

Exploration of potential roles of paraptosis-related prognostic genes in multiple myeloma development and experimental validation

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Abstract

Background

Paraptosis-associated disturbances may contribute to tumor microenvironmental remodeling in multiple myeloma (MM). This study developed a paraptosis-related gene (P-RG) signature to estimate MM prognosis and to identify possible therapeutic implications.

Methods

Transcriptomic cohorts were analyzed together with 66 P-RGs. Prognosis-related candidates were derived through differential expression screening, protein-protein interaction network analysis, univariate Cox regression, proportional hazards assumption testing, and machine-learning selection. A risk score was generated and assessed in discovery and validation cohorts. Clinical variables were examined to establish a prognostic nomogram. Functional enrichment, immune microenvironment, and drug sensitivity analyses were conducted, and RT-qPCR was used for expression validation.

Results

MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB were selected as prognostic genes and were all risk factors for MM (hazard ratio > 1). The risk model effectively separated patients with different survival outcomes, and a nomogram incorporating risk score and stage also achieved favorable prognostic performance. Immune analyses linked the signature to Type 2 T helper cells, natural killer (NK) cells, and differential CD274/CD276 expression. Chemokine-, cytokine-, and receptor-related pathways were also associated with MM prognosis. Drug sensitivity results suggested that the model was relevant to treatment response, whereas RT-qPCR confirmed broad dysregulation of the paraptosis-related signature in MM, especially increased EIF2S1 and HSPB8 expression.

Conclusion

This study defined MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB as MM prognostic genes and indicated their potential involvement in disease progression and survival prediction, providing references for prognostic evaluation and therapeutic exploration in MM.

1 Introduction

Multiple myeloma (MM) is a plasma cell malignancy in which abnormal clonal plasma cells accumulate within the bone marrow niche. It represents the second most frequent hematologic cancer worldwide, and its increasing incidence places a considerable burden on patients[1][2]. The disease is clinically diverse, with typical manifestations including osteolytic damage, anemia, renal impairment, hypercalcemia, and infection susceptibility, all of which impair patient quality of life[3]. Modern treatments, including proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and autologous stem cell transplantation, have improved disease control[4]. Nevertheless, MM is rarely curable because relapse and drug resistance commonly occur. Prognosis also varies markedly among patients. Although the International Staging System (ISS) remains useful in clinical practice, it does not fully capture the molecular heterogeneity that drives MM progression. More refined biomarkers and individualized stratification tools are therefore required[1].

The concept of regulated cell death has moved beyond classical apoptosis and now includes several non-apoptotic death programs. Paraptosis is one such mechanism, characterized by extensive cytoplasmic vacuolization and dependence on newly synthesized proteins[5][6]. Increasing evidence connects paraptosis-related genes (P-RGs) with tumor initiation, stress adaptation, organelle dysfunction, and therapeutic response in multiple cancers, including breast cancer and glioma[5][7]. However, the relevance of P-RGs to MM biology and outcome remains insufficiently defined. Because endoplasmic reticulum stress and mitochondrial dysfunction are closely tied to myeloma-cell survival and drug resistance, examining paraptosis and its molecular regulators may reveal additional pathways involved in MM progression and may uncover candidate therapeutic targets[7][2].

Public high-throughput transcriptomic resources provide an efficient basis for investigating P-RGs in MM. Datasets deposited in the GEO and UCSC Xena contain gene-expression profiles with corresponding clinical annotations, allowing cross-cohort mining and validation at relatively low cost. Statistical and machine-learning approaches, such as Cox proportional hazards modeling and LASSO regression, further allow molecular features to be integrated with survival information[8][9]. These methods can generate multigene signatures with potential value for prognostic assessment.

Accordingly, this work used a multi-step computational strategy to identify differentially expressed P-RGs in MM, establish and validate a key-gene prognostic model, and evaluate the links between the model, immune infiltration, and drug sensitivity. By combining independent MM transcriptomic cohorts, this study aimed to provide a molecular tool for survival prediction and to clarify how paraptosis-associated mechanisms may intersect with immune regulation in MM. Such analysis may contribute to personalized treatment decisions and extend current understanding of MM from the perspective of regulated cell death.

2 Materials and methods

2.1 Data collection

GSE39754 [GPL5175; plasma cell transcriptomic data from 170 MM patients and six control samples] and GSE4581 [GPL570; plasma cell transcriptomic data from 414 MM patients with survival information] were obtained from the GEO database(<https://www.ncbi.nlm.nih.gov/geo/>). MMRF-COMMPASS, containing plasma cell transcriptomic profiles from 764 MM patients, of whom 763 had survival information, was downloaded from the UCSC Xena(<https://xena.ucsc.edu/>). GSE39754 was used for differentially expressed gene (DEG) discovery, whereas GSE4581 and MMRF-COMMPASS were applied to construct and validate the risk model. In addition, 66 P-RGs were collected from a previous study (Table S1)[10].

2.2 Identification and correlation analysis of differentially expressed P-RGs

DEGs between MM and control samples in GSE39754 were screened with the limma package (v 3.58.1)[11] under the thresholds $p < 0.05$ and $|\log_2FC| > 0.5$. A volcano plot was drawn with ggplot2 (v 1.0)[12], and the ten most upregulated and ten most downregulated DEGs were annotated. ComplexHeatmap (v 2.18.0) [13] was used to display the same top-ranked DEGs in a heatmap. The overlap between the DEG list and the 66 P-RGs was obtained with ggvenn (v 1.2.2) [14] to define differentially expressed P-RGs. Pairwise correlations among

these P-RGs were then calculated using the psych package (v 2.4.3)[15], with $|cor| > 0.3$ and $p < 0.05$ as the screening criteria.

2.3 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses

Biological functions and pathways associated with differentially expressed P-RGs were examined by GO and KEGG enrichment using clusterProfiler (v 4.10.1; $p_{adj} < 0.05$)[16]. The top ten GO terms and top five KEGG terms were summarized in bar plots generated with ggplot2 (v 1.0).

2.4 Identifying candidate genes via protein-protein interaction (PPI) network

To examine potential protein-level connections, all differentially expressed P-RGs were submitted to the STRING with an interaction score > 0.4 . Isolated nodes were removed before network visualization in Cytoscape software (v 3.9.1)[17].[18] Genes showing stronger network connectivity were retained as candidate genes for subsequent mechanistic exploration of MM development.

2.5 Identification of prognostic genes

Candidate genes were next evaluated for prognostic relevance. In 763 MMRF-COMMPASS patients with survival data, univariate Cox regression was conducted with the coxph function of the survival package (v 3.7.0)[19]. Genes with $p < 0.05$ and $HR \neq 1$ were considered survival-related, and 95% confidence intervals (CIs) were calculated. The PH assumption was tested using cox.zph in the same package, and genes passing the criterion $p > 0.05$ were retained as prognosis-related genes. Forestplot (v 2.0.1)[20] was used to visualize the results. Finally, glmnet (v 4.1.1) was applied for LASSO regression[21]. The final prognostic genes and their LASSO coefficients were obtained at the minimum lambda value.

