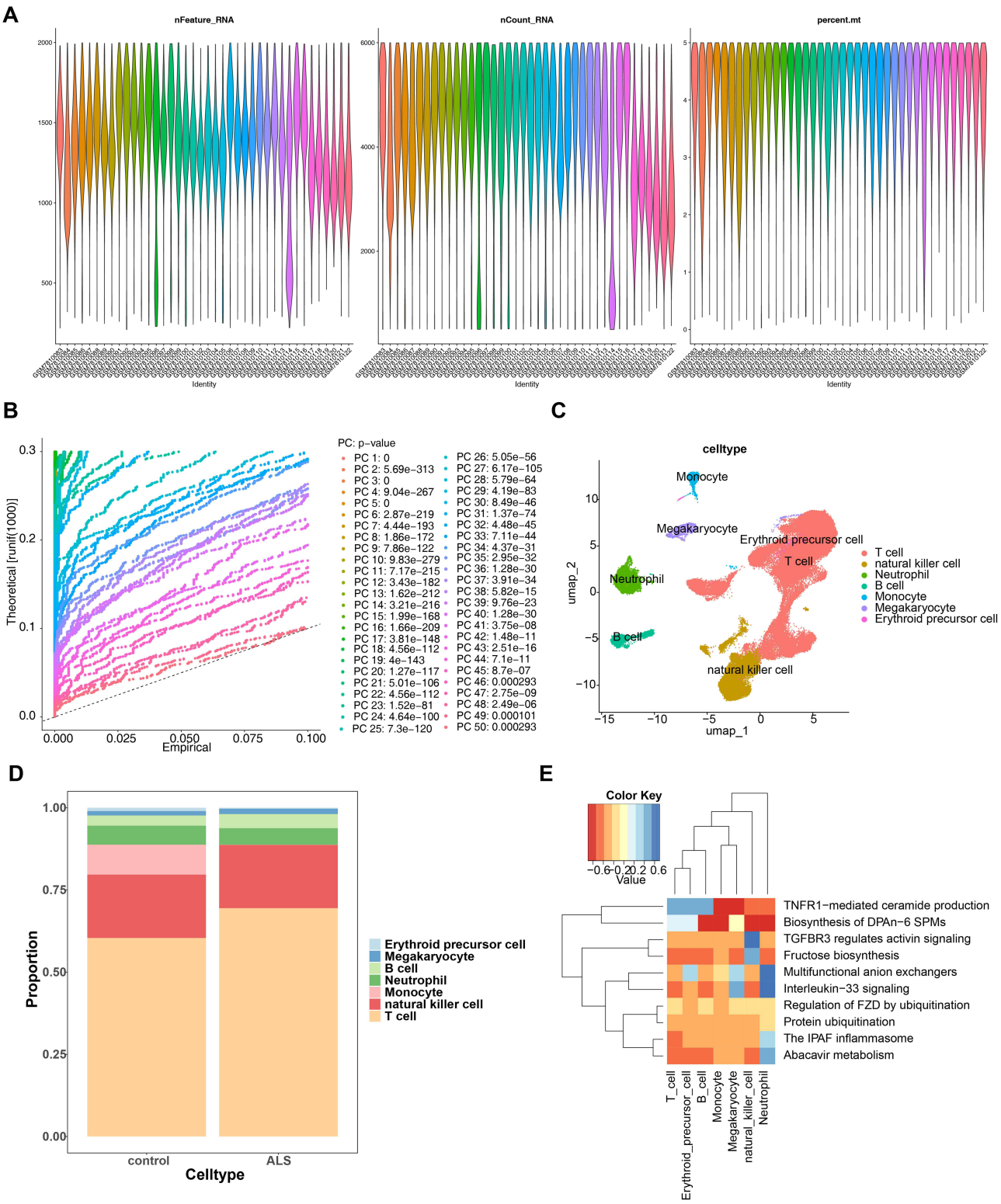

Figure 7 Single-cell data preprocessing and clustering analysis. (**A**) Post–QC distributions of detected genes (nFeature_RNA), transcript counts (nCount_RNA), and mitochondrial fraction (percent.mt). (**B**) Identification of the top 30 principal components (PCs) based on a significance threshold of p<0.05. (**C**) Uniform manifold approximation and projection (UMAP) embedding of single cells, delineating seven annotated immune phenotypes, each color corresponding to a distinct cell type. (**D**) Comparative composition of cell types between ALS and control cohorts. (**E**) Functional enrichment analysis of each cell cluster.

