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Integrating single-cell and bulk RNA profiles to uncover glutamine metabolism's role in prognosis and immune dynamics in multiple myeloma

Fei Zhao[1] and Feifei Che[2*]

Abstract

Objective Multiple myeloma (MM) exhibits significant heterogeneity, leading to variable treatment responses and poor clinical outcomes. Glutamine metabolism-related genes (GMRGs) represent critical regulators of tumor biology, yet their prognostic and therapeutic significance in MM remains unexplored. This study aims to identify GMRG-driven tumor signatures and establish their clinical utility as prognostic biomarkers, therapeutic targets and enhancers of drug sensitivity.

Methods Integrated transcriptomic and single-cell sequencing analyses of public multi-omics cohorts enabled systematic identification of GMRGs in MM through weighted co-expression network analysis coupled with univariate Cox proportional hazards modeling. Clinically prioritized GMRGs showing elevated expression in patient specimens were functionally validated through proliferation assays and pharmacological sensitivity profiling.

Results Integrated multi-omics analysis combining single-cell sequencing with bulk transcriptomic profiling and prognostic screening identified 51 prognostic GMRGs, with 10 core signature genes selected for model construction. The risk stratification system demonstrated robust prognostic capacity validated across multiple independent MM cohorts. Pathway enrichment revealed significant involvement in immune system, cell cycle and tumor signaling. MM patient validation identified DLD, SFT2D2, and UBA2 as significantly upregulated genes that promote tumor growth through enhancement of proliferation. Mechanistic investigations via shRNA-mediated knockdown established that DLD and UBA2 silencing significantly enhanced therapeutic efficacy of MM inhibitors.

Conclusion Multicohort-validated GMRGs (DLD/UBA2) drive MM progression and MM inhibitor responses. Clinical upregulation and functional silencing confirm dual therapeutic potential as prognostic biomarkers and drug-sensitizing targets.

Keywords Multiple myeloma, Glutamine metabolism-related genes (GMRGs), Prognosis, Immune infiltration, Biomarker

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Introduction

Multiple myeloma (MM) represents a heterogeneous plasma cell malignancy characterized by diverse cytogenetic abnormalities and molecular pathogenesis. Despite significant progress in molecularly targeted chemotherapeutics, MM remains an incurable disease with variable clinical outcomes, posing substantial challenges in therapeutic management [1, 2]. The median overall survival spans from weeks to over a decade, with five-year survival rates approaching 40% [3], underscoring the critical need for refined prognostic biomarkers to guide risk-adapted therapeutic strategies. Such biomarkers could optimize treatment selection and enhance clinical outcomes through personalized therapeutic paradigms [4].

Glutamine (Gln), a conditionally essential amino acid serving as a metabolic substrate for nucleotide synthesis, lipid biosynthesis, energy production via glutaminolysis, has emerged as a key metabolic vulnerability in cancer biology. Tumor cells frequently exhibit glutamine addiction, characterized by oncogenic reliance on exogenous Gln to sustain proliferation and survival through glutamate (Glu) conversion and oxidative phosphorylation [5, 6]. This metabolic phenotype renders glutamine metabolism an attractive therapeutic target, with pharmacological inhibitors of glutamine transporters (e.g.,ASCT2/SLC1A5) demonstrating preclinical efficacy in limiting tumor growth [7–9].

MM cells display distinct metabolic profiles compared to healthy counterparts, notably demonstrating reduced serum Gln levels [10]. Functional studies reveal that MM pathogenesis critically depends on glutamine homeostasis, with enhanced expression of glutamine transporters facilitating oncogenic glutamine uptake [9]. This metabolic adaptation drives MM cell proliferation through glutamine synthetase (GS)-mediated Gln biosynthesis from glutamate and ammonium [11, 12].These findings position glutamine metabolism as a potential therapeutic axis in MM.

The clinical relevance of glutamine metabolism-related genes (GMRGs) warrants systematic investigation as prognostic biomarkers and therapeutic targets. Notably, proteasome inhibitors (mainstays in MM therapy) induce metabolic stress that may synergize with glutamine-targeted interventions to overcome chemoresistance. Elucidating the clinical significance of GMRGs could yield novel biomarkers for therapeutic stratification and inform combination strategies to enhance proteasome inhibitor efficacy in relapsed/refractory MM.

Single-cell RNA sequencing (scRNA-seq) has revolutionized our ability to decipher cellular heterogeneity in hematologic malignancies, particularly by resolving transcriptional programs linked to therapeutic resistance and immune evasion [13, 14]. These subpopulations may serve as prospective treatment targets and be employed in the building of risk profiles. For instance, Zhao et al. identified a novel predictive biomarker for multiple myeloma using scRNA-Seq [15]. Building upon these findings, our study postulates that GMRGs critically influence MM prognosis. We aim to characterize key GMRGs associated with clinical outcomes and develop a robust prognostic model to enhance risk assessment and guide precision therapy.

In this investigative study, we acquired multi-omics data from the Gene Expression Omnibus (GEO) repository, including single-cell transcriptomic profiles and bulk RNA-seq datasets from MM patients. Through combined single-cell trajectory inference and weighted gene co-expression network analysis (WGCNA), we identified dysregulated GMRGs associated with MM pathogenesis. Machine learning-driven Least Absolute Shrinkage and Selection Operator (LASSO) regression (least absolute shrinkage and selection operator) was subsequently applied to construct a GMRG-based risk signature. This integrative approach addresses a critical knowledge gap in MM biology by establishing the first glutamine metabolism-centric prognostic framework.

Materials and methods

Data preprocessing

Transcriptomic expression profiles and clinical data of MM were retrieved from the Gene Expression Omnibus (GEO) database. The single-cell RNA sequencing dataset GSE118900, comprising 597 CD138-positive plasma cells from 15 individuals, was analyzed to characterize cellular GMRGs. Raw data were normalized using log2 transformation, and samples/genes with missing values were excluded to ensure analytical robustness. The GSE136337 dataset served as the training cohort for prognostic model development, while GSE4581 was designated as the independent validation set.