2.6 Construction and evaluation of a risk model

For the 763 MM patients in MMRF-COMMPASS, the risk score was calculated with the following formula, where $coef$ indicates the LASSO coefficient and β_{gene} indicates the expression level of each prognostic gene.

$$riskScore = \sum (\beta_{gene} * coef)$$

According to the optimal cutoff value, all MM samples were assigned to the high-risk group (HRG) or low-risk group (LRG). Risk distribution and survival status were shown in a Sankey diagram. Overall survival between HRG and LRG was compared by the log-rank test, and Kaplan-Meier (K-M) curves were plotted with survminer (v 0.4.9)[22]. Time-dependent ROC curves were generated with survivalROC (v 1.0.3)[23]; an area under the ROC curve (AUC) greater than 0.6 was regarded as acceptable predictive performance. The same model was further tested in GSE4581.

2.7 Identification of independent prognostic factors

The relationships between risk score and clinicopathological factors, including ISS tumor stage, age, and gender, were analyzed in 763 MMRF-COMMPASS samples using Wilcoxon and Kruskal-Wallis tests ($p < 0.05$).

Risk score, stage, age, and gender were then evaluated for association with overall survival (OS) by univariate Cox regression in the survival package (v 3.7.0), using $p < 0.05$ and $HR \neq 1$ as criteria, followed by PH assumption testing ($p > 0.05$). Multivariate Cox regression with the same thresholds was used to determine which of these four variables independently predicted OS.

2.8 Construction and evaluation of a nomogram

Independent prognostic variables were incorporated into a nomogram using the rms package (v 6.5.0)[24]. Calibration curves were produced with rms to examine agreement between predicted and observed outcomes. DCA, implemented with ggDCA (v 1.2)[25], was used to evaluate the clinical benefit of the nomogram.

2.9 Gene set enrichment analysis (GSEA)

GSEA was used to explore biological processes and pathways associated with MM development in the 763 MMRF-COMMPASS samples. DESeq2 (v 1.38.0) first assessed differences between HRG and LRG[26]. Correlation coefficients were then calculated with the cor function in the psych package (v 2.4.3). The reference gene set c2.cp.kegg.v2023.1.Hs.symbols.gmt was obtained from the Molecular Signatures Database and ordered according to descending cor values. ClusterProfiler (v 4.10.1) was applied for enrichment testing, with $|NES| > 1$ and $p_{adj} < 0.05$ as criteria. The five most significant pathways were displayed as ridge plots.

2.10 Immune microenvironment analysis

In the 763 MMRF-COMMPASS samples, single-sample GSEA (ssGSEA) was used to estimate the abundances of 28 immune cell types in HRG and LRG[27]. Immune cells not meeting $p < 0.05$ were excluded. Stacked charts and box plots were generated with ggplot2 (v 1.0) to show immune composition and intergroup differences. Spearman analysis in psych (v 2.4.3) was then used to evaluate correlations among differential immune cells and between these cells and prognostic genes, with $|cor| > 0.3$ and $p < 0.05$. Immune score, stromal score, and ESTIMATE score were calculated with ESTIMATE (v 1.0.13)[28], and differences between HRG and LRG were tested at $p < 0.05$. The expression of IDO1, VTCN1, PDCD1LG2, CD276, and CD274 was also compared between risk groups ($p < 0.05$). These results were visualized as box charts with ggplot2 (v 1.2).

2.11 Drug sensitivity analysis

To assess treatment relevance, 138 MM-related drugs were collected from the Genomics of Drug Sensitivity in Cancer. IC50 values for these drugs in 763 MM samples were estimated using pRRophetic (v 0.5)[29]. Drugs with IC50 differences between HRG and LRG were identified at $p < 0.05$, and box plots were generated with ggplot2 (v 1.2).

2.11 RT-qPCR

Five paired frozen tissue specimens, including ten control samples and five MM samples, were obtained from Tangdu Hospital for RT-qPCR verification of prognostic gene expression. The study was approved by the Ethics Committee of Tangdu Hospital, and written informed consent was obtained from all patients. Total RNA was isolated from the 10 samples with TRIzol (Ambion, Austin, USA) according to the manufacturer's protocol. cDNA synthesis was performed using the SureScript-First-strand-cDNA-synthesis-kit (Servicebio, Wuhan, China). RT-qPCR was conducted with 2xUniversal Blue SYBR Green qPCR Master Mix (Servicebio, Wuhan, China), and primer sequences are provided in Table S3. GAPDH served as the internal reference. Relative expression was

calculated by the $2^{-\Delta\Delta C_t}$ method. GraphPad Prism 5 was used for visualization and statistical testing, with t-test applied to calculate p values.

2.12 Statistical analysis

All analyses were carried out in R programming language (v 4.2.2)[30]. Between-group differences were evaluated with the log-rank test or Wilcoxon test, and $p < 0.05$ was defined as statistically significant.

3 Results

3.1 26 candidate genes affected MM through multiple pathways

A total of 8,112 DEGs were detected at the initial screening stage, including 3,041 upregulated and 5,071 downregulated genes. These genes indicated marked transcriptional divergence between MM and control samples. OLR1 showed the strongest reduction, whereas TNFRSF17 exhibited the largest expression change in MM samples (Fig. 1A and Table S2). Intersection with the P-RG list identified 36 P-RGs with differential expression during MM development (Fig. 1B and Table S3). Among them, MYLK had the strongest negative association with EIF2S1 ($\text{cor} = -0.337$ and $p < 0.01$) and the strongest positive association with CSF1 ($\text{cor} = 0.437$ and $p < 0.01$) (Fig. 1C and Table S4-5). Enrichment analysis linked the candidate genes to the oxytocin signaling pathway and endoplasmic reticulum (ER)-related processes, including regulation of unfolded protein and ER-nucleus signaling pathway (Fig. 1D-E and Table S6-7). After PPI network filtration, 26 genes remained for subsequent investigation of MM mechanisms. MYLK interacted only with PRKACG, whereas CDK4 showed interactions with PPP3CA, ITPR3, CASP3, CASP7, and PHB (Fig. 1F and Table S8). These interaction patterns may help explain how these genes participate in MM and may inform personalized therapeutic exploration.

3.2 Risk model showed good prediction performance of prognostic genes for MM

Prognostic modeling was performed using 763 MMRF-COMMPASS patients. Univariate Cox analysis first identified MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, CASP3, and PHB as MM risk factors ($\text{HR} > 1$ and $p < 0.05$) (Fig. 2A and Table 1). LASSO regression further reduced the gene set to MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB when lambda reached its minimum value (0.01011795) (Fig. 2B). The final formula was $\text{riskScore} = 0.203 \times \text{MYLK} + 0.579 \times \text{EIF2S1} + 0.103 \times \text{CDK4} + 0.189 \times \text{PPP3CA} + 0.098 \times \text{HSPB8} + 0.373 \times \text{PHB}$ (Table 2). Using 4.753453 as the cutoff, 381 patients were classified as HRG and 382 as LRG. Patients with lower risk scores contained a larger proportion of surviving cases (Fig. 2C), and LRG showed a significantly higher survival probability than HRG ($p < 0.0001$) (Fig. 2D). The AUC values for 1-, 2-, and 3-year survival were all greater than 0.6, supporting the predictive value of the model (Fig. 2E). External validation in GSE4581 produced similar results. With 16.21127 as the cutoff, 207 patients were assigned to HRG and 207 to LRG (Fig. 2F). Lower risk scores again corresponded to more surviving patients, LRG had better survival than HRG ($p = 0.00088$) (Fig. 2G), and the AUC values indicated favorable model performance (Fig. 2H). These results suggest that the risk score may aid prognosis estimation and individualized management in MM.