https://doi.org/10.2147/JIR.S585872

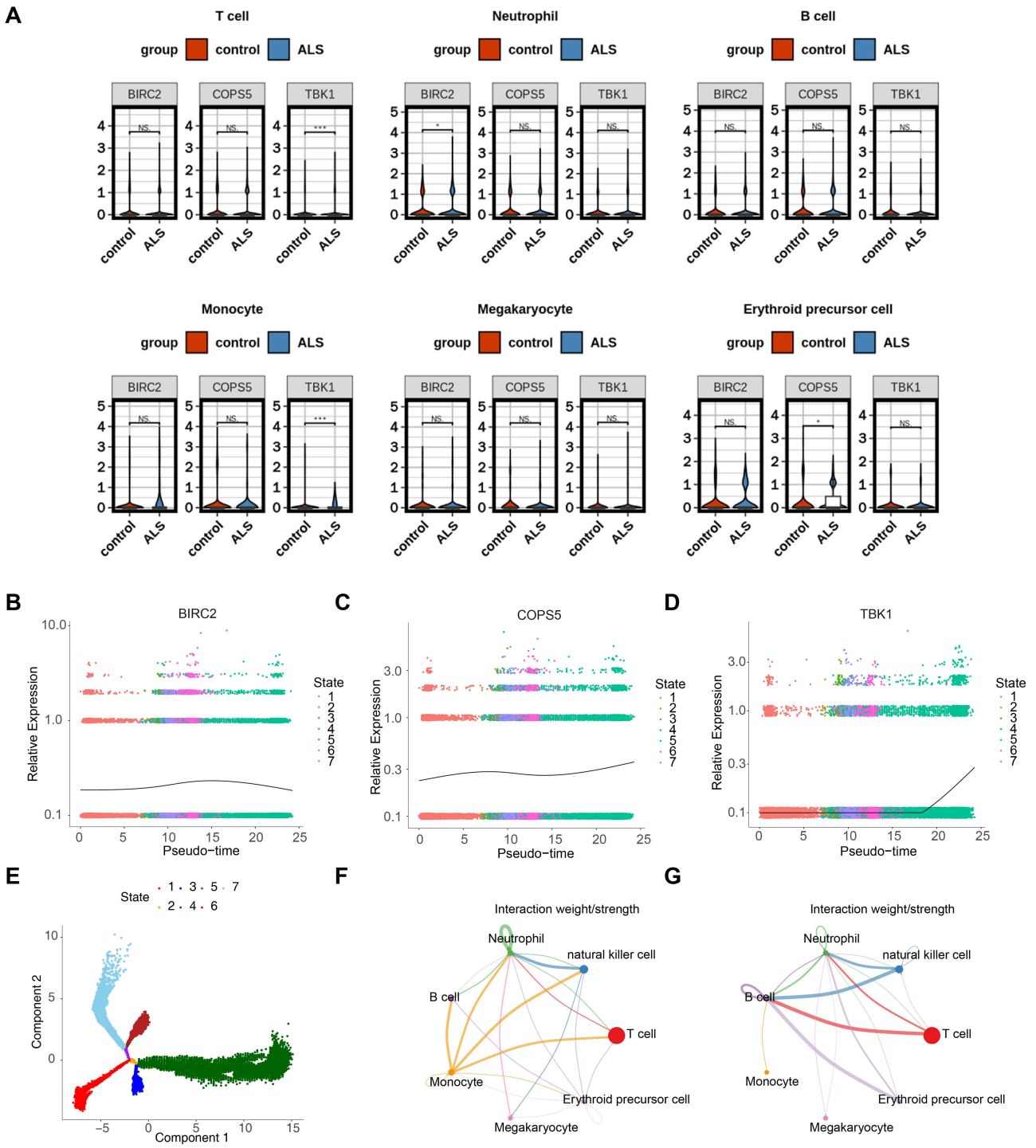

Figure 8 Single-cell RNA-seq analysis of T cell ubiquitination-related differentially expressed genes (TURDEGs). (**A**) Violin plots comparing core TURDEGs expression within T cell subsets between ALS and control samples, *p < 0.05, *** p < 0.001, Ns, no significance. (**B**–**D**). Smoothed pseudotime expression profiles of *BIRC2* (**B**), *COPS5* (**C**), and *TBK1* (**D**); the abscissa represents pseudotime and the ordinate represents relative expression levels, with color coding for differentiation phases. (**E**) Gene expression kinetics along the T cell maturation trajectory; colors designate discrete developmental stages. (**F**) Aggregate interaction weight/strength of T cell–derived ligand–receptor pairs in normal groups. (**G**) Aggregate interaction weight/strength of T cell-derived ligand–receptor pairs in ALS.

exacerbate neuroinflammation via the NF-κB pathway and has been implicated in the pathogenesis of Alzheimer's disease, Parkinson's disease, and ALS.[31,32] COPS5, the catalytic subunit of the COP9 signalosome, modulates the activity of Cullin–RING E3 ligases (CRLs), thereby regulating key cellular processes such as proteostasis, RNA metabolism, and inflammatory signaling. Dysregulation of *COPS5* is associated with neurodegenerative disorders.[33,34] Targeting the COPS5/NEDDylation axis