Identification of glutamine metabolism-related genes

The single-cell dataset GSE118900 [16] was processed using the Seurat R package (v4.4.0). Quality control thresholds included retention of cells with $<20\%$ mitochondrial gene content and >200 detected genes. Following normalization via the "NormalizeData" function, the top 2,000 highly variable genes were identified using "FindVariableFeatures". Cell type annotation using SingleR [17] confirmed all cells as B/plasma lineage. GMRGs were curated from the GeneCards database (correlation score cutoff: >15.0), yielding 226 candidate genes. Cellular metabolic activity was quantified via the AUCell R package [18], with cells stratified into high/low glutamine metabolism (GM) groups based on median AUC values. Differentially expressed genes (scRNA-diff-Marker) between GM groups were identified using the "FindAllMarkers" function.

Weighted gene co-expression network analysis

Co-expression networks were constructed using WGCNA (v1.73) [19]. A soft threshold power ($\beta = 10$) was selected to achieve scale-free topology ($R^2 = 0.9$). The dynamic tree cut algorithm identified gene modules, with a merging threshold of 0.25 to minimize module redundancy. Among nine identified modules, the green (positively correlated) and blue (negatively correlated) modules showing strongest associations with GM were retained. Intersection of module-derived genes with scRNA-diff-Marker defined the final GMRG set.

Prognostic model construction and validation

A 51-gene signature was derived from the intersection of single-cell sequencing and WGCNA module analyses. Prognostic relevance was determined through univariate Cox regression. LASSO regression (glmnet R package v4.1-8) with L1-norm regularization was applied to transcriptomic data to identify glutamine metabolism-related features. The optimal λ value (corresponding to 10 variables) was selected via 10-fold cross-validation using minimum mean squared error criteria.

Risk scores were calculated for each MM patient using modeling coefficients, with stratification into high/low-risk groups by median cutoff. Survival analysis was performed using the survival R package across two independent cohorts: GSE136337 ($n = 426$) and GSE4581 ($n = 414$). Statistical significance was confirmed by log-rank tests (both cohorts < 0.05). Time-dependent receiver operating characteristic (ROC) curves were generated with the survivalROC package to evaluate model performance, quantified through area under the curve (AUC) values.

Functional enrichment profiling

Single-sample gene set enrichment analysis (ssGSEA) and Gene set variation analysis (GSVA) were respectively employed to calculate Enrichment scores and pathway scores. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed using clusterProfiler(v4.12.6) [20], with reference gene sets obtained from MsigDB (https://www.gsea-msigdb.org/gsea/index.jsp).

Immune microenvironment and drug sensitivity assessment

Immune cell infiltration levels were quantified via CIBERSORT [21] analysis of the GSE136324 cohort. Drug sensitivity predictions were derived from the Genomics of Drug Sensitivity in Cancer (GDSC) using oncoPredict [22], with half-maximal inhibitory concentrations (IC50) estimated through ridge regression modeling of cancer cell line pharmacogenomic data.

qRT-PCR assessment of key gene expression in MM patients

This study received ethical approval from the Hospital Ethics Committee (No.2023 – 577). Bone marrow aspirates were collected from 11 newly diagnosed MM patients (age range: 18–65 years) and 9 age-matched healthy donors between January 20, 2024 and March 20, 2024. Exclusion criteria included: (1) prior anticancer therapy; (2) concurrent malignancies; (3) severe comorbidities (e.g., cardiac/pulmonary insufficiency). All participants provided written informed consent in accordance with Declaration of Helsinki guidelines. At each time point, 1–2 cm long bone marrow core samples were collected by experienced physicians following strict standard operating procedures to ensure sample quality.

CD138 + plasma cells were isolated from mononuclear cells using flow cytometry (FACSAria III, BD Biosciences). Total RNA was extracted with TRIzol reagent (Thermo Fisher), reverse-transcribed using PrimeScript RT Master Mix (Takara Bio), and quantified via qRT-PCR on a QuantStudio 5 system (Applied Biosystems) with SYBR Premix Ex Taq (Takara Bio). The $2^{-\Delta\Delta CT}$ method was employed for relative quantification using GAPDH as endogenous control. Primer sequences are detailed in Supplementary Table S3.

Cell proliferation assays

U266 myeloma cells (ATCC® TIB-196™) were transfected with siRNA targeting three model genes using Lipofectamine 3000 (Thermo Fisher). For proliferation analysis, 5×10^3 cells/well were seeded in 96-well plates. Cell viability was monitored daily for 5 days using CCK-8 reagent (Beyotime Biotechnology, C0037), with absorbance measured at 450 nm after 2 h incubation at 37 °C (SpectraMax i3x, Molecular Devices).

Drug sensitivity testing was performed with three investigational compounds: AZD8186_1918, Doramapimod_1042, and AZD6482_2169. Cells were exposed to serial drug dilutions (0–10 μM) for 2 h at 37 °C. The absorbance at 450 nm (OD450) was measured using a microplate reader. Dose-response curves were analyzed using mixed-effects models (lme4 package in R) with fixed factors (concentration, treatment) and random intercepts for experimental batches.

Statistical analysis

All anaiyses were conducted in R 4.2.2. Normality was assessed by Shapiro-Wilk test. Continuous variables were compared using Student's t-test (parametric) or Wilcoxon rank-sum test (non-parametric). Two-tailed P value < 0.05 was considered statistically significant.

Results

Single-cell profiling reveals glutamine metabolic heterogeneity in MM

Quality-controlled single-cell RNA sequencing data (GSE118900, $n = 15$ MM patients) underwent normalization and principal component analysis using Seurat. Following t-distributed stochastic neighbor embedding (t-SNE), cells segregated into six transcriptionally distinct clusters (Fig. 1A). Automated cell type annotation via SingleR confirmed all clusters as B-lineage cells (plasma cell/B cell identity; Fig. 1B). The AUCell algorithm quantified GMRG activity scores across single cells (Fig. 1C). Median-based stratification separated cells into high and low glutamine metabolism subgroups (Fig. 1D). Differential expression analysis identified scRNA-diff-Marker genes distinguishing these subgroups.