Table 1
PH assumption test

Genes	p
MYLK	0.592574456
EIF2S1	0.939814263
CDK4	0.396679014
PPP3CA	0.769101202
HSPB8	0.535494479
CASP3	0.818848197
PHB	0.323319112

Table 2
lasso-coef

	MYLK	EIF2S1	CDK4	PPP3CA	HSPB8	PHB
coef	0.202757713	0.579399097	0.102988509	0.189225377	0.097914121	0.373084082

3.3 Stage and risk score had good prediction performance for MM

Risk score varied significantly among ISS stages, including comparisons between stage I and II and between stage I and III ($p < 0.05$) (Fig. 3A). Both stage and risk score satisfied the univariate and multivariate Cox regression criteria and passed the PH assumption test, identifying them as independent prognostic factors for MM (Fig. 3B-C and Table 3). Their HR values were also above 1, confirming their risk-factor status. A nomogram based on stage and risk score showed that higher total points indicated greater predicted MM risk, and the estimated risk rose with overall survival time (Fig. 3D). Calibration curves were close to the ideal slope of 1, indicating good agreement between predicted and observed outcomes (Fig. 3E). DCA further showed that the nomogram yielded a higher net benefit than other models (Fig. 3F). Therefore, this model may support more precise clinical stratification and earlier intervention for high-risk patients.

Table 3
PH assumption test

PH assumption test	
phe	p
stage	0.130768298
age	0.607878238
gender	0.726706506
risk Score	0.480527615

3.4 Multiple signaling pathways affected the MM development

GSEA revealed 41 pathways related to MM development. Chemokines, cytokines and their receptors, and hematopoietic cells were among the prominent pathway categories (Fig. 4 and Table S9). These findings indicate that MM progression may involve a broad regulatory network. Chemokines and cytokines mediate communication among cells and may affect both immune-cell activity and tumor-cell behavior. Hematopoietic cells, as important components of the bone marrow niche where MM develops, may also interact directly or indirectly with malignant plasma cells.

3.5 Prognostic genes affected MM development through immune microenvironment

Immune infiltration analysis detected 26 immune cell types with significant differences between HRG and LRG, including macrophages, natural killer (NK) cells, and mast cells ($p < 0.05$) (Fig. 5A-B). Macrophages showed a strong positive association with myeloid-derived suppressor cells (MDSC) ($\text{cor} = 0.9$ and $p < 0.05$) (Fig. 5C), implying coordinated immune activity within the MM microenvironment. Further correlation analysis found that Type 2 T helper cells were positively associated with PPP3CA ($\text{cor} = 0.322$ and $p < 0.0001$), whereas NK cells were negatively associated with PHB ($\text{cor} = -0.357$ and $p < 0.001$) (Fig. 5D). These patterns suggest that PPP3CA and PHB may influence MM prognosis through distinct immune-related mechanisms. Immune score, stromal score, and ESTIMATE score were all significantly different between HRG and LRG ($p < 0.05$) (Fig. 5E), indicating different immune and stromal compositions between risk groups. CD274 and CD276 were also upregulated in HRG (Fig. 5F), suggesting possible roles in immune escape and prognostic stratification. These results may provide clues for immunomodulatory treatment development in MM.

3.6 Totally 108 drugs had significant differences of IC50 in MM development

The drug sensitivity analysis showed that 108 drugs had significantly different IC50 values between HRG and LRG. The ten most significant candidates were Etoposide, PF.4708671, GEMCITABINE, QS11, DOXORUBICIN, A.443654, CCT018159, Vinorelbine, AZD.0530, and Mitomycin.C ($p < 0.05$) (Fig. 6 and Table S10). These findings suggest potential treatment-related differences between the two risk groups. Drug-response information combined with the risk score may help refine future predictive models and support personalized therapeutic decision-making in MM.

3.7 Expression validation

Compared with the NC group, EIF2S1, CDK4, PPP3CA, and HSPB8 were significantly increased in the MM group (all $P < 0.01$) (Fig. 7). EIF2S1 displayed the greatest elevation, reaching approximately a 6-fold higher level in MM samples than in NC samples. By contrast, MYLK was significantly reduced in MM samples compared with NC samples ($P < 0.01$), making it the only downregulated gene in the MM cohort. These results indicated that the paraptosis-related gene signature was widely altered in MM, with particularly prominent upregulation of EIF2S1 and HSPB8.

4 Discussion

MM arises from the clonal expansion of malignant plasma cells in the bone marrow and produces complications such as bone destruction, anemia, renal dysfunction, and infection susceptibility. Although proteasome inhibitors, immunomodulatory drugs, and autologous stem cell transplantation have improved outcomes, relapse and drug resistance still prevent durable cure in many patients[31][32][33]. In this study, transcriptomic datasets from public resources were integrated to identify MM-related P-RGs, namely MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB, and to develop a prognostic risk model. The model maintained predictive performance in an external cohort and was associated with immune infiltration and drug sensitivity profiles. These observations indicate that paraptosis-related molecular changes may be connected with the MM immune microenvironment and therapeutic response, supporting the potential clinical relevance of P-RG-based biomarkers.

The discovery of 36 differentially expressed P-RGs, followed by PPI network construction, provides a framework for interpreting the molecular basis of MM pathogenesis. MYLK and CDK4 showed distinct interaction patterns, suggesting that they may affect MM through different biological processes. Enrichment in the oxytocin signaling pathway and unfolded protein response (UPR) supports a relationship with ER stress and protein-homeostasis imbalance, both of which are relevant to MM progression. The negative correlation between MYLK and EIF2S1 and the positive correlation between MYLK and CSF1 suggest that these genes may participate in stress-response regulation and communication with the microenvironment. Comparable mechanisms have been described in contexts where abnormal ER stress enables tumor adaptation and resistance to therapy[34][35].

The prognostic signature composed of MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB performed consistently across cohorts, supporting the biological relevance of these genes in MM[36]. Under normal conditions, EIF2S1 (Eukaryotic translation initiation factor 2 subunit 1) is a component of the eIF2 complex, which binds initiator tRNA (Met-tRNA^{iMet}) and transports it to the small ribosomal subunit to start protein translation[37][38]. Kinase-mediated phosphorylation of EIF2S1 alters GTP affinity and reduces translation initiation efficiency[39][40]. Therefore, inhibiting kinases associated with EIF2S1 may reduce EIF2S1 phosphorylation and restrain abnormal protein translation in myeloma cells. The high risk coefficient of EIF2S1 further suggests that it may act as an important node linking ER stress, translational control, and apoptosis-related regulation in MM[41].