https://doi.org/10.2147/JIR.S585872

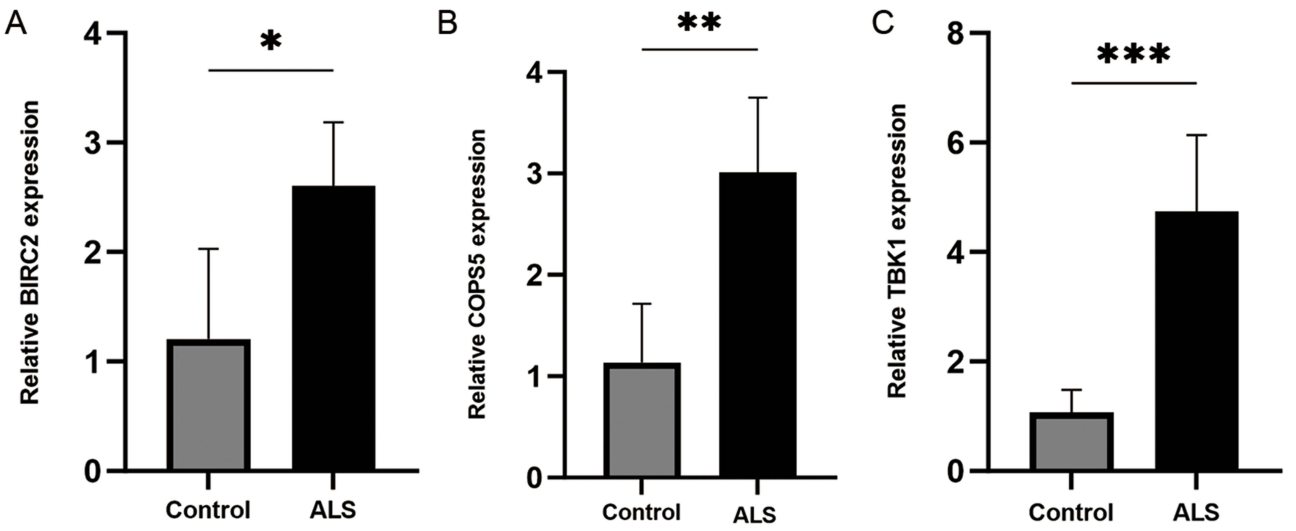

Figure 9 Reverse transcriptase-polymerase chain reaction validation of *BIRC2* (**A**), *COPS5* (**B**), and *TBK1* (**C**). Data are presented as relative transcript levels, with statistical significance indicated by asterisks: *p<0.05, **p<0.01, and ***p<0.001.

has been shown to reduce microglial and endothelial inflammation, maintain blood–brain barrier integrity, and provide neuroprotection, highlighting its therapeutic potential.[35] Given COPS5's role in regulating CRLs, impairment of these ligases may lead to defective protein turnover and muscle atrophy, contributing to ALS pathogenesis.[36,37] TNK-binding kinase 1 (*TBK1*) is a multifunctional IKK-family serine/threonine kinase featuring kinase, ubiquitin-like, and dimerization domains.[38] It facilitates autophagic clearance of protein aggregates and damaged mitochondria through receptor phosphorylation and modulates mTOR-dependent immune responses.[39] TBK1's ubiquitin-dependent activation serves as a key checkpoint for initiating mitophagy.[40] Epidemiological studies have identified *TBK1* variants in approximately 1.81% of ALS and frontotemporal dementia (FTD) cases, with haploinsufficiency confirmed as a pathogenic mechanism in ALS.[41] These findings suggest that TURDEGs contribute to ALS onset and progression through the modulation of UPS and neuroinflammation. Further investigation into these genes and their associated pathways could provide a deeper understanding of ALS pathogenesis and support targeted interventions aimed at correcting UPS imbalance and alleviating immune-inflammatory dysregulation.

Notably, RBCK1 was initially identified as a potential core gene by several machine learning algorithms. As an E3 ubiquitin ligase, RBCK1 plays a role in regulating inflammatory responses, a key pathological feature of ALS.[42] Its preliminary identification provides indirect support for the validity of our bioinformatic framework. However, to enhance the robustness and translational relevance of the prediction model, stringent selection criteria were applied, requiring consistent and significant differential expression across two independent transcriptomic datasets. Since RBCK1 did not meet the prespecified threshold in the validation dataset, it was excluded from the final core gene set. While this approach may have excluded some potentially relevant genes, it strengthened the reliability of BIRC2, COPS5, and TBK1 as candidate biomarkers for ALS. Further studies involving larger cohorts and specific ALS subtypes are necessary to clarify RBCK1's biological and clinical significance.

Functional enrichment analyses of the TURDEGs revealed significant association with pathways related to ubiquitination, autophagy, and NOD-like receptor signaling, suggesting that these processes may contribute to ALS pathogenesis through their involvement in T-cell function. Specifically, BIRC2, regulated by E3 ubiquitin ligases, is implicated in inflammatory pathway regulation and ubiquitination events within NOD-like receptor signaling.[43,44] This suggests a potential role in T-cell survival and activation.[45,46] COPS5, the catalytic subunit of the COP9 signalosome,[47] regulates the deneddylation and participates in T-cell activation.[48,49] This is consistent with our finding that COPS5 is negatively correlated with Treg abundance, a key regulator of autophagy and innate immunity.[50] COPS5's involvement in downstream of NOD-like receptor signaling further supports its potential role in T-cell regulation.[51] In the context of ALS, the aberrant expression of these TURDEGs may contribute to dysfunction in the UPS, leading to the accumulation of abnormal proteins such as mutant SOD1 and TDP-43.[13] This dysregulation could also impair axonal transport and DNA