Construction of a prognostic model related to glutamine metabolism

WGCNA was performed to identify glutamine metabolism-related modules. A soft threshold power of 10 was selected to ensure a scale-free topology (Fig. 2A). Nine gene modules were generated by grouping related modules (Fig. 2B). Risk scores showed the strongest negative correlation with the green module (R = -0.46, $p < 0.001$) and the strongest positive correlation with the blue module ($R = 0.64$, $p < 0.001$) (Fig. 2C). The intersection of differential genes identified from these two modules with the high-low AUC group difference analysis was defined as GMRGs (Fig. 2D). Univariate Cox analysis was performed to screen GMRGs for prognostic correlation with MM (Table S1). Subsequently, We performed LASSO regression analysis to model prognosis in the training cohort (Fig. 2E). The risk model incorporated 10 GMRGs

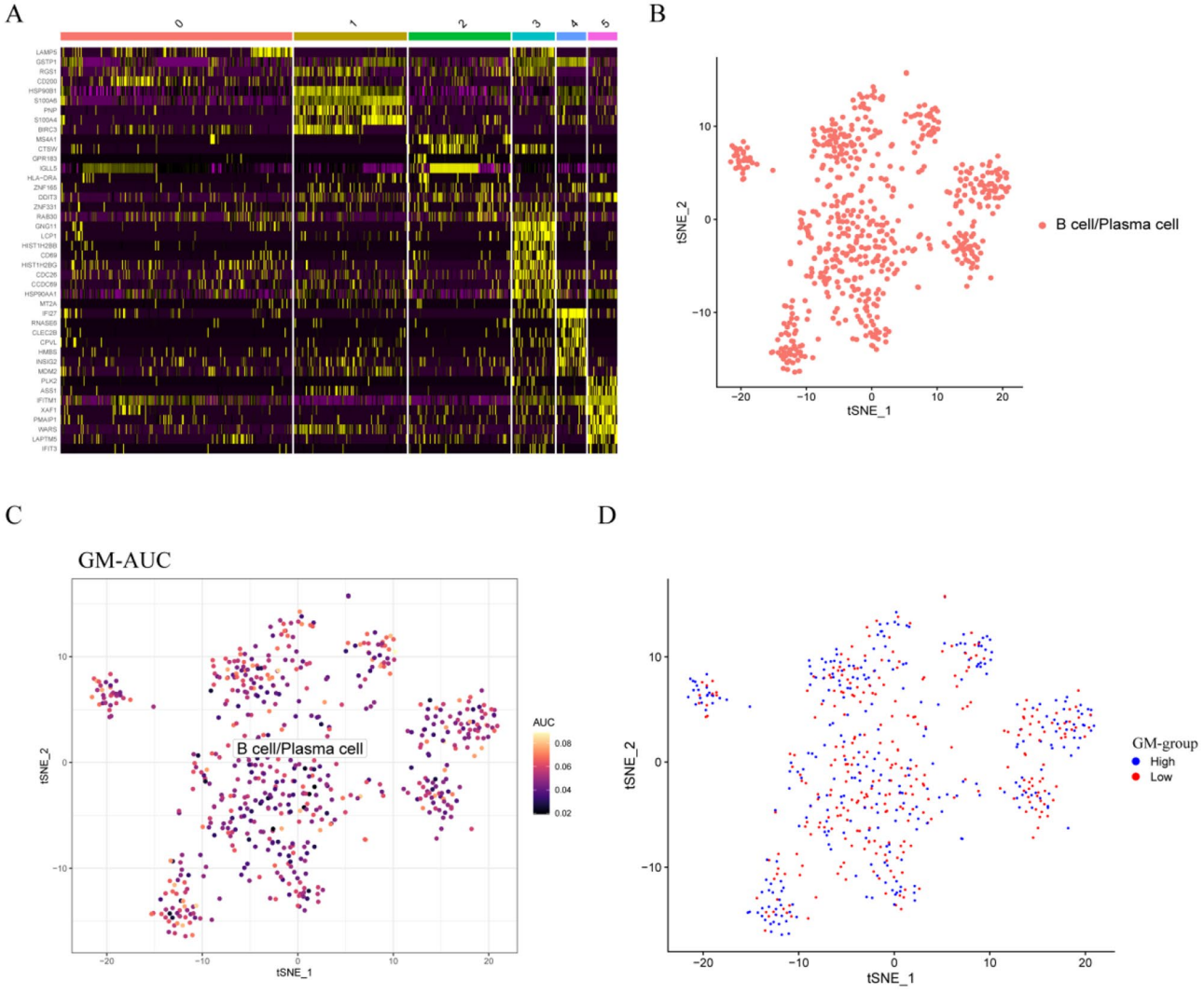

Fig. 1 Single-cell characterization of glutamine metabolic heterogeneity in MM. (**A**) Heatmap of cluster-defining marker genes across six clusters transcriptional subgroups. (**B**) t-SNE projection demonstrating B-cell/plasma cell identity via SingleR annotation. (**C**) GM-AUCell scoring distribution of glutamine metabolic activity. (**D**) Bimodal stratification into high/low metabolic subgroups (median cutoff)