PHB (Prohibitin) is a conserved multifunctional protein mainly distributed in mitochondria and the nucleus. Within the nucleus, PHB participates in transcriptional regulation and gene-expression control, with JAK2 as one representative target[42]. Through interactions with multiple transcription factors, PHB can influence downstream transcriptional programs[43]. In this study, PHB showed an inverse relationship with natural killer (NK) cell infiltration, suggesting potential involvement in mitochondrial regulation and immune evasion. Evidence connecting PHB expression with tumor-cell survival and immune modulation also supports its value as a possible therapeutic target in MM[44].

The combined prognostic value of the paraptosis-based risk score and ISS stage suggests that molecular and clinical indicators describe different but complementary aspects of MM. This type of combined stratification is consistent with approaches that use molecular signatures together with conventional clinical indices to improve prediction. In the present model, the gene signature mainly represented tumor-intrinsic processes, especially ER stress and cell-cycle regulation, whereas ISS more directly reflected tumor burden and organ dysfunction. The nomogram based on these two factors showed good calibration and net clinical benefit, indicating possible

translational utility. Such integrated tools may enable more detailed patient classification by linking disease biology with clinical severity.

Immune microenvironment analysis demonstrated that high- and low-risk groups had different immune infiltration patterns and immune checkpoint profiles. The positive association between macrophages and myeloid-derived suppressor cells (MDSCs) suggests an immunosuppressive niche that may favor immune escape and contribute to MM progression or reduced immunotherapy response[45]. The negative relationship between PHB and NK cells, together with the positive relationship between PPP3CA and Th2 cells, indicates that certain prognostic genes may regulate immune surveillance and evasion within the MM microenvironment. Increased CD274 (PD-L1) and CD276 expression in high-risk patients further supports the involvement of checkpoint-related immune escape. These findings describe a paraptosis-linked immune landscape in MM and imply that this cell death program may intersect with immune regulation.

Drug sensitivity analysis identified 108 drugs with significant differences between HRG and LRG. Among the most notable compounds were Etoposide, AZD.0530, GEMCITABINE, and other agents identified by screening. AZD.0530 is a small-molecule tyrosine kinase inhibitor that suppresses selected kinase activities[46]. In this study, AZD.0530 showed a lower IC50 in LRG than in HRG, although its therapeutic value in MM needs further verification. Mechanistically, inhibition of receptor tyrosine kinase signaling by AZD-0530 may weaken downstream proliferative pathways in myeloma cells, including the Ras-MAPK pathway. Reduced transmission through these pathways may restrict myeloma-cell proliferation. Experimental studies are required to confirm whether the predicted drug responses can guide individualized treatment selection.

In summary, this study developed and validated a prognostic model based on six paraptosis-related genes, MYLK, EIF2S1, CDK4, PPP3CA, HSPB8, and PHB. The model stratified MM patients by survival risk and identified risk score, together with ISS stage, as an independent prognostic factor. The analysis also characterized immune features of high-risk MM, including altered immune infiltration and increased checkpoint expression. These findings provide additional insight into MM pathophysiology and may support future individualized treatment strategies, particularly those involving immunotherapy and chemotherapy.

Several limitations remain. First, the study was largely based on public datasets, including GEO and MMRF, and functional validation such as in vitro and in vivo experiments was not performed. Retrospective data may also include batch effects and variation in sample collection or processing, which could influence robustness and generalizability. Second, available clinical information was mainly restricted to survival outcomes. Thus, the performance of the model in specific treatment settings could not be fully evaluated because variables such as treatment history, recurrence, and comorbidities were incomplete. Future work should validate the biological functions of these key genes, test the model prospectively in clinical cohorts, and explore therapeutic strategies derived from this framework to advance precision medicine for MM.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of IEC OF INSTITUT E for National Drug Clinical Trials,Tangdu

Hospital,Fourth MilitaryMedical University (protocol code K-HG-202608-21 and 2026-08-26 of approval). Written informed consent has been obtained from the patient.

Consent to publish

Not applicable

Funding Declaration

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Data Availability

Data Availability StatementThe datasets analysed in the present study are available in the Gene Expression Omnibus (GEO) repository under accession number GSE39754 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39754>) and GSE4581 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE4581>), as well as GDC MMRF-COMMPASS is available in the UCSC Xena platform (<https://xenabrowser.net/datapages/?cohort=GDC%20MMRF-COMMPASS>).

Clinical trial number

not applicable.