repair pathways.[14,52] Furthermore, ubiquitin imbalance may alter the immune microenvironment by affecting T-cell migration, differentiation, and proliferation.[53,54] Autophagy, which is impaired in ALS, is closely linked to proteotoxic stress and mitochondrial dysfunction.[55] Likewise, aberrant activation of NOD-like receptors, particularly those associated with motor neuron degeneration, has been linked to neuroinflammation in ALS.[56] Mitochondrial dysfunction and defective mitophagy, recognized contributors to neurodegeneration, may also play a role in these processes.[55,57] In summary, BIRC2, COPS5, and TBK1 appear to influence the immune microenvironment and T-cell dysfunction in ALS through their role in ubiquitination-dependent proteostasis, autophagic processes, and NOD-like receptor-mediated inflammatory signaling. Ubiquitination imbalance may disrupt T-cell signaling, autophagy defects may impair T-cell metabolic adaptation and Treg survival, and persistent activation of NOD-like receptor signaling may promote a pro-inflammatory shift in $CD4^+$ memory T cells. These findings support a potential link between UPS dysregulation and T-cell dysfunction, offering new insights into the immunopathological mechanisms underlying ALS.

T cell–mediated immunity is increasingly recognized as crucial to ALS pathogenesis.[54] CIBERSORT analysis of peripheral blood in this study identified a significant expansion of $CD4^+$ memory–activated T cells, alongside a marked depletion of Tregs, corroborating previous findings.[58] To further explore the molecular basis of these alterations in T-cell subsets, the correlations between the core TURDEGs and immune-cell infiltration were examined. The expression levels of COPS5 and TBK1 were found to be inversely correlated with Treg abundance and positively associated with $CD4^+$ memory–resting T cells. Tregs, as key mediators of immunosuppression, are strongly linked to disease progression when their numbers are reduced.[59] In contrast, CD4+ memory-resting T cells are vital for maintaining the peripheral immune system's reserve capacity.[60] In this study, the significant negative correlation between Tregs and memory-resting T cells suggests a shift in T-cell homeostasis in ALS. This shift may be regulated by the core TURDEGs, potentially influencing T-cell activation thresholds and functional polarization. COPS5 may modulate T-cell activation thresholds by regulating CRL activity.[49] Similarly, TBK1 has been implicated in T-cell activation through mTOR-dependent immune responses,[61] suggesting that increased expression of these genes may impair Treg maintenance under inflammatory conditions.[62] Furthermore, BIRC2 may regulate T-cell survival by stabilizing NF-κB signaling,[63] with its positive correlation to resting memory T cells indicating a role in maintaining T-cell quiescence and limiting excessive activation. These observations suggest that the core TURDEGs are associated with the T-cell subset imbalance seen in ALS, characterized by a reduction in Tregs and an accumulation of resting memory T cells, contributing to a pro-inflammatory immune microenvironment. Notably, the pro-inflammatory cytokine milieu in ALS not only promotes the transition of glial cells to a neurotoxic phenotype but also enhances the cytotoxic effects of infiltrating T cells and NK cells via direct ligand–receptor interactions.[64] The reduction in Tregs numbers further weakens immunosuppressive capacity, potentially creating a vicious cycle that exacerbates neuroinflammation.

Supporting the central role of T cells, single-cell transcriptomic analysis identified T cells as the predominant immune subset in the peripheral blood of patients with ALS, highlighting their potential pathogenic relevance. Pseudotime trajectory analysis revealed dynamic modulation of *BIRC2, COPS5*, and *TBK1* during T-cell differentiation, suggesting their involvement in maturation and effector functions. Additionally, Cell-Chat analysis indicated significantly enhanced T cell–neutrophil crosstalk in ALS, implying that dysregulated intercellular signaling may exacerbate neurodegeneration. These findings position core TURDEGs-mediated T-cell dysfunction as central contributors to ALS pathophysiology, with aberrant immune cell interactions acting as potential accelerants of disease progression. Future studies should elucidate the molecular mechanisms underlying these genes and assess their therapeutic potential.

A multigene nomogram based on core TURDEGs outperformed single-gene models, demonstrating superior net clinical benefit in both decision-curve and clinical-impact analyses while effectively stratifying individuals at high risk for ALS. However, the model yielded an AUC of 0.60, indicating only modest discriminative ability and limited overall predictive performance. This relatively low performance may partly reflect the exploratory nature of the model, which was derived from peripheral blood transcriptomic data. Therefore, its principal value may reside not in diagnostic optimization, but in highlighting the potential coordinated role of core genes in ALS pathogenesis. Additionally, regulatory network analysis revealed the upstream mechanisms governing these core genes. GATA2 merged as a central hub within the regulatory interactome, directly regulating the transcription of all three core TURDEGs by binding to their promoter regions. As a GATA transcription factor, GATA2 has been implicated in neurodegeneration

through its modulation of DNA damage responses, immune signaling, and cellular senescence.[65–67] Based on the functional roles of the core genes, candidate therapeutic agents with potential clinical relevance were identified. In silico drug–gene interaction profiling highlighted adavosertib, a WEE1 kinase inhibitor, as the top candidate for targeting *TBK1*. By restoring cell-cycle checkpoint control, adavosertib may mitigate neuronal DNA damage and prevent aberrant cell-cycle reentry.[68] MRS2211, a selective $P2Y_{13}$ antagonist, merged as the highest-scoring compound for *BIRC2*, suggesting its potential therapeutic efficacy in attenuating glial overactivation, reduction of neuroinflammation and oxidative stress, and enhancing neuromuscular junction integrity.[69]