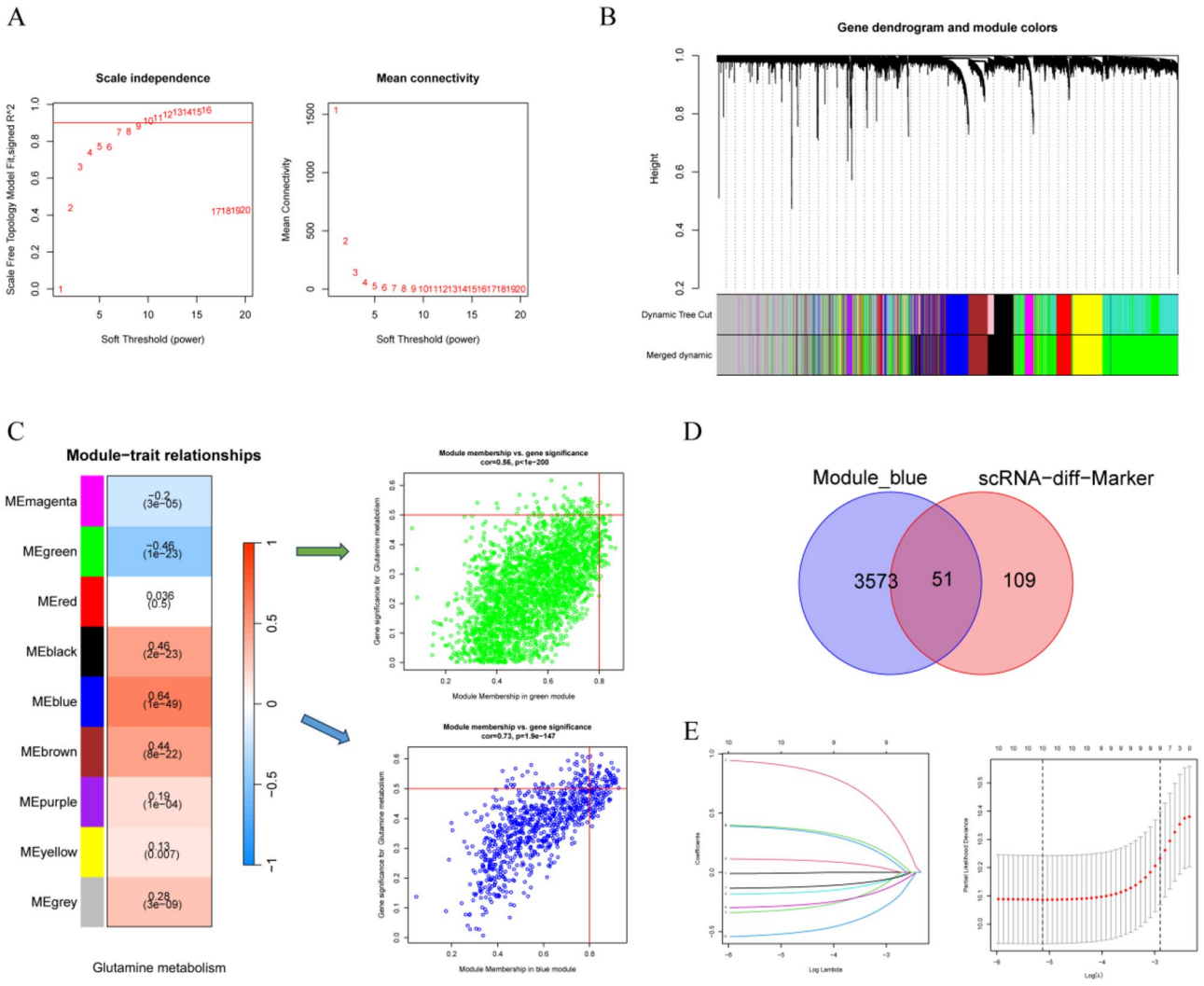

Fig. 2 Construction of a prognostic model related to glutamine metabolism. (**A**) Scale-free topology model fit for WGCNA soft-threshold selection. (**B**) Gene dendrogram and module colors of WGCNA. (**C**) Module-trait relationships highlighting glutamine-associated green/blue modules. (**D**) Venn diagram intersecting MEblue module with scRNA-diff-Marker. (**E**) LASSO coefficient trajectories were used to develop prognostic models

(ANKRD28, DLD, FAM114A2, HSP90AA1, KIF13B, NFAT5, PGM3, SFT2D2, UBA2, UBA5), with detailed regression coefficients provided in Table S2.

Prognostic association of risk scores and clinical characteristics

Survival analysis validated the prognostic reliability of the glutamine metabolism-derived risk model. Both training and validation cohorts exhibited significantly reduced overall survival in high-risk MM patients (Fig. 3A-B). Univariate Cox regression identified age, albumin, B2M, LDH, ISS stage, R-ISS stage, and risk score as significant prognostic predictors (Fig. 3C). However, multifactorial Cox analysis demonstrated that only age, B2M, ISS stage and risk score could be independent prognostic factors (Fig. 3D). The risk score model achieved superior predictive performance for 1- to 3-year survival (AUC > 0.7; Fig. 3E), outperforming conventional indicators including age, B2M, ISS stage (Fig. 3F). These results suggest that risk scores are superior molecular indicators over traditional pathologic indicators.

Immune microenvironment and drug sensitivity profiling

Tumor microenvironment (TME) analysis revealed distinct immune infiltration patterns between risk groups. High-risk patients showed increased infiltration of CD4 + memory-activated T cells and mast cells, alongside decreased macrophage M2 polarization and dendritic cell activation ($p < 0.05$; Fig. 4A). Pathway enrichment analysis further indicated upregulation of antigen-presenting cell (APC) co-stimulation pathway and downregulation of HLA-mediated antigen presentation, immune checkpoint signaling, and type I interferon responses in the high-risk group ($p < 0.05$; Fig. 4B).