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Figures

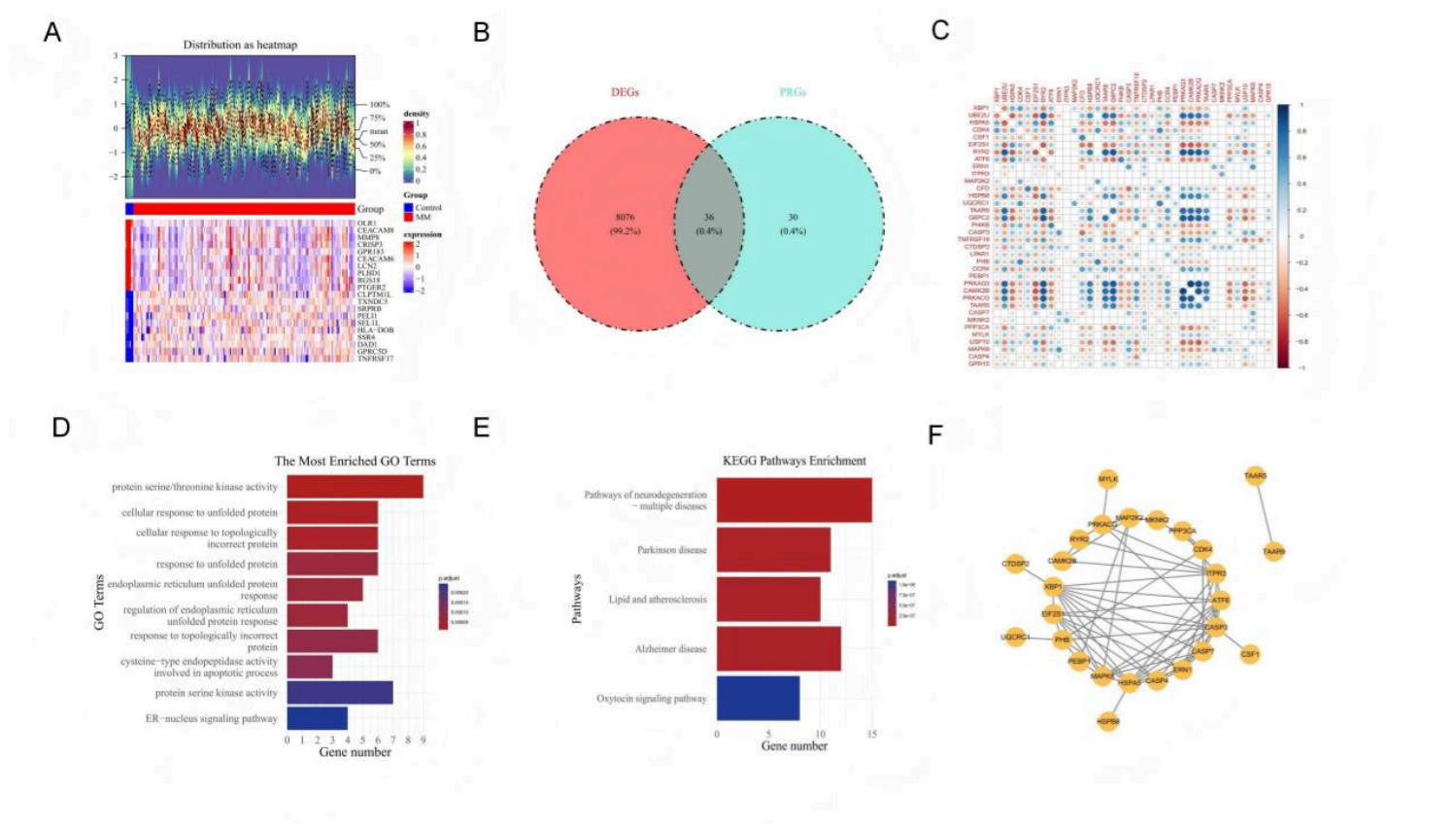


Figure 1

Identification and functional enrichment analysis of PRG-related DEGs in MM.

(A) Expression distribution heatmap of all genes in MM and control samples. (B) Venn diagram showing the overlap between differentially expressed genes (DEGs) and pyroptosis-related genes (PRGs). (C) Correlation heatmap of PRGs, with color indicating Pearson correlation coefficients. (D) Top enriched Gene Ontology (GO) terms for PRG-related DEGs. (E) Top enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for PRG-related DEGs. (F) Protein-protein interaction (PPI) network of the identified PRGs.

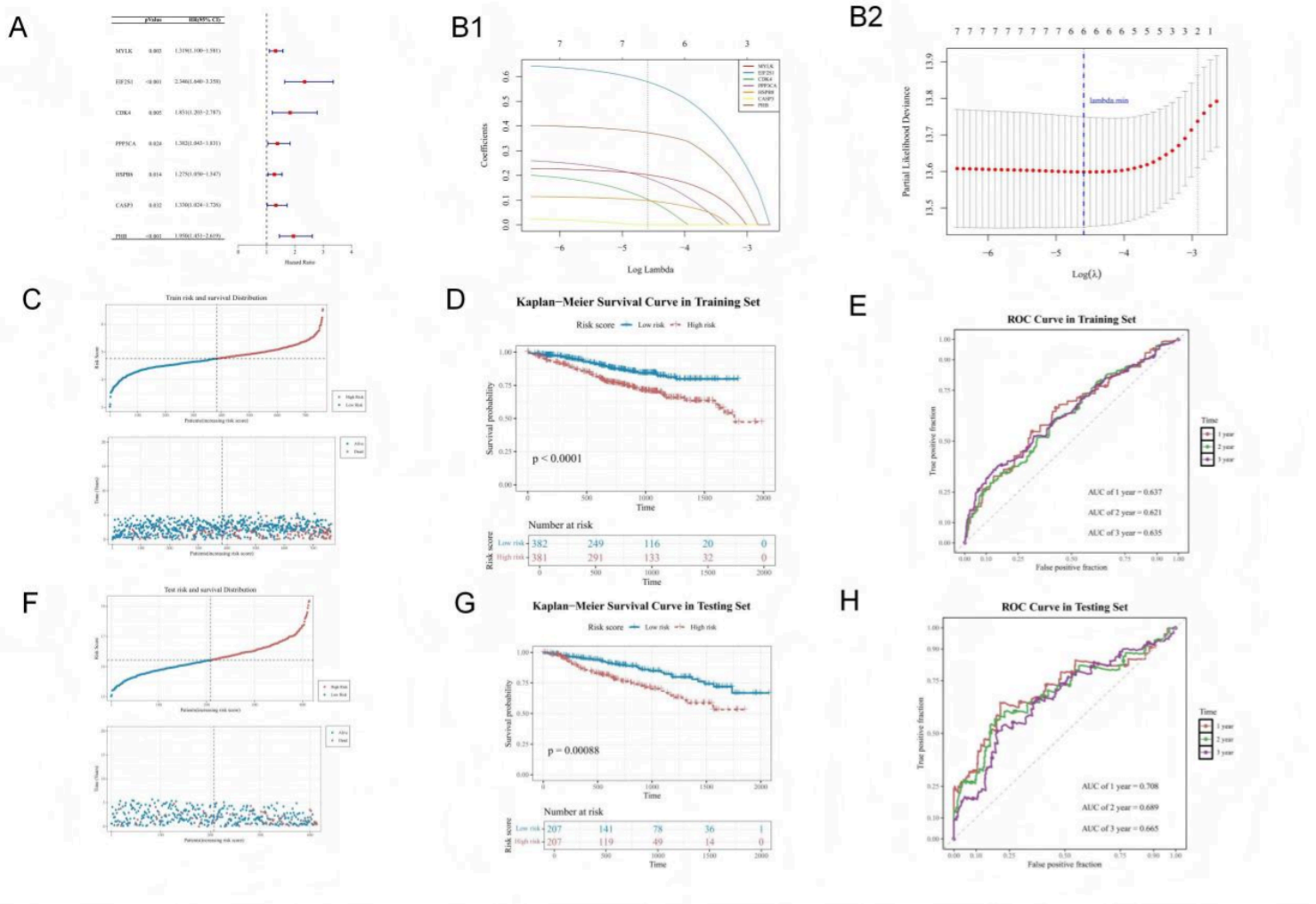


Figure 2

Construction and validation of the pyroptosis-related gene signature for multiple myeloma (MM).

(A) Forest plot showing the hazard ratios (HR) and 95% confidence intervals (CI) of prognostic PRGs identified by univariate Cox regression analysis.

(B1-B2) LASSO regression analysis for selecting prognostic genes. (B1) Coefficient profiles of genes at different log(λ) values. (B2) Partial likelihood deviance for cross-validation, with the optimal λ indicated by the dashed line.

(C) Risk score distribution and survival status of patients in the training set. Top: risk score curve; bottom: scatter plot of survival status.

(D) Kaplan-Meier survival curves comparing overall survival between low- and high-risk groups in the training set ($p < 0.0001$).

(E) Time-dependent ROC curves for 1-, 2-, and 3-year survival prediction in the training set.

(F) Risk score distribution and survival status of patients in the testing set.

(G) Kaplan-Meier survival curves comparing overall survival between low- and high-risk groups in the testing set ($p = 0.00088$).

(H) Time-dependent ROC curves for 1-, 2-, and 3-year survival prediction in the testing set.

