

Identification of PANoptosis-Related Biomarkers in Hypertrophic Cardiomyopathy: Insights from Multi-Omics Analysis

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Background: Hypertrophic cardiomyopathy (HCM) is a common inherited cardiomyopathy characterized by ventricular hypertrophy, fibrosis, and increased risk of sudden cardiac death. However, the underlying molecular pathways contributing to its progression remain incompletely defined. PANoptosis, a newly defined inflammatory form of programmed cell death integrating pyroptosis, apoptosis, and necroptosis, has been implicated in cardiac injury and may represent a convergent mechanism linking inflammation and myocardial remodeling, but remains uninvestigated in HCM.

Methods: Transcriptomic profiles from HCM and control hearts were analyzed to identify differentially expressed PANoptosis-related genes. A nine-gene diagnostic panel was constructed using a comprehensive multi-algorithm machine learning framework integrating ensemble, kernel-based, and regularized regression models, and validated in external cohorts. Molecular subtypes were identified through consensus clustering. Immune infiltration, functional enrichment, and ceRNA regulatory networks were evaluated. Single-nucleus RNA sequencing localized gene expression to specific cardiac cell types. Cell-cell communication analysis explored intercellular signaling. Experimental validation was performed in a murine HCM model using echocardiography, histology, and RT-qPCR. Molecular docking assessed therapeutic potential of candidate compounds. Finally, molecular docking and target prediction were applied to explore potential therapeutic compounds acting on the PANoptosis axis.

Results: Nine PANoptosis-related genes (S100A9, GADD45A, IER3, STAT3, SFRP1, PHLDA1, JAK2, MYC, S100A8) showed high diagnostic performance (AUC > 0.95). Two molecular subtypes displayed distinct immune and metabolic signatures. PANoptosis genes correlated with T cells, macrophages, and dendritic cells. CellChat analysis revealed PDGF-mediated signaling between cardiomyocytes and fibroblasts. Key genes exhibited cell-type-specific expression. In vivo validation confirmed gene expression trends. Moreover, folic acid and tretinoin exhibited favorable docking affinity with core targets, suggesting potential therapeutic relevance.

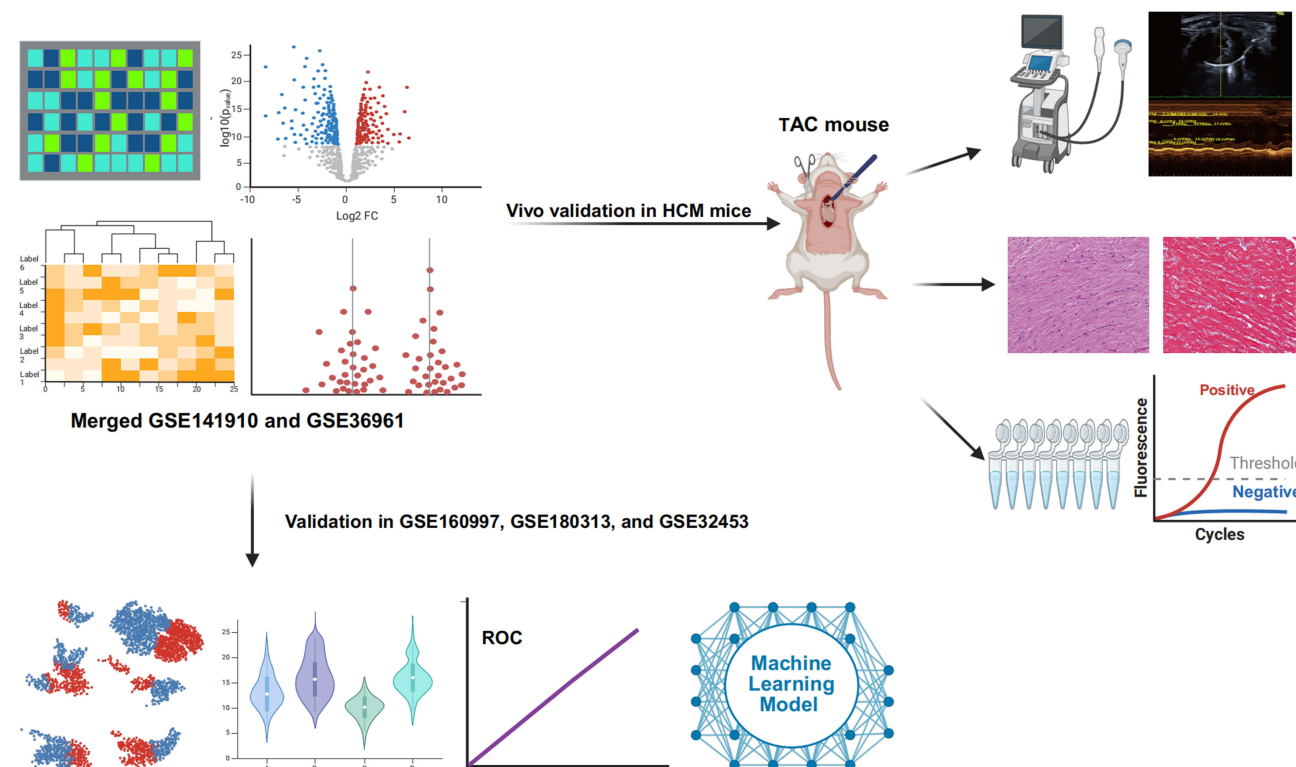
Conclusion: This study provides the first systematic evidence linking PANoptosis to the molecular pathogenesis of HCM. PANoptosis contributes to HCM pathogenesis and immune remodeling, and the identified biomarkers demonstrate translational potential as diagnostic indicators and therapeutic targets. The integrated analysis highlights novel PANoptotic signaling axes that may guide future precision diagnosis and intervention strategies for HCM.