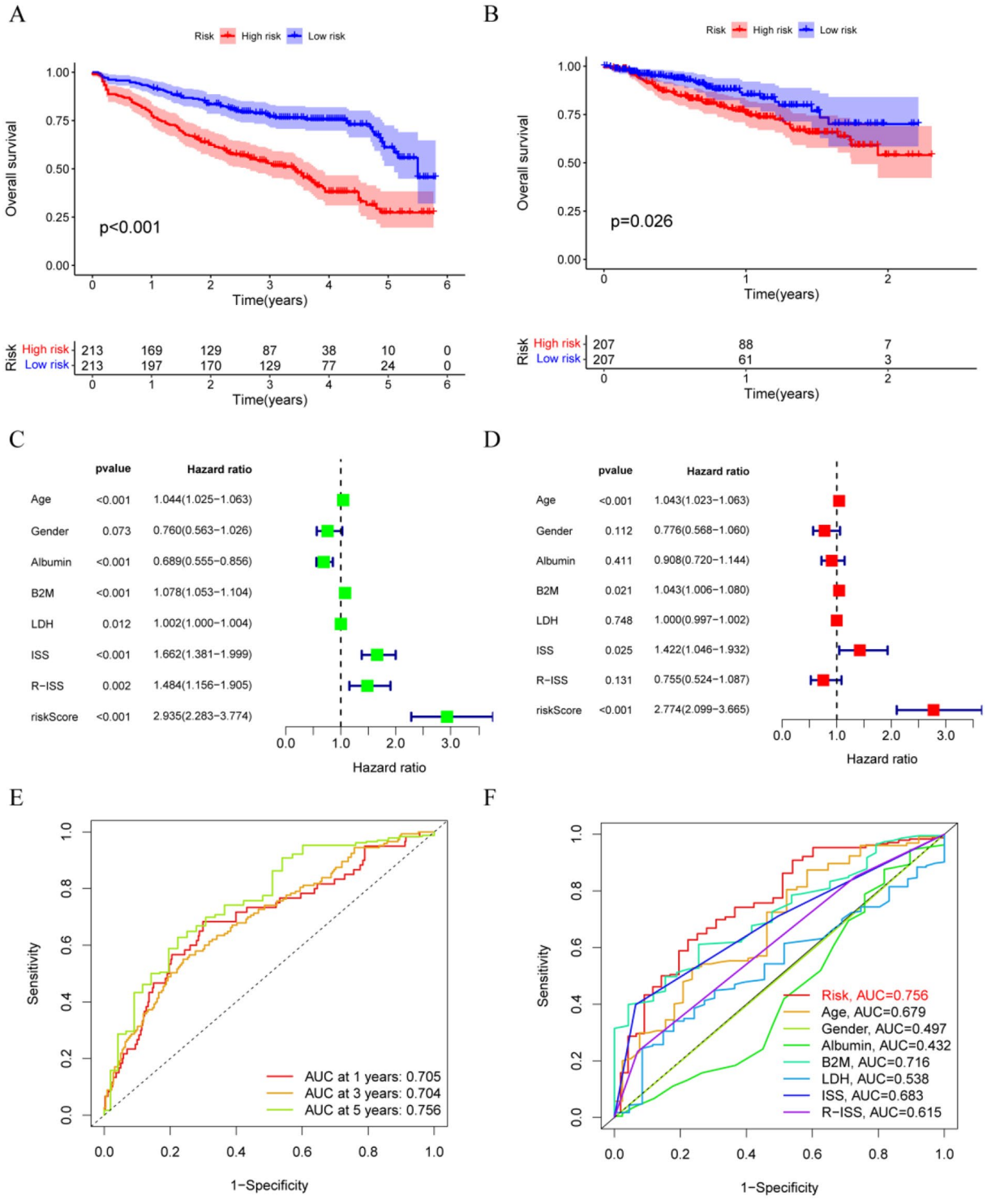

Fig. 3 Prognostic performance of glutamine metabolism-derived risk scores. Kaplan-Meier survival curves comparing overall survival between high- and low-risk MM patients in (**A**) training (GSE136337) and (**B**) validation (GSE4581) cohorts. (**C**) Forest plot of univariate Cox regression for clinical variables and risk scores. (**D**) Multifactorial Cox regression identifying independent prognostic factor. (**E**) Time-dependent ROC curves evaluating 1-, 3-, and 5-year survival prediction. (**F**) Comparative AUC analysis between risk scores and conventional prognostic indicators

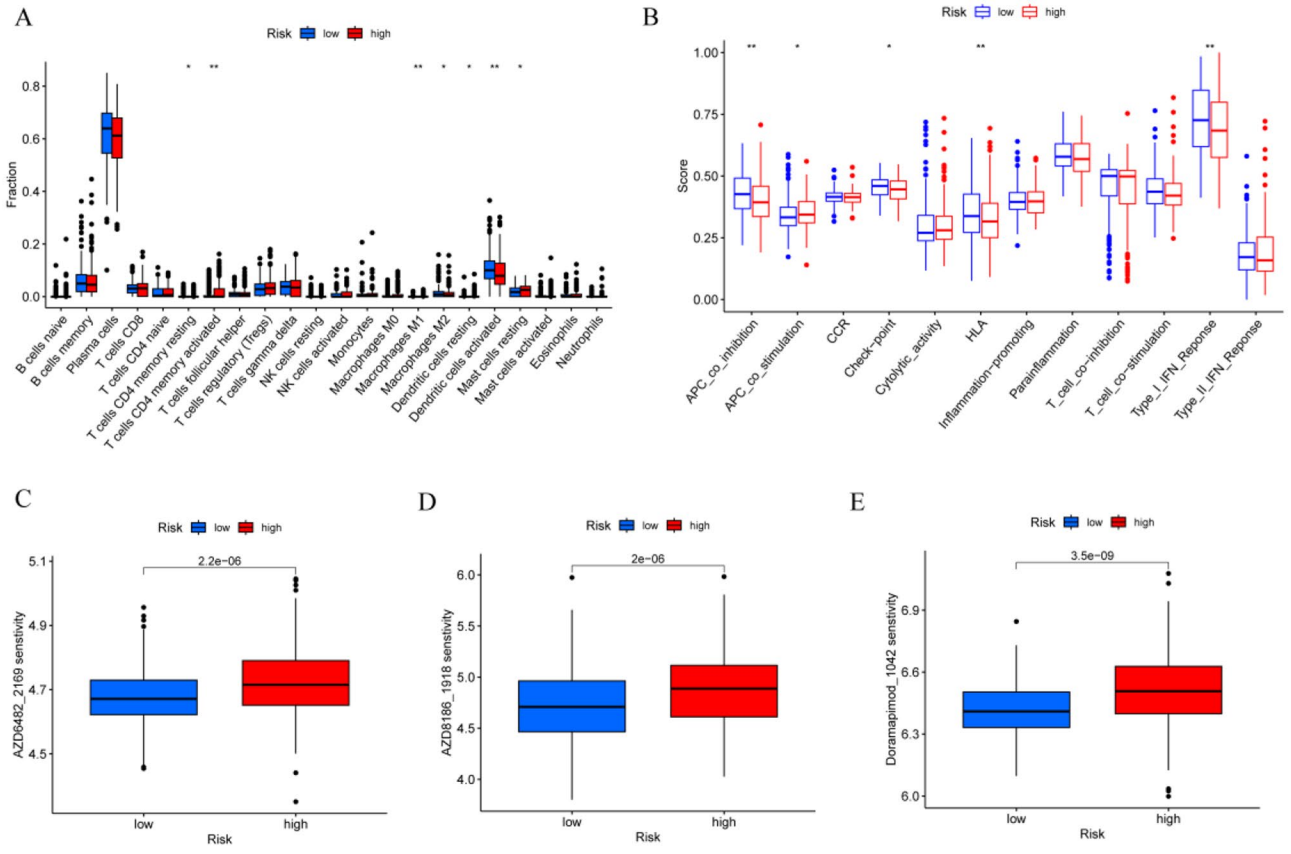

Fig. 4 Immune microenvironment and drug sensitivity profiling. (**A**) Differences infiltration levels of immune cell subsets between risk groups. (**B**) Enrichment scores of immune-related pathways across risk groups. (**C-E**) Predicted drug sensitivity profiles for (**C**) AZD6482_2169, (**D**) AZD8186_1918, and (**E**) Doramapimod_1042 in high versus low risk patients

Drug sensitivity prediction via oncoConnect identified three compounds with preferential efficacy in high-risk patients: AZD6482_2169, AZD8186_1918, and Doramapimod_1042 ($p < 0.05$; Fig. 4C-E).