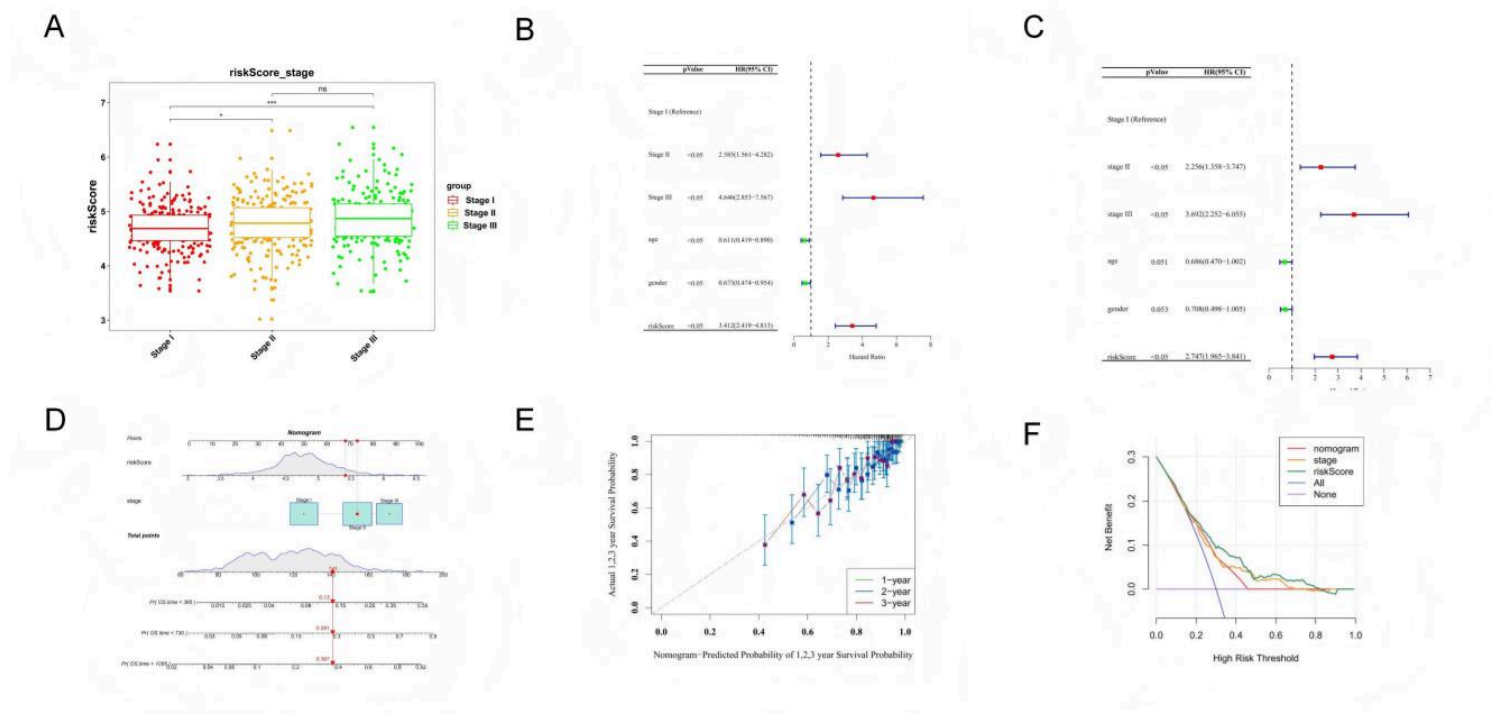


Figure 3

Clinical correlation and prognostic performance of the risk score and nomogram model.

(A) Box plot showing the distribution of risk scores across different clinical stages (Stage I–III). Statistical significance is indicated by asterisks (* $p < 0.05$, *** $p < 0.001$; ns, not significant).

(B-C) Forest plots of univariate (B) and multivariate (C) Cox regression analyses, evaluating the prognostic significance of the risk score and clinical variables (stage, age, gender). Hazard ratios (HR) and 95% confidence intervals (CI) are shown.

(D) Nomogram integrating the risk score and clinical stage to predict 1-, 2-, and 3-year overall survival probabilities.

(E) Calibration curves of the nomogram for 1-, 2-, and 3-year survival prediction, comparing predicted and actual survival probabilities.

(F) Decision curve analysis (DCA) showing the net benefit of the nomogram, risk score, and clinical stage models across different threshold probabilities.