Several limitations should be acknowledged. First, all transcriptomic analyses were based on peripheral blood samples, so caution is warranted when extrapolating these findings to pathological processes within the CNS, particularly motor neuron degeneration. Second, the risk prediction model was developed using cross-sectional data, which precludes causal inference and limits its applicability for predicting individual disease trajectories. Third, the moderate size of the public cohorts and the relatively small qPCR validation cohort may limit the generalizability of the findings. Additionally, this study relied primarily on transcriptomic data, lacking protein-level validation and functional experiments, which limits the mechanistic understanding of how core TURDEGs contribute to T-cell dysfunction and UPS dysregulation in ALS. The drug prediction results were also based on bioinformatic associations and require experimental validation. Finally, potential confounding factors, such as sex, were not fully addressed. Future studies involving larger, multicenter, prospective cohorts, along with mechanistic investigations using ALS animal models and iPSC-derived neuron–glia co-culture systems, are needed to validate these findings and evaluate candidate therapies targeting the T cell–ubiquitination axis.

Conclusions

This study represents the first systematic characterization of T cell-associated ubiquitination networks in ALS. By integrating bulk and single-cell transcriptomic data, this study defined stage-dependent expression patterns of core genes and demonstrated enhanced T cell–neutrophil crosstalk. *TBK1* and *BIRC2* were identified as therapeutic targets, offering mechanistic insights for potential interventions. Prospective functional studies and validation in larger longitudinal cohorts are essential to confirm these findings and clarify their translational value for early diagnosis and therapy.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China (2025-E0557).

Abbreviations

ALS, Amyotrophic lateral sclerosis; WGCNA, Weighted gene co-expression network analysis; GSEA, Gene Set Enrichment Analysis; CNS, Central nervous system; TBK1, TANK-binding kinase 1; Treg, Regulator T cells; UPS, Ubiquitin–proteasome system; DCA, Decision curve analysis; CIC, Clinical impact curves; DGIdb, Drug–Gene Interaction Database; PCA, Principal component analysis; UMAP, Uniform manifold approximation and projection; NK, Natural killer; TF, Transcription Factors; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TRUDEGs, T cell-related ubiquitination-related differentially expressed genes; TRGs, T cell–related genes; TURGs, T cell-ubiquitination-related genes; DEGs, Differentially Expressed Genes; URGs, Ubiquitination-related genes.

Data Sharing Statement

The datasets utilized in this research are accessible through online repositories. All data analyzed during this study are presented in the main text and the Supplementary Materials of this article. Additional source data supporting the findings of this study are available upon reasonable request from the corresponding author.

Informed Consent Statement

Written informed consent was obtained from all participants after they were informed of the purpose of the study.

Acknowledgments

We would like to extend our sincere gratitude to Yuanyuan Zhu, Zhongyin Liang, and Yunji Leng from Becreative Lab Co. Ltd. (Beijing, China) for their assistance in bio-informatics analysis.

Author Contributions

Ailing Jiang: Investigation, Data curation, Formal analysis, Methodology, Software, Writing—original draft. Yanzhen Huang: Formal analysis, Investigation, Data curation, Validation. Xianting Que: Software, Data curation. Chonglin Li: Software, Visualization. Ziqun Lin: Investigation, Validation. Wen Huang: Conceptualization, Supervision, Writing—review and editing. All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was funded by "The National Natural Science Foundation of China, grant number 82460254" and "The Natural Science Foundation of Guangxi Province (CN), grant number 2023GXNSFAA026247.

Disclosure

The authors declare no conflicts of interest.