Keywords: hypertrophic cardiomyopathy, PANoptosis, diagnostic biomarkers, machine learning

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disorder, affecting approximately 1 in 200 individuals globally.¹ Clinically, HCM is characterized by asymmetric left ventricular hypertrophy, myocyte

Graphical Abstract



disarray, and interstitial fibrosis, often accompanied by diastolic dysfunction, arrhythmias, and an elevated risk of sudden cardiac death, particularly among young adults. Although mutations in sarcomeric genes such as MYH7, MYBPC3, and TNNT2 are well-established as causative,^{2,3} substantial phenotypic variability among mutation carriers suggests that additional molecular mechanisms contribute to disease progression and clinical outcomes. Recent studies have also identified desmosomal gene variants—particularly in desmoplakin (DSP)—as emerging contributors to atypical or arrhythmogenic forms of HCM. DSP mutations disrupt intercellular adhesion, promote myocardial fibrosis, and predispose to ventricular arrhythmias, thereby expanding the genetic and pathological spectrum of HCM.^{4,5} Beyond genotype-phenotype relationships, there remains a need for dynamic molecular indicators that capture active disease biology and inform clinical decision-making.

Recent research has emphasized the pivotal role of inflammation and regulated cell death pathways in cardiac remodeling associated with HCM.^{6,7} PANoptosis, a newly characterized inflammatory form of programmed cell death that integrates features of pyroptosis, apoptosis, and necroptosis, has emerged as a central mechanism in a range of pathological conditions, including infectious diseases, malignancies, and autoinflammatory syndromes.⁸ PANoptosis is executed via multiprotein complexes termed PANoptosomes and is known to amplify cytokine release and exacerbate tissue injury. Mechanistically, PANoptosis differs from single-pathway death programs by coordinating caspase-dependent apoptotic signals, gasdermin-mediated pyroptotic pore formation, and RIPK-driven necroptotic execution within one regulatory scaffold, thereby enabling inflammatory amplification and cross-talk that are not explained by any individual pathway alone. While PANoptosis has been increasingly recognized as a key inflammatory cell death pathway in cardiovascular contexts—such as macrophage-mediated plaque instability in atherosclerosis,⁹ vascular smooth muscle cell dysfunction under ischemia-induced mitochondrial stress,¹⁰ and cardiomyocyte death in acute myocardial infarction¹¹—its role in HCM remains largely unexplored, and no diagnostic markers related to this pathway have

been systematically identified to date. Positioning PANoptosis within the broader spectrum of cardiomyocyte death thus provides a biologically plausible framework to interrogate inflammatory remodeling in HCM.