Gene Set Enrichment Analysis

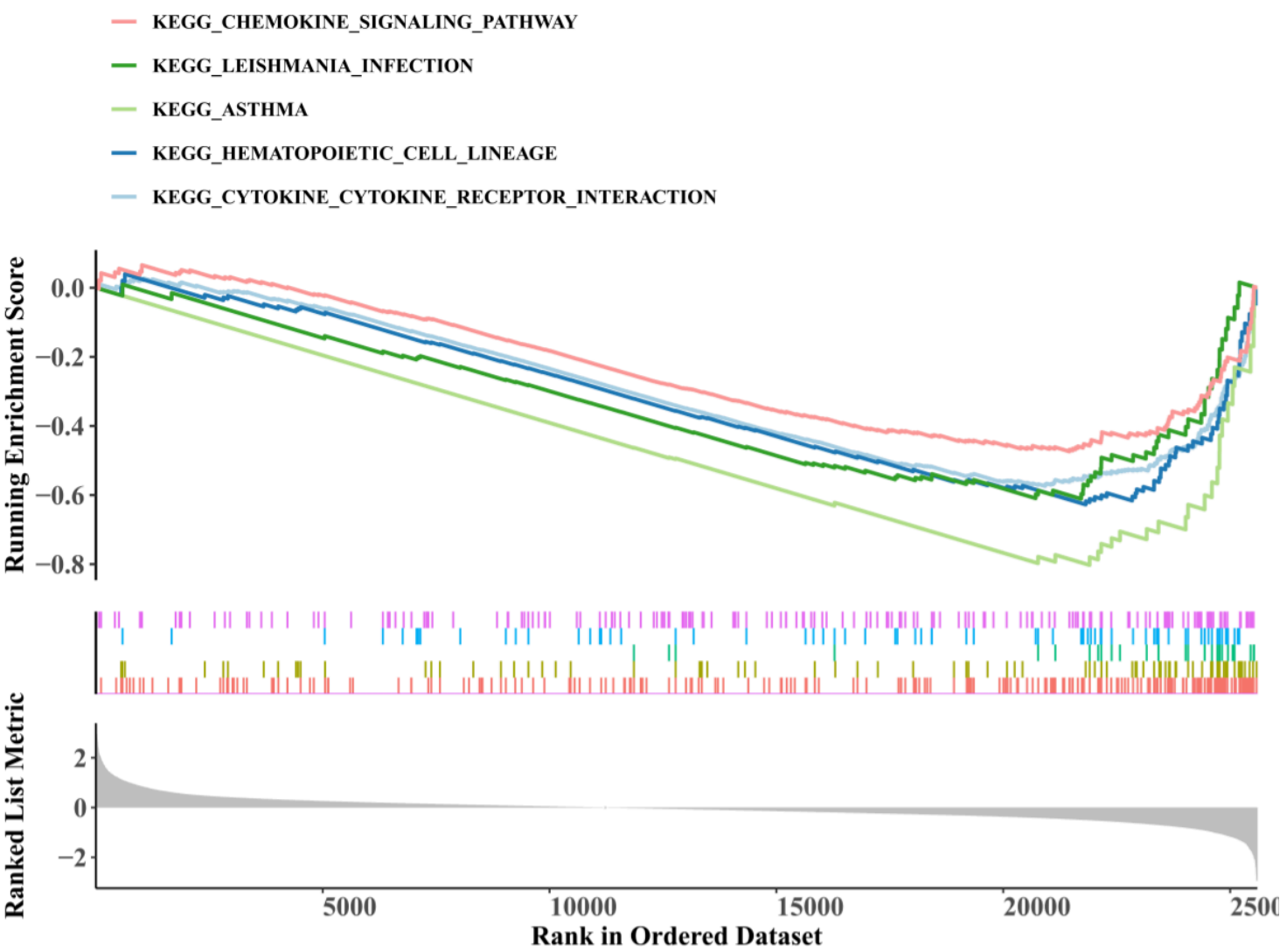


Figure 4
Gene Set Enrichment Analysis (GSEA) of the risk signature.

GSEA plots showing the top five downregulated KEGG pathways in the high-risk group, The running enrichment score (top), gene set positions (middle), and ranked list metric (bottom) are displayed for each pathway.

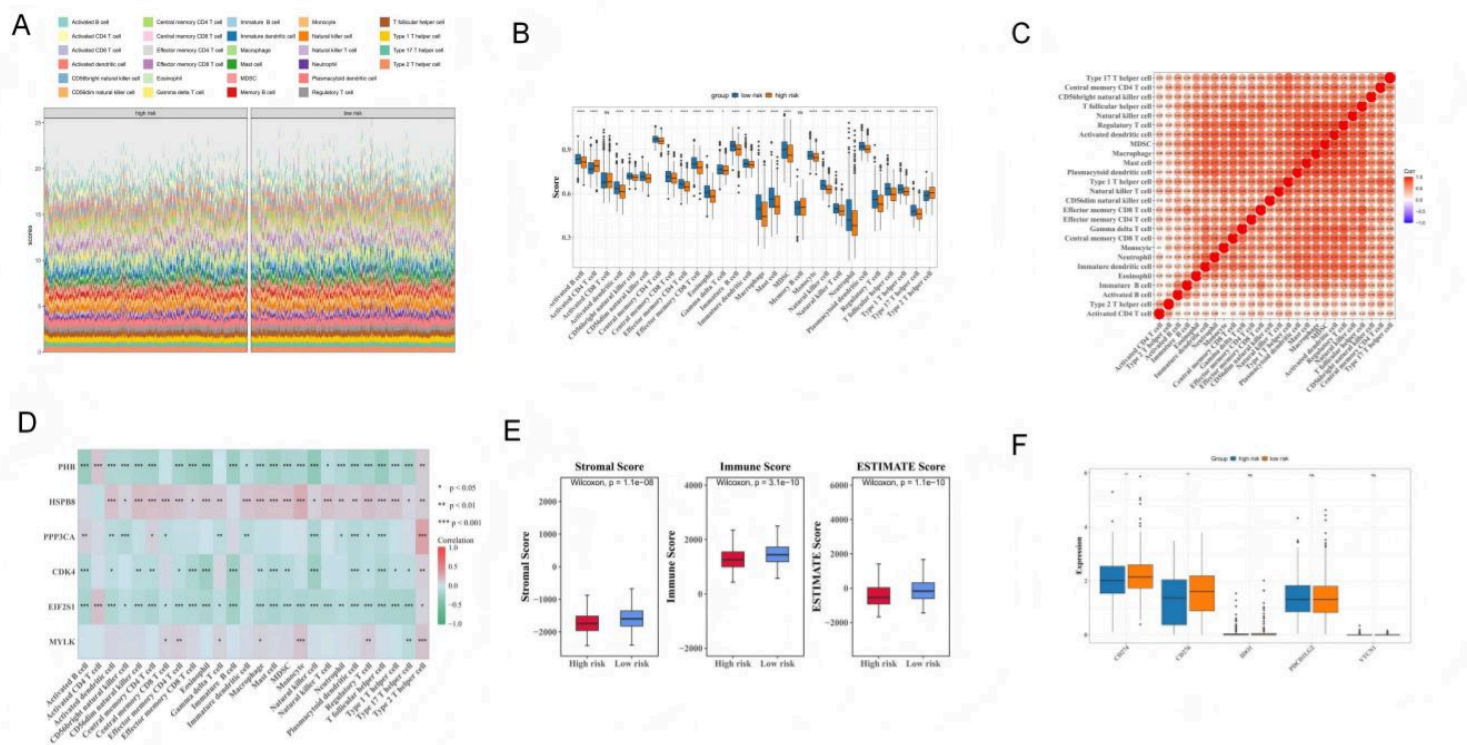


Figure 5

Tumor immune microenvironment analysis based on the pyroptosis-related risk signature. (A) Landscape of immune cell infiltration in high- and low-risk groups. (B) Box plots comparing the infiltration levels of 22 immune cell types between high- and low-risk groups. (C) Correlation heatmap showing the associations between different immune cell types (red = positive correlation, blue = negative correlation). (D) Correlation heatmap between risk signature genes and immune cell infiltration. (E) Comparison of Stromal Score, Immune Score, and ESTIMATE Score between high- and low-risk groups using the Wilcoxon test. (F) Box plots showing the expression levels of immune checkpoint genes (CD274, CD276, HAVCR1, PDCD1LG2, and VTCN1) in high- vs. low-risk groups.