References

1. Wolfson C, Gauvin DE, Ishola F, Oskoui M. Global prevalence and incidence of amyotrophic lateral sclerosis: a systematic review. *Neurology*. 2023;101(6):e613–e623. doi:10.1212/WNL.0000000000207474
2. O'Neill K, Shaw R, Bolger I, et al. ALS molecular subtypes are a combination of cellular and pathological features learned by deep multiomics classifiers. *Cell Rep*. 2025;44(3):115402. doi:10.1016/j.celrep.2025.115402
3. Catanese A, Rajkumar S, Sommer D, et al. Multiomics and machine-learning identify novel transcriptional and mutational signatures in amyotrophic lateral sclerosis. *Brain*. 2023;146(9):3770–3782. doi:10.1093/brain/awad075
4. Tzeplaeff L, Wilfling S, Requardt MV, Herdick M. Current State and Future Directions in the Therapy of ALS. *Cells*. 2023;12(11):1523. doi:10.3390/cells12111523
5. Ramachandran S, Grozdanov V, Leins B, et al. Low T-cell reactivity to TDP-43 peptides in ALS. *Front Immunol*. 2023;14:1193507. doi:10.3389/fimmu.2023.1193507
6. Yazdani S, Seitz C, Cui C, et al. T cell responses at diagnosis of amyotrophic lateral sclerosis predict disease progression. *Nat Commun*. 2022;13(1):6733. doi:10.1038/s41467-022-34526-9
7. Cao W, Fan D. Neutrophils: a subgroup of neglected immune cells in ALS. *Front Immunol*. 2023;14:1246768. doi:10.3389/fimmu.2023.1246768
8. Yu W, He J, Cai X, Yu Z, Zou Z, Fan D. Neuroimmune crosstalk between the peripheral and the central immune system in amyotrophic lateral sclerosis. *Front Aging Neurosci*. 2022;14:890958. doi:10.3389/fnagi.2022.890958
9. Pan Y, Nicolazzo JA. Altered blood-brain barrier and blood-spinal cord barrier dynamics in amyotrophic lateral sclerosis: impact on medication efficacy and safety. *Br J Pharmacol*. 2022;179(11):2577–2588. doi:10.1111/bph.15802
10. Zaccai S, Nemirovsky A, Lerner L, et al. CD4 T-cell aging exacerbates neuroinflammation in a late-onset mouse model of amyotrophic lateral sclerosis. *J Neuroinflamm*. 2024;21(1):17. doi:10.1186/s12974-023-03007-1
11. Thome AD, Thonhoff JR, Zhao W, et al. Extracellular vesicles derived from ex vivo expanded regulatory T cells modulate in vitro and in vivo inflammation. *Front Immunol*. 2022;13:875825. doi:10.3389/fimmu.2022.875825
12. Lambert-Smith IA, Saunders DN, Yerbury JJ. Proteostasis impairment and ALS. *Prog Biophys Mol Biol*. 2022;174:3–27. doi:10.1016/j.pbiomolbio.2022.06.001
13. Hoshino T, Mukai A, Yamashita H, et al. NDRG1 upregulation by ubiquitin proteasome system dysfunction aggravates neurodegeneration. *Mol Brain*. 2024;17(1):77. doi:10.1186/s13041-024-01150-1
14. Farrawell NE, Bax M, McAlary L, et al. ALS-linked CCNF variant disrupts motor neuron ubiquitin homeostasis. *Hum Mol Genet*. 2023;32(14):2386–2398. doi:10.1093/hmg/ddad063
15. Kim SH, Nichols KD, Anderson EN, et al. Axon guidance genes modulate neurotoxicity of ALS-associated UBQLN2. *eLife*. 2023;12:e84382. doi:10.7554/eLife.84382
16. Qiao G, Zhao Y, Li Z, et al. T cell activation threshold regulated by E3 ubiquitin ligase Cbl-b determines fate of inducible regulatory T cells. *J Immunol*. 2013;191(2):632–639. doi:10.4049/jimmunol.1202068
17. Lutz-Nicoladoni C, Wolf D, Sopper S. Modulation of immune cell functions by the E3 ligase Cbl-b. *Front Oncol*. 2015;5:58. doi:10.3389/fonc.2015.00058
18. Gu J, Chen C, He P, Du Y, Zhu B. Unraveling the immune regulatory functions of USP5: implications for disease therapy. *Biomolecules*. 2024;14(6):683. doi:10.3390/biom14060683
19. Shi Y, Zhu R. Analysis of damage-associated molecular patterns in amyotrophic lateral sclerosis based on ScRNA-seq and bulk RNA-seq data. *Front Neurosci*. 2023;17:1259742. doi:10.3389/fnins.2023.1259742