Pathway characterization of risk stratification groups

Comparative transcriptomic analysis between high- and low-risk groups identified 72 differentially expressed genes (DEGs). GO enrichment revealed significant associations with mitotic processes, including chromosome segregation and microtubule binding, alongside protein kinase regulator activity (Fig. 5A). pathway analysis demonstrated predominant enrichment in cell cycle regulation, p53 signaling, and cellular senescence pathways (Fig. 5B). GSVA quantification of KEGG gene sets further confirmed enhanced activity in pyrimidine metabolism, cell cycle, DNA replication, and RNA polymerization within high-risk patients (Fig. 5C).

Clinical validation of prognostic signature genes

The expression levels of ANKRD28, DLD, FAM114A2, HSP90AA1, KIF13B, NFAT5, PGM3, SFT2D2, UBA2, and UBA5 were detected by qRT-PCR in 20 bone marrow samples, including 9 controls and 11 newly diagnosed MM patients. As shown in Fig. 6A, DLD, SFT2D2, and UBA2 were upregulated in MM, which may be closely associated with disease progression, while HSP90AA1 expression showed no intergroup difference. CCK-8 proliferation assays demonstrated that siRNA-mediated silencing of **DLD**, **SFT2D2**, or **UBA2** significantly attenuated tumor cell growth (Fig. 6B-D). We investigated the effects of gene knockdown (DLD, UBA2, and SFT2D2) on the sensitivity of tumor cells to three drugs: AZD8186_1918, Doramapimod_1042, and AZD6482_2169. The results showed that DLD and UBA2 play critical roles in mediating the sensitivity of tumor cells to these drugs (Fig. 7A-B), whereas SFT2D2 has a relatively minor impact (Fig. 7C).

Discussion

The association between glutamine metabolism and MM pathogenesis has been extensively investigated, yet clinical translation remains limited. As the second most prevalent hematologic malignancy, MM poses significant therapeutic challenges due to chemoresistance and relapse [23]. Glutamine, an essential amino acid

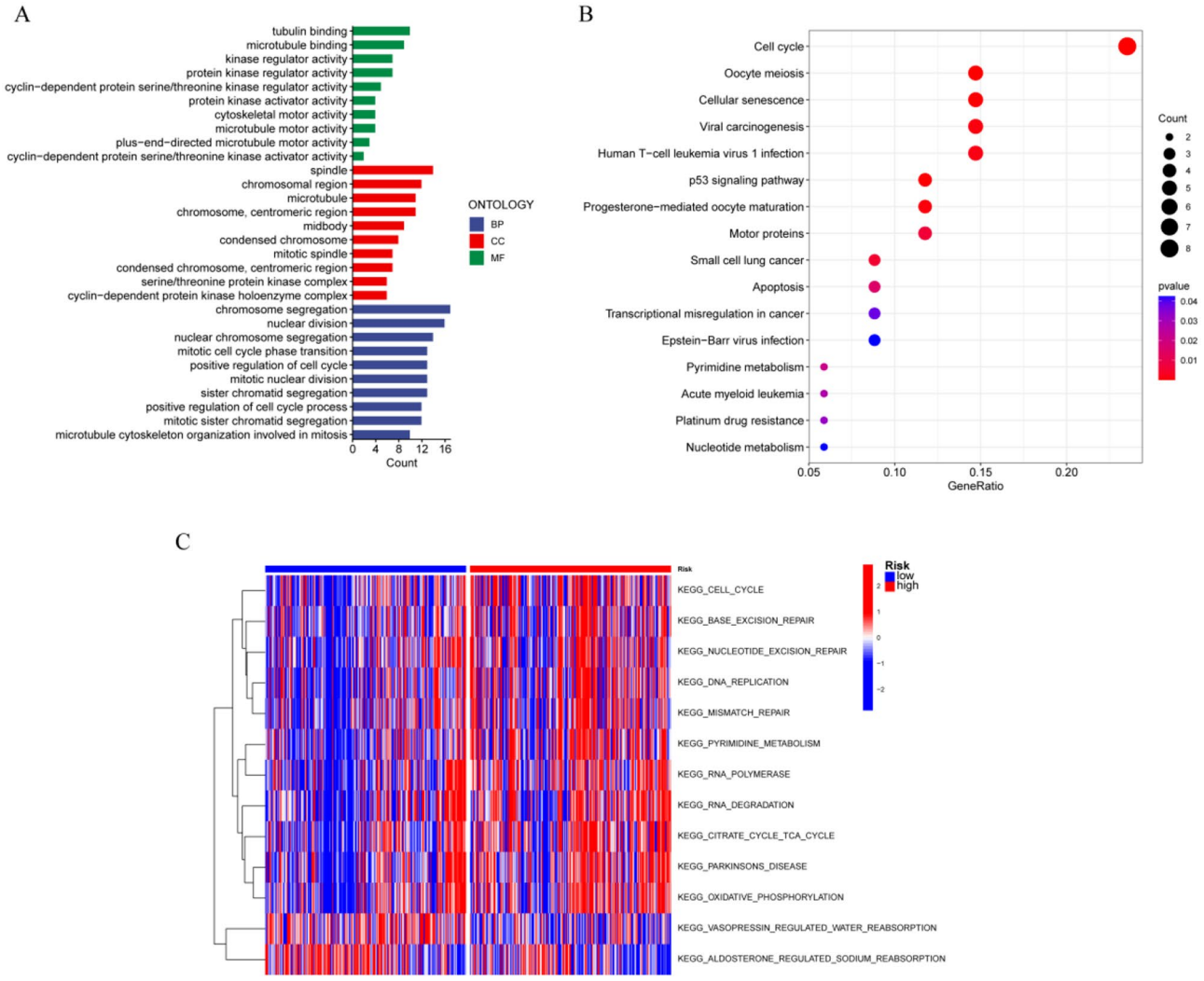

Fig. 5 Pathway dysregulation associated with risk stratification. (**A**) GO as well as (**B**) KEGG enrichment analysis of differential genes in high- and low-risk patient groups. (**C**) Heatmap for GSVA enrichment analysis of KEGG gene set in high and low risk patient groups

in cellular metabolism, is involved in various biological processes, including energy production, nucleotide synthesis, and antioxidant responses [24]. MM cells are reported to be highly dependent on glutamine metabolism [25, 26], with human myeloma cell lines (HMCLs) exhibiting pronounced sensitivity to glutamine depletion, indicating their addiction to this amino acid [9]. This dependence may stem from the significantly higher glutamine demand of MM cells. By regulating the glutamine metabolic pathway, MM cells can obtain sufficient energy and metabolic intermediates necessary for proliferation.