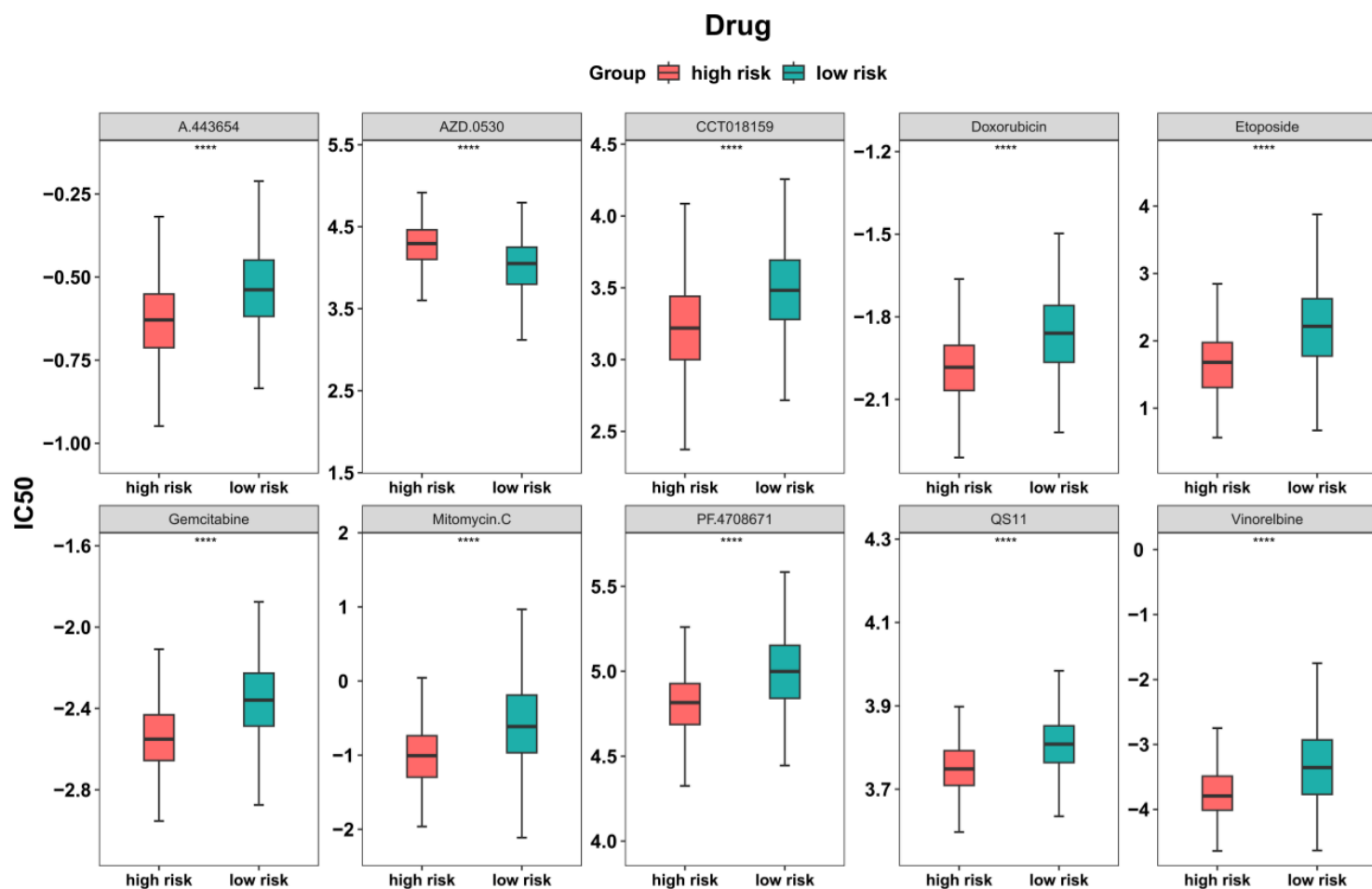


Figure 6

Drug sensitivity analysis between high- and low-risk groups.

Box plots showing the IC50 values of 10 anti-cancer drugs (A.443654, AZD.0530, CCT018159, Doxorubicin, Etoposide, Gemcitabine, Mitomycin.C, PF.4708671, QS11, and Vinorelbine) in high-risk (red) and low-risk (cyan) groups. The **** symbol indicates $p < 0.0001$.

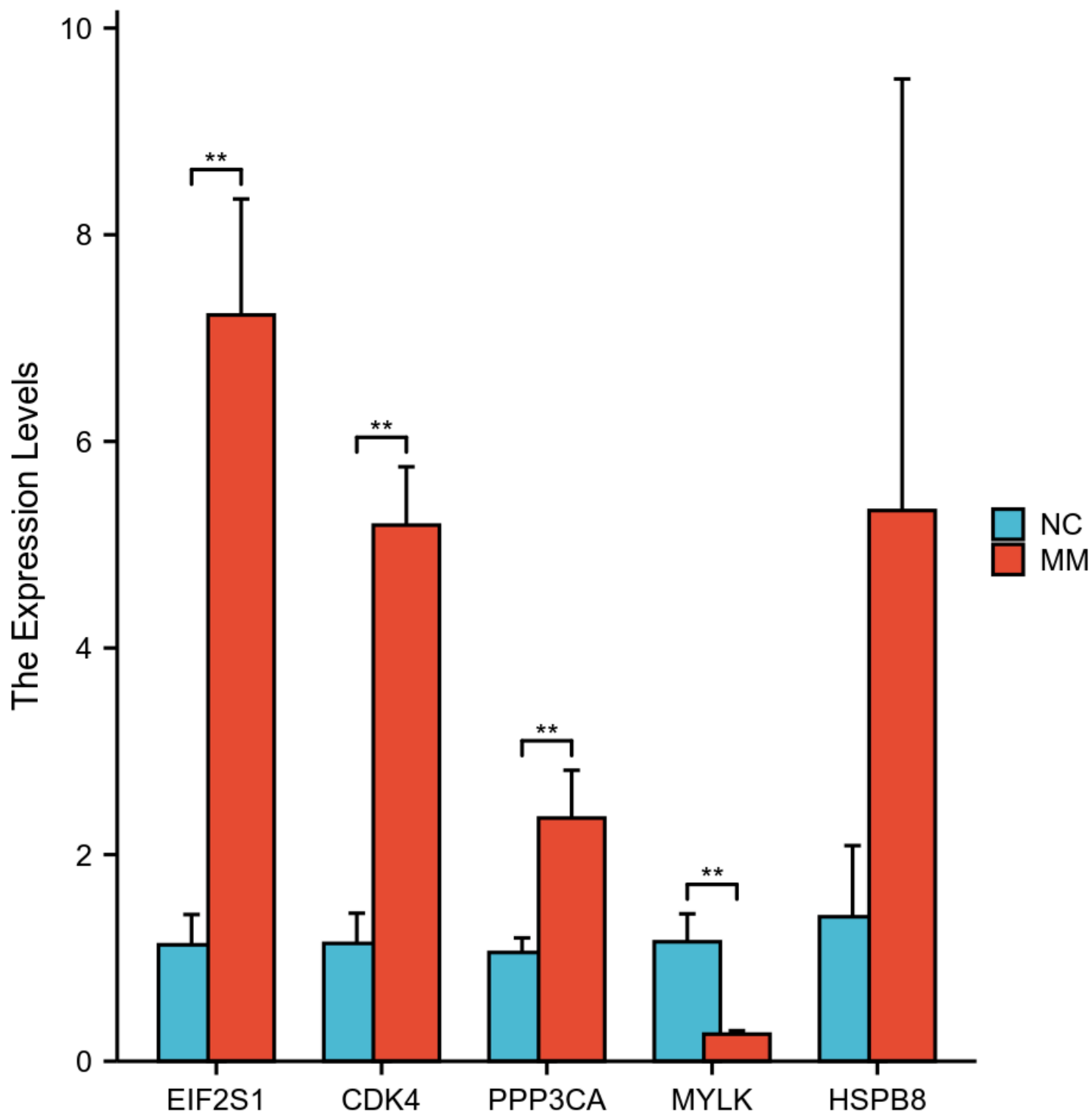


Figure 7

Validation of the expression levels of signature genes in MM patients.

Supplementary Files

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