20. Itou T, Fujita K, Okuzono Y, et al. Th17 and effector CD8 T cells relate to disease progression in amyotrophic lateral sclerosis: a case control study. *J Neuroinflamm*. 2024;21(1):331. doi:10.1186/s12974-024-03327-w
21. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47. doi:10.1093/nar/gkv007
22. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–287. doi:10.1089/omi.2011.0118
23. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics*. 2013;29 (14):1830–1831. doi:10.1093/bioinformatics/btt285
24. Van Calster B, Wynants L, Verbeek JFM, et al. Reporting and interpreting decision curve analysis: a guide for investigators. *Eur Urol*. 2018;74 (6):796–804. doi:10.1016/j.eururo.2018.08.038
25. Yu G, Li F, Qin Y, Bo X, Wu Y, Wang S. GOSemSim: an R package for measuring semantic similarity among GO terms and gene products. *Bioinformatics*. 2010;26(7):976–978. doi:10.1093/bioinformatics/btq064
26. Tang Q, Nie F, Kang J, Chen W. mRNALocater: enhance the prediction accuracy of eukaryotic mRNA subcellular localization by using model fusion strategy. *Mol Ther*. 2021;29(8):2617–2623. doi:10.1016/j.ymthe.2021.04.004
27. Jin S, Plikus MV, Nie Q. CellChat for systematic analysis of cell-cell communication from single-cell transcriptomics. *Nat Protoc*. 2025;20 (1):180–219. doi:10.1038/s41596-024-01045-4
28. Griss J, Viteri G, Sidiropoulos K, Nguyen V, Fabregat A, ReactomeGSA HH. Efficient multi-omics comparative pathway analysis. *Mol Cell Proteom*. 2020;19(12):2115–2125. doi:10.1074/mcp.TIR120.002155
29. Varfolomeev E, Blankenship JW, Wayson SM, et al. IAP antagonists induce autoubiquitination of c-IAPs, NF-κB activation, and TNFα-dependent apoptosis. *Cell*. 2007;131(4):669–681. doi:10.1016/j.cell.2007.10.030
30. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol*. 2017;17(9):559–572. doi:10.1038/nri.2017.49
31. Kaushik A, Parashar S, Ambasta RK, Kumar P. Ubiquitin E3 ligases assisted technologies in protein degradation: sharing pathways in neurodegenerative disorders and cancer. *Ageing Res Rev*. 2024;96:102279. doi:10.1016/j.arr.2024.102279
32. M-m M, Zhao J, Liu L, C-y W. Identification of cuproptosis-related genes in Alzheimer's disease based on bioinformatic analysis. *Eur J Med Res*. 2024;29(1):495. doi:10.1186/s40001-024-02093-y
33. Yang Y, Song R, Gao Y, Yu H, Wang S. Regulatory mechanisms and therapeutic potential of JAB1 in neurological development and disorders. *Mol Med*. 2023;29(1):80. doi:10.1186/s10020-023-00675-w
34. Rivellini C, Porrello E, Dina G, et al. JAB1 deletion in oligodendrocytes causes senescence-induced inflammation and neurodegeneration in mice. *J Clin Invest*. 2022;132(3):e145071. doi:10.1172/JCI145071
35. Tian Y, Milic J, Monasor LS, et al. The COP9 signalosome reduces neuroinflammation and attenuates ischemic neuronal stress in organotypic brain slice culture model. *Cell Mol Life Sci*. 2023;80(9):262. doi:10.1007/s00018-023-04911-8
36. Du WX, Goodman CA, Gregorevic P. Deubiquitinases in skeletal muscle-the underappreciated side of the ubiquitination coin. *Am J Physiol*. 2024;327(6):C1651–C1665. doi:10.1152/ajpcell.00553.2024
37. Hughes DC, Goodman CA, Baehr LM, Gregorevic P, Bodine SC. A critical discussion on the relationship between E3 ubiquitin ligases, protein degradation, and skeletal muscle wasting: it's not that simple. *Am J Physiol*. 2023;325(6):C1567–C1582. doi:10.1152/ajpcell.00457.2023
38. Tu D, Zhu Z, Zhou Alicia Y, et al. Structure and ubiquitination-dependent activation of TANK-binding kinase 1. *Cell Rep*. 2013;3(3):747–758. doi:10.1016/j.celrep.2013.01.033
39. Li X, Cheng K, Shang M-D, et al. MARCH1 negatively regulates TBK1-mTOR signaling pathway by ubiquitinating TBK1. *BMC Cancer*. 2024;24 (1):902. doi:10.1186/s12885-024-12667-y
40. Saha B, Olsvik H, Williams GL, et al. TBK1 is ubiquitinated by TRIM5α to assemble mitophagy machinery. *Cell Rep*. 2024;43(6):114294. doi:10.1016/j.celrep.2024.114294
41. Zhao B, Jiang Q, Lin J, et al. TBK1 variants in Chinese patients with amyotrophic lateral sclerosis: genetic analysis and clinical features. *Eur J Neurol*. 2023;30(10):3079–3089. doi:10.1111/ene.15973
42. Guo J, Sun D, Zhang J, et al. The E3 ubiquitin ligase RBCK1: implications in the tumor immune microenvironment and antiangiogenic therapy of glioma. *Comput Struct Biotechnol J*. 2023;21:5212–5227. doi:10.1016/j.csbj.2023.10.020
43. Zhang S-L, Zhang S-J, Li L, et al. NAP1L1 regulates BIRC2 ubiquitination modification via E3 ubiquitin ligase UBR4 and hence determines hepatocellular carcinoma progression. *Cell Death Discov*. 2024;10(1):154. doi:10.1038/s41420-024-01927-2
44. Yao P, Li D. Multi-omics dissection of the role of ubiquitination modification in post-stroke neuroinflammation and function damage. *Synapse*. 2026;80(2):e70039. doi:10.1002/syn.70039
45. Jing L, Zheng D, Sun X, Shi Z. DBDPE upregulates NOD-like receptor signaling to induce NLRP3 inflammasome-mediated HAECs pyroptosis. *Environ Pollut*. 2023;318(1):120882. doi:10.1016/j.envpol.2022.120882
46. Zhao Z, Xu B, Wang S, et al. Tfh cells with NLRP3 inflammasome activation are essential for high-affinity antibody generation, germinal centre formation and autoimmunity. *Ann Rheum Dis*. 2022;81(7):1006–1012. doi:10.1136/annrheumdis-2021-221985
47. Wicker CA, Izumi T. Analysis of RNA expression of normal and cancer tissues reveals high correlation of COP9 gene expression with respiratory chain complex components. *BMC Genom*. 2016;17(1):983. doi:10.1186/s12864-016-3313-y
48. Faull SV, Lau AMC, Martens C, et al. Structural basis of Cullin 2 RING E3 ligase regulation by the COP9 signalosome. *Nat Commun*. 2019;10 (1):3814. doi:10.1038/s41467-019-11772-y
49. Bonnevier JL, Zhang R, Mueller DL. E3 ubiquitin ligases and their control of T cell autoreactivity. *Arthritis Res Ther*. 2005;7(6):233–242. doi:10.1186/ar1842
50. Qin Y, Qiu D, Zhang Q. HNF1A regulates the crosstalk between innate immune responses and MAFLD by mediating autophagic degradation of TBK1. *Autophagy*. 2022;19(3):1026–1027. doi:10.1080/15548627.2022.2110728
51. Yang Y, Lang X, Sun S, et al. NLRP2 negatively regulates antiviral immunity by interacting with TBK1. *Eur J Immunol*. 2018;48(11):1817–1825. doi:10.1002/eji.201847589
52. Giannakou M, Akrani I, Tsoka A, et al. Discovery of novel inhibitors against ALS-related SOD1(A4V) aggregation through the screening of a chemical library using Differential Scanning Fluorimetry (DSF). *Pharmaceuticals*. 2024;17(10):1286. doi:10.3390/ph17101286