Recent studies suggest that inhibiting the glutamine metabolic pathway could represent a novel therapeutic approach for treating multiple myeloma. Targeting glutamine metabolism enhances the binding of BIM to BCL-2, thereby triggering synthetic lethality in response to Vincristine [27]. Consequently, continued research into the molecular mechanisms and prognostic indicators of glutamine metabolism in MM may help identify distinct MM subgroups, ultimately improving prospects for precision medicine in this disease.

To our knowledge, this study is the first to establish a novel survival risk profile using genes associated with glutamine metabolism. This profile demonstrated robust performance in both training and external validation sets, with the area under the ROC curve for 1–3 years exceeding 0.7, indicating good accuracy and discriminative power. Prognostic outcomes significantly differed between high-risk and low-risk patients. Drug response analyses suggest that AZD6482_2169, AZD8186_1918, and Doramapimod_1042 may provide greater therapeutic benefits for high-risk patients. Notably, AZD8186 is a potent and selective inhibitor of PI3Kβ/δ, and AZD6482 selectively inhibits p110β and PI3Kβ. Inhibition of PI3Kβ activity effectively suppresses tumor cell proliferation, migration, and survival. Similarly, Doramapimod,

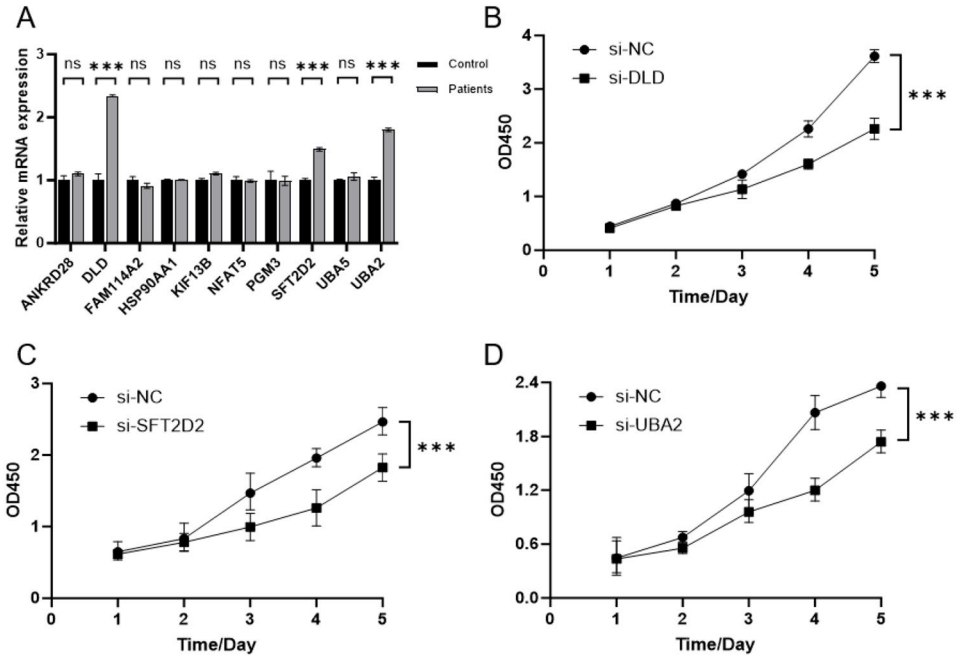

Fig. 6 Experimental validation of model-centered genes in clinical samples. (**A**) qRT-PCR quantification of 10 prognostic genes (ANKRD28, DLD, FAM114A2, HSP90AA1, KIF13B, NFAT5, PGM3, SFT2D2, UBA2, and UBA5) in control ($n=9$) versus MM ($n=11$) samples. (**B-D**) Cell proliferation rates following siRNA-mediated knockdown of (**B**) DLD, (**C**) SFT2D2, and (**D**) UBA2 (***$p<0.01$ vs. Scramble)

a p38 MAPK inhibitor, plays a critical role in cellular stress responses, immune responses, and tumorigenesis and metastasis. By inhibiting p38 MAPK, tumor cell resistance and metastasis can be reduced, potentially enhancing therapeutic efficacy by modulating the tumor microenvironment.

Functional investigations reveal that high-risk patients exhibit increased activity in cell cycle progression, the p53 signaling pathway, and oxidative phosphorylation. The p53 protein, known as the guardian of the genome [28], plays a vital role in maintaining genomic stability. In response to DNA damage, p53 coordinates multiple DNA damage response mechanisms to protect the genome [29]. p53 activates the expression of DNA repair proteins such as DDB2 and XPC [30], influencing cell fates such as apoptosis, senescence, or tumorigenesis [31]. By repressing CDK and cyclin B, which are essential for mitotic entry, p53 is implicated in G2/M phase blocking, reducing the likelihood of gene mutations and preventing the activation of carcinogenic genes [32, 33].

In addition to its role in glucose metabolism, p53 regulates various other cellular processes, including glutamine catabolism. GLS2 mediates the influence of p53 on glutamine catabolism [34]. p53 boosts GLS2 expression, leading to increased synthesis of glutamate and α-ketoglutarate within the cell. This enhancement supports mitochondrial oxidative phosphorylation and ATP production. Concurrently, GLS2 elevates cellular levels of the antioxidant glutathione by increasing intracellular glutamate, a precursor for antioxidant glutathione, thus lowering cellular ROS levels. Therefore, p53 plays a pivotal role in regulating glutamine metabolism, which is critical for tumor cell survival and proliferation.

An important finding of our study is that in patients with MM, high glutamine metabolic activity is strongly associated with reduced expression of HLA, immune checkpoints, APC co-inhibition, and interferon type I signaling. This aligns with the work of Puchades-Carrasco et al., which demonstrates that MM patients exhibit unique metabolic profiles compared to healthy controls. These associations may reflect how tumor cells evade immune system surveillance through metabolic reprogramming. Cancer cells exploit nutritional stress to generate an immunosuppressive microenvironment, subsequently affecting the function of tumor-infiltrating lymphocytes [35]. The heightened metabolic demands of tumor cells and activated T lymphocytes may instigate competition for glutamine within the TME [36, 37]. This competition can lead to tumor cells outcompeting T cells for local glutamine, thereby altering the properties of tumor-infiltrating lymphocytes. In this context, glutamine depletion not only promotes tumor cell proliferation and survival but also limits T cell-mediated antitumor immunity [38]. Consequently, glutamine may influence tumor antigen presentation pathways and promote reduced expression of immune checkpoint genes, facilitating immune evasion.