Given the clinical burden of HCM and the limitations of current genetic markers in capturing disease activity, there is a growing need to identify dynamic molecular signatures associated with inflammatory signaling and cell death. Increasing evidence links immune activation and persistent inflammation to myocardial fibrosis and adverse remodeling in HCM, suggesting a more mechanistic role beyond a secondary response.^{6,12–14} Notably, elevated levels of interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) have been correlated with disease severity and poor prognosis,^{14,15} supporting the hypothesis that PANoptotic signaling may be involved in HCM pathogenesis. Against this backdrop, biomarker discovery targeting PANoptosis has potential translational utility for clinical risk stratification (eg, identifying inflammation-dominant phenotypes), early diagnosis, and prioritization of patients for immuno-inflammatory or anti-fibrotic interventions.

In this study, we performed integrative transcriptomic profiling of HCM using public datasets (GSE141910 and GSE36961), identifying PANoptosis-related gene signatures through differential analysis and a multi-algorithm machine learning framework integrating ensemble, kernel-based, and regularized regression models (eg, Random Forest, XGBoost, SVM, and LASSO). A robust nine-gene diagnostic panel (GADD45A, SFRP1, S100A9, S100A8, IER3, STAT3, PHLDA1, JAK2, and MYC) was derived from the intersection of PANoptosis-related differentially expressed genes using an integrative multi-algorithm machine learning framework and subsequently validated across three independent external cohorts (GSE160997, GSE180313, and GSE32453). Experimental validation in a TAC-induced murine HCM model confirmed the mRNA expression trends of these markers. Furthermore, single-nucleus RNA sequencing revealed their distinct cellular localization across cardiomyocytes, fibroblasts, and immune cells. CellChat-based analysis uncovered a cardiomyocyte–fibroblast signaling axis potentially mediated by PDGF, underscoring PANoptotic crosstalk in myocardial remodeling. Collectively, these data provide initial, hypothesis-generating evidence implicating PANoptosis in HCM and nominate candidate biomarkers and signaling axes with direct relevance to patient stratification and therapeutic development, warranting prospective protein-level validation and functional studies.

Materials and Methods

Data Sources

Gene expression datasets GSE141910,¹⁶ GSE36961, GSE160997,¹⁷ GSE180313,¹⁸ and GSE32453 were retrieved from the Gene Expression Omnibus (GEO) database. To reduce batch effects, datasets GSE141910 and GSE36961 were merged and corrected using the “sva” package (v4.3.2) in R. This combined dataset included 197 control samples and 137 HCM samples, which served as the primary training set. The datasets GSE160997 (controls = 5, HCM = 19), GSE180313 (controls = 8, HCM = 14), and GSE32453 (controls = 5, HCM = 8) were used as independent validation cohorts. Sample-size distributions and metadata are summarized in [Supplementary Table 1](#).

Differential Expression Analysis and Intersection with PANoptosis-Related Genes

Differentially expressed genes (DEGs) were identified based on the criteria of false discovery rate (FDR) < 0.05 (Benjamini–Hochberg adjustment) and $|\log_2\text{FC}| \geq 0.5$ using the limma package (v3.58.1). The results of the differential analysis were visualized using heatmaps and volcano plots. Subsequently, the identified DEGs were intersected with a curated list of PANoptosis-related genes obtained from published literature and databases¹⁹ ([Supplementary Table 2](#)) to identify key genes involved in PANoptosis that were dysregulated in HCM. Unless otherwise stated, statistical tests were two-sided.

GO and KEGG Functional Enrichment Analysis

GO (Gene Ontology) enrichment analysis of PANoptosis-related intersecting gene set was performed using the ClusterProfiler R package (v4.8.3),^{20,21} covering three categories: biological process, cellular component, and molecular function. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis was also conducted. The significance threshold was set at an adjusted p-value (p.adjust) < 0.05 using the Benjamini-Hochberg correction. The

enrichment results were visualized as bar plots showing the number of genes and statistical significance, with color coding based on the GO category. Gene universe/background was defined as all expressed genes after preprocessing; redundant terms were reduced with simplify at a cutoff of 0.7.

Machine Learning Model Construction and Diagnostic Gene Selection

A total of 101 machine learning model combinations were constructed using various algorithmic pipelines, including random forest (RF), extreme gradient boosting (XGBoost), glmBoost, support vector machines (SVM), and regularization techniques such as LASSO, ridge regression, and elastic net. Ten-fold cross-validation with stratification was used within the training set for hyperparameter tuning; nested resampling was applied when comparing model families to mitigate overfitting. Feature selection strategies included stepwise regression (forward, backward, and both directions) and principal component-based methods. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC) across the training set and three external validation datasets (GSE160997, GSE180313, and GSE32453). Confusion matrices were used to assess prediction accuracy. Differential expression analysis of candidate genes was visualized using volcano plots, and top-ranking genes were selected based on their model contribution and statistical significance. To assess feature stability, we computed selection frequencies across resamples and retained genes with stability $\geq 70\%$.

Consensus Clustering Analysis of PANoptosis-Related Genes

Consensus clustering analysis was performed using the “ConsensusClusterPlus (v1.60.0)”²² R package to identify molecular subtypes based on the expression profiles of PANoptosis-related genes. The optimal number of clusters (k) was determined based on the cumulative distribution function (CDF) curves, delta area plot, and consensus heatmap. Principal component analysis (PCA) was used to evaluate the separation between clusters. A heatmap was generated to display the expression patterns of PANoptosis genes across clusters. Boxplots were used to compare the expression levels of selected key genes between the subtypes. To characterize functional differences between the identified subtypes, gene set variation analysis (GSVA) was performed using the “GSVA” and “limma” R packages.²³ GO and KEGG gene sets were used as the reference pathways. Differentially enriched pathways between clusters were identified based on GSVA scores, with significant results visualized using bar plots. Subtype robustness was assessed by subsampling (80% samples, 1,000 iterations).

Construction and Validation of a PANoptosis-Related Nomogram Model

A nomogram was constructed using the “rms” (v6.7-0) R package to quantitatively predict the risk of HCM based on the expression levels of 9 PANoptosis-related diagnostic genes. Model calibration was evaluated using calibration curves, and decision curve analysis (DCA) was conducted to assess clinical utility. The diagnostic performance of each gene was evaluated using receiver operating characteristic (ROC) curves in both the training and three independent validation datasets (GSE160997, GSE180313, GSE32453). We report optimism-corrected C-statistics via bootstrap (1,000 iterations) and present calibration-in-the-large and calibration slope.

Immune Infiltration Correlation Analysis

Expression profiles were integrated with immune infiltration scores to systematically evaluate correlations with core genes. Immune infiltration was quantified using the CIBERSORT (LM22 signature; 1,000 permutations)²⁴ and MCPcounter (v1.2.0)²⁵ algorithms, and the data were uniformly normalized. Spearman correlation coefficients were calculated, and bubble plots were used to visualize the associations across different cell types. For selected genes, the top immune cell correlations were further displayed as lollipop plots, showing the magnitude and significance of each association. P-values for multiple gene-cell correlations were adjusted with Benjamini-Hochberg.

Construction of a ceRNA Regulatory Network Based on Core Genes

To explore the post-transcriptional regulatory mechanisms of PANoptosis-related diagnostic genes, a competing endogenous RNA (ceRNA) network was constructed. First, miRNA-mRNA interactions were predicted using

miRDB, TargetScan, and miRTarBase. Next, lncRNA–miRNA interactions were obtained from StarBase and LncBase databases. Overlapping miRNAs and lncRNAs were then integrated with the PANoptosis signature genes to establish a lncRNA–miRNA–mRNA network. The final ceRNA network was visualized using Cytoscape (v3.10). Only interactions supported by ≥ 2 databases were retained; where applicable, adjusted p-values < 0.05 were required for inclusion.

Prediction of Drug Targets and Molecular Docking

To identify therapeutic compounds targeting PANoptosis-related genes, drug–gene interaction data were obtained from CTD.²⁶ Bubble plots and interaction networks were generated using R and CTD data. Protein structures were sourced from PDB or predicted via AlphaFold2; ligand structures were retrieved from PubChem and energy-minimized. Docking was conducted using AutoDock Vina, and interactions were visualized with PyMOL (v2.5) and Discovery Studio Visualizer (2021) to identify key binding residues and interactions. Protein preparation involved addition of hydrogens and Gasteiger charges; grid boxes encompassed known or predicted binding pockets; docking exhaustiveness was set to 16; top-ranked poses were selected by binding affinity and interaction plausibility. PDB identifiers and docking energies are provided in [Supplementary Table 4](#).

Animal Experiments

Eight-week-old male C57BL/6 mice were obtained from the Experimental Animal Center of Inner Mongolia Medical University (License No. SYXK 2024–0002). All procedures were conducted in accordance with the “Guidelines of the Chinese Animal Research Council” and the “Guide for the Care and Use of Laboratory Animals.” Mice were housed under standard conditions ($22 \pm 2^\circ\text{C}$, $55\% \pm 5\%$ humidity, 12 h light/dark cycle) with ad libitum access to food and water. After one week of acclimatization, mice were randomly divided into two groups: a control group ($n = 8$) and an HCM model group ($n = 8$). A transverse aortic constriction (TAC)²⁷ procedure was performed to induce HCM in male C57BL/6 mice, following protocols approved by the Animal Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University. Mice were anesthetized via intraperitoneal injection of pentobarbital sodium (50 mg/kg) and maintained in a supine position on a heating pad to ensure body temperature stability. After hair removal and disinfection of the neck and thoracic region using 75% ethanol, a tracheotomy was conducted to enable mechanical ventilation with a rate of approximately 120 breaths per minute. A left thoracotomy was performed at the second intercostal space to access the thoracic cavity. Under a stereomicroscope, the thymus was carefully separated to expose the aortic arch. A 6–0 silk suture was passed beneath the arch between the brachiocephalic and left common carotid arteries. A slipknot was tied around both the artery and a 27-gauge blunted needle placed in parallel. The knot was then tightened, and the needle quickly withdrawn to create a defined constriction of approximately 0.4 mm in diameter. Following the procedure, the thymus was repositioned, and the chest wall and skin were closed using 6–0 polypropylene sutures. Mice received 0.2 mL of intraperitoneal penicillin to prevent infection. In sham-operated controls, the surgical procedure was identical except that the aortic ligation was omitted. This model induces significant left ventricular outflow tract obstruction and results in compensatory myocardial hypertrophy within four weeks post-surgery. After four weeks and following echocardiographic assessment, mice were anesthetized with 2% isoflurane and euthanized via cervical dislocation. Heart tissues were then harvested, rinsed with cold PBS, and processed for histological staining and RT-qPCR analysis.

Echocardiographic Assessment of Cardiac Function

Mice were anesthetized using 3% isoflurane and secured in the supine position for imaging. Transthoracic echocardiography was conducted using a high-resolution ultrasound system (FUJIFILM VisualSonics, Canada) to assess cardiac structure and function. Key indicators of HCM included interventricular septal thickness during diastole (IVSD) and systole (IVSS), left ventricular mass (LV mass), left ventricular ejection fraction (LVEF), and left ventricular fractional shortening (LVFS). For accuracy, each parameter was measured over three consecutive cardiac cycles, and the mean value was used for statistical analysis. Image acquisition and analysis were performed by an operator blinded to group allocation.

Histopathological Analysis of Myocardial Tissue

Cardiac tissues were fixed in 4% paraformaldehyde for 24 hours, then processed through graded ethanol dehydration, embedded in paraffin, and sliced into 4 μm sections. For histological analysis, sections were dewaxed, rehydrated, and stained with hematoxylin (5 min), followed by acid–alcohol differentiation and ammonia water treatment for nuclear bluing. Eosin was applied for cytoplasmic staining (1–2 min), after which sections were dehydrated, cleared in xylene, and mounted for microscopic examination. To evaluate collagen deposition and fibrosis, Masson’s trichrome staining was carried out. Sections were sequentially treated with hematoxylin, acid fuchsin, phosphomolybdic acid, and aniline blue, then dehydrated and observed under a light microscope.

Reverse Transcription–Quantitative PCR (RT-qPCR)

Total RNA was extracted from left ventricular myocardial tissue using TRIzol reagent (Invitrogen, USA) according to the manufacturer’s protocol. RNA purity and concentration were measured using an N50 microvolume spectrophotometer (Implen, Germany). High-quality RNA (1 μg per sample) was used as input for first-strand cDNA synthesis, performed using a commercial reverse transcription kit (TransGen Biotech, Beijing, China) following the supplier’s instructions. Quantitative PCR (qPCR) was subsequently conducted using a SYBR Green PCR Master Mix (Selleck, USA) on a standard 96-well platform. Each reaction (total volume of 20 μL) contained 10 μL of 2 $\times$ SYBR mix, 1 μL of forward primer, 1 μL of reverse primer, 2 μL of cDNA template, and 6 μL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 30s, followed by 40 amplification cycles consisting of denaturation at 95°C for 5 s and annealing/extension at 60°C for 30s. Primer pairs specific to each gene were designed and synthesized by Suzhou Jinweizhi Biotechnology Co., Ltd. β -actin was used as the endogenous control to normalize gene expression levels. The relative mRNA expression of target genes—including S100A9, GADD45A, IER3, STAT3, SFRP1, PHLDA1, JAK2, MYC, and S100A8—was quantified using the $2^{-\Delta\Delta\text{Ct}}$ method. All reactions were performed in triplicate, and results were presented as mean $\pm$ standard deviation. Primer sequences are listed in [Supplementary Table 3](#). Outliers (>3 SD from the group mean) were pre-specified and excluded before statistical testing.

Single-Nucleus Transcriptomic Analysis and Gene Localization

Publicly available processed single-nucleus RNA-seq data were downloaded from the Broad Institute’s Single Cell Portal (https://singlecell.broadinstitute.org/single_cell), project ID: SCP1303.²⁸ Data from patients with HCM and normal donors (NF) were analyzed using the Seurat R package (v4.3.0).²⁹ After normalization and quality control, dimensionality reduction was performed using t-distributed stochastic neighbor embedding (t-SNE). Cell type annotation was based on canonical marker genes and verified using the SingleR (v2.2.0) package and Human Primary Cell Atlas reference, and samples were visualized by condition and donor identity. Cell type-specific gene expression was confirmed by Z-score-scaled heatmaps of known marker genes. Spatial distribution of PANoptosis-related diagnostic genes (GADD45A, SFRP1, S100A9, S100A8, IER3, STAT3, PHLDA1, JAK2, and MYC) was assessed using density-based feature plots.

Cell-Cell Communication Analysis

A CellChat object was constructed using the CellChatDB.human database to identify potential ligand–receptor pairs and to estimate intercellular communication probabilities.³⁰ All ligand–receptor pairs were identified using CellChatDB.human, and both communication probability and pathway-specific probability were computed. Key signaling pathways were subsequently visualized at multiple levels to evaluate the contribution of sender and receiver cells and to display the expression patterns of key genes. Only interactions with probability >0.05 and supported by expressed ligand–receptor pairs in both sender and receiver were reported.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9.0 and R 4.3.3. Data are presented as mean $\pm$ standard deviation (SD). For two-group comparisons, unpaired, two-tailed Student’s *t*-test was used after confirming normality (Shapiro–Wilk) and homoscedasticity (Levene’s test); when assumptions were violated, the Mann–Whitney *U*-test was

applied. For >2 groups, one-way ANOVA with Tukey's post hoc test or Kruskal–Wallis with Dunn's post hoc test (BH-adjusted) was used as appropriate. Correlations (immune infiltration) were assessed with Spearman's rho unless specified. A p-value < 0.05 was considered statistically significant ($p < 0.05$; $p < 0.01$; ns = not significant). Multiple testing was controlled using the Benjamini–Hochberg procedure where applicable. Effect sizes and 95% confidence intervals are reported for key comparisons. Residual heterogeneity from public datasets was mitigated by ComBat and verified by PCA before and after correction.

Result

Identification of Differentially Expressed Genes and Functional Enrichment Related to PANoptosis in HCM Patients

To investigate the transcriptomic alterations associated with HCM, we integrated two gene expression datasets, GSE141910 and GSE36961, and performed batch effect correction to minimize inter-dataset variation (Figure 1A and B). The overall expression profiles of DEGs in HCM and control myocardial tissues were visualized by hierarchical clustering in a heatmap, which demonstrated a clear distinction between the two groups and consistent expression patterns within each group (Figure 1C). These DEGs were further illustrated using a volcano plot, with representative upregulated genes such as SMOC2, THBS4, and FRZB, and downregulated genes including SERPINA3, CD163, and SLCO4A1 (Figure 1D). To explore the potential involvement of PANoptosis—a newly defined form of inflammatory programmed cell death—in HCM, we intersected the HCM-related DEGs with a curated PANoptosis gene set. As shown in the Venn diagram (Figure 1E), 30 overlapping genes were identified, accounting for 2.8% of total DEGs. This overlap, although modest in proportion, points to a discrete PANoptosis-linked transcriptomic component within HCM. To gain insight into the functional significance of the DEGs, GO and KEGG pathway enrichment analyses were conducted (Figure 1F). KEGG analysis identified significant enrichment in pathways such as the JAK–STAT signaling pathway, IL-17 signaling, AGE–RAGE signaling, and Th17 cell differentiation, many of which have established roles in cardiac fibrosis, immune activation, and apoptotic processes. Together, these findings support an immune–cell death signaling axis in HCM and provide a biologically plausible context for subsequent PANoptosis-focused analyses. Notably, several enriched pathways (JAK–STAT, IL-17, AGE–RAGE) have established links to fibroblast activation, extracellular matrix remodeling, and myocardial fibrosis, suggesting that PANoptosis-related inflammation may contribute to structural remodeling in HCM.

Machine Learning Model Construction and Evaluation

To identify robust PANoptosis-related diagnostic gene signatures for HCM, we employed an integrated machine learning approach incorporating multiple algorithms and validation datasets. A total of 81 modeling strategies (screened from 101 candidate pipelines) were tested, combining algorithms such as RF, Lasso, SVM, XGBoost, and ElasticNet, evaluated across both training and three independent validation cohorts (GSE160997, GSE180313, GSE32453). Among all algorithms tested, the RF model exhibited the highest and most consistent diagnostic performance across datasets. The RF model achieved AUC = 1.000 in the training, GSE160997, and GSE180313 cohorts, and remained robust in GSE32453 (AUC = 0.975) (Figure 2A and B). These ROC curves were generated using RF-derived risk scores for each cohort, confirming strong discriminative ability and cross-cohort generalizability. We acknowledge that the near-perfect AUC values may partly reflect model overfitting due to the limited sample size and potential cohort homogeneity. To minimize this risk, all preprocessing and feature-selection steps were confined to the training partition, and model hyperparameters were optimized through 10-fold stratified cross-validation and resampling iterations. The slightly lower AUC observed in GSE32453 suggests that the model retains robust generalization rather than artifact-driven performance. Confusion matrices confirmed high classification accuracy, with minimal false positives and no false negatives in most cohorts (Figure 2C). Feature selection across models identified nine key genes, including S100A9, S100A8, MYC, JAK2, SFRP1, GADD45A, STAT3, PHLDA1, and IER3, all of which have known roles in inflammation, apoptosis, and myocardial injury (Figure 2D). We further assessed feature stability across resampling iterations and observed high retention frequencies for the nine-gene panel, supporting robustness. Nonetheless, given the retrospective design and

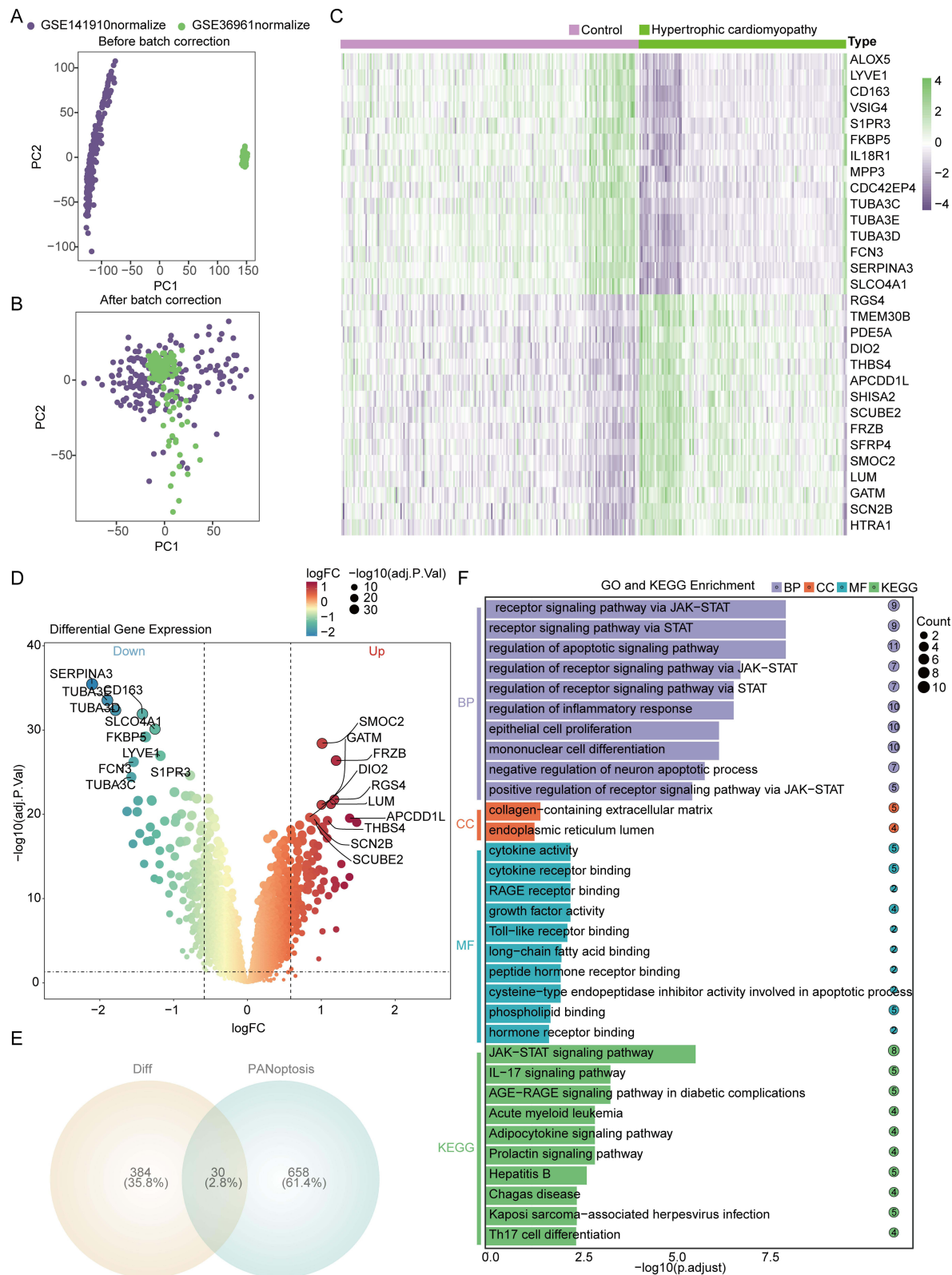


Figure 1 Batch correction, differential expression analysis, and functional enrichment in HCM. **(A)** PCA plot showing clear batch effects between GSE141910 and GSE36961 before correction. **(B)** PCA plot demonstrating effective integration after batch correction using the sva package. **(C)** Heatmap of representative differentially expressed genes (DEGs) between control and HCM samples. **(D)** Volcano plot displaying upregulated (red) and downregulated (blue) DEGs in HCM. **(E)** Venn diagram showing the intersection between DEGs and PANoptosis-related genes. **(F)** GO and KEGG pathway enrichment of DEGs, highlighting immune response and apoptotic signaling pathways.