53. Hong Z, Liu F, Zhang Z. Ubiquitin modification in the regulation of tumor immunotherapy resistance mechanisms and potential therapeutic targets. *Exp Hematol Oncol*. 2024;13(1):91. doi:10.1186/s40164-024-00552-0
54. Rolfes L, Schulte-Mecklenbeck A, Schreiber S, et al. Amyotrophic lateral sclerosis patients show increased peripheral and intrathecal T-cell activation. *Brain Commun*. 2021;3(3):fcab157. doi:10.1093/braincomms/fcab157
55. Magrì A, Lipari CLR, Risiglione P, et al. ERK1/2-dependent TSPO overactivation associates with the loss of mitophagy and mitochondrial respiration in ALS. *Cell Death Dis*. 2023;14(2):122. doi:10.1038/s41419-023-05643-0
56. Murdock BJ, Zhao B, Webber-Davis IF, et al. Early immune system changes in amyotrophic lateral sclerosis correlate with later disease progression. *Med*. 2025;6(8):100673. doi:10.1016/j.medj.2025.100673
57. Zhang H, Gao C, Yang D, et al. Urolithin a improves motor dysfunction induced by copper exposure in SOD1G93A transgenic mice via activation of mitophagy. *Mol Neurobiol*. 2024;62(6):6922–6937. doi:10.1007/s12035-024-04473-1
58. Kaur K, Chen P-C, Ko M-W, et al. The potential role of cytotoxic immune effectors in induction, progression and pathogenesis of Amyotrophic Lateral Sclerosis (ALS). *Cells*. 2022;11(21):3431. doi:10.3390/cells11213431
59. Graber DJ, Cook WJ, Sentman M-L, Murad-Mabaera JM, Sentman CL. Human CD4+CD25+ T cells expressing a chimeric antigen receptor against aberrant superoxide dismutase 1 trigger antigen-specific immunomodulation. *Cytotherapy*. 2024;26(2):126–135. doi:10.1016/j.jcyt.2023.11.007
60. Zheng L, Han X, Yao S, et al. The CD8α-PILRα interaction maintains CD8+ T cell quiescence. *Science*. 2022;376(6596):996–1001. doi:10.1126/science.aaz8658
61. Runde AP, Mack R, Sj PB, Zhang J. The role of TBK1 in cancer pathogenesis and anticancer immunity. *J Exp Clin Cancer Res*. 2022;41(1):135. doi:10.1186/s13046-022-02352-y
62. Garg G, Muschaweckh A, Moreno H, et al. Blimp1 prevents methylation of Foxp3 and loss of regulatory T cell identity at sites of inflammation. *Cell Rep*. 2019;26(7):1854–1868.e1855. doi:10.1016/j.celrep.2019.01.070
63. He J, Huang X, Xing D, et al. UBE2B drives NF-κB signaling and gastric cancer progression through BIRC2-mediated K63-linked ubiquitination of TRAF1. *Mol Cancer Res*. 2026;1557. doi:10.1158/1541-7786.MCR-25-0884
64. Garofalo S, Cocozza G, Bernardini G, et al. Blocking immune cell infiltration of the central nervous system to tame neuroinflammation in amyotrophic lateral sclerosis. *Brain Behav Immun*. 2022;105:1–14. doi:10.1016/j.bbi.2022.06.004
65. Liu L, Peng Y, Liu W, Xu J, Li D, Li X. GATA-binding protein 4 promotes neuroinflammation and cognitive impairment in Aβ1-42 fibril-infused rats through small nucleolar RNA host gene 1/miR-361-3p axis. *Chin J Physiol*. 2023;66(1):14–20. doi:10.4103/cjop.CJOP-D-22-00057
66. Aktar A, Heit B. Role of the pioneer transcription factor GATA2 in health and disease. *J Mol Med*. 2023;101(10):1191–1208. doi:10.1007/s00109-023-02359-8
67. Choi K, Lee J, Kim G, Lim Y, Kang HJ. Recovery of synaptic loss and depressive-like behavior induced by GATA1 through blocking of the neuroinflammatory response. *Front Cell Neurosci*. 2024;18:1369951. doi:10.3389/fncel.2024.1369951
68. Kok JR, Palminha NM, Dos Santos Souza C, El-Khamisy SF, Ferraiuolo L. DNA damage as a mechanism of neurodegeneration in ALS and a contributor to astrocyte toxicity. *Cell Mol Life Sci*. 2021;78(15):5707–5729. doi:10.1007/s00018-021-03872-0
69. Pérez-Sen R, Queipo MJ, Morente V, Ortega F, Delicado EG, Miras-Portugal MT. Neuroprotection mediated by P2Y 13 nucleotide receptors in neurons. *Comput Struct Biotechnol J*. 2015;13:160–168. doi:10.1016/j.csbj.2015.02.002