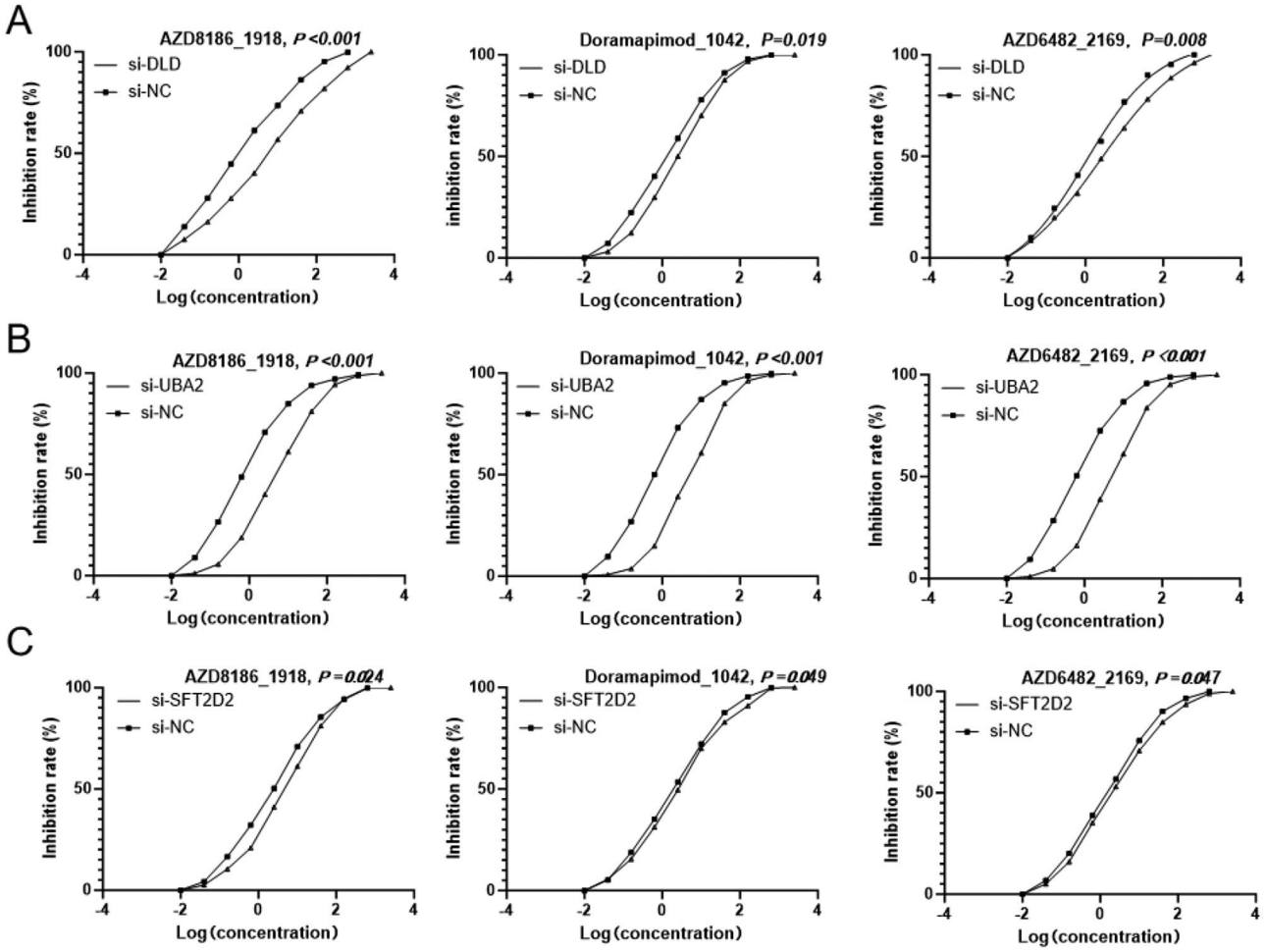

Fig. 7 Pharmacological consequences of gene suppression. Drug sensitivity changes following (**A**) DLD, (**B**) UBA2, and (**C**) SFT2D2 knockdown. IC50 values were calculated for AZD8186_1918, Doramapimod_1042, and AZD6482_2169 using dose-response curves

Three limitations of this investigation must be acknowledged. First, the prognostic signature was derived solely from retrospective analysis of public datasets (GSE136337 and GSE4581), which inherently introduces selection bias due to uncontrolled confounding variables (e.g., treatment heterogeneity, undefined comorbidities). Second, the integration of bulk RNA-seq data with single-cell profiles may amplify technical variability, as batch effects between platforms were not systematically corrected. Third, although we validated three candidate genes (DLD, SFT2D2, UBA2) in clinical samples, functional validation of the entire GMRG network remains incomplete. These limitations restrict the generalizability of our findings and underscore the necessity for prospective multi-center studies with standardized sample collection protocols.

In conclusion, through single-cell sequencing and gene expression profiling, we identified a robust gene signature associated with glutamine metabolism that can serve as an independent biomarker for predicting survival in MM patients. These findings enhance our understanding of glutamine metabolism in MM and support the development of stratified treatment and prognostic strategies. Additionally, this research addresses a gap in identifying therapeutic targets within the glutamine metabolism pathway in multiple myeloma.

Future research should prioritize three key areas: (1) Prospective validation of the GMRG signature in treatment-naïve cohorts using flow cytometry-based metabolic phenotyping; (2) Experimental validation of glutamine-mediated immune evasion mechanisms through CRISPR-mediated Glutaminase knockdown models; (3) Development of GMRG-targeted combination therapies in immunocompetent MM xenografts.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14239-0.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Not applicable.

Author contributions
Conception and design of the work (Conceptualization by Feifei Che) Acquisition, analysis, or interpretation of data (Formal analysis by Fei Zhao) Drafting the work or revising it critically for important intellectual content (Writing - Original Draft by Fei Zhao, Writing - Review & Editing by Feifei Che).

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Data availability
The data that support the findings of this study are available from the corresponding author, [FC], upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Sichuan Provincial People's Hospital and Sichuan Academy of Medical Sciences (2023-577). All patients gave Written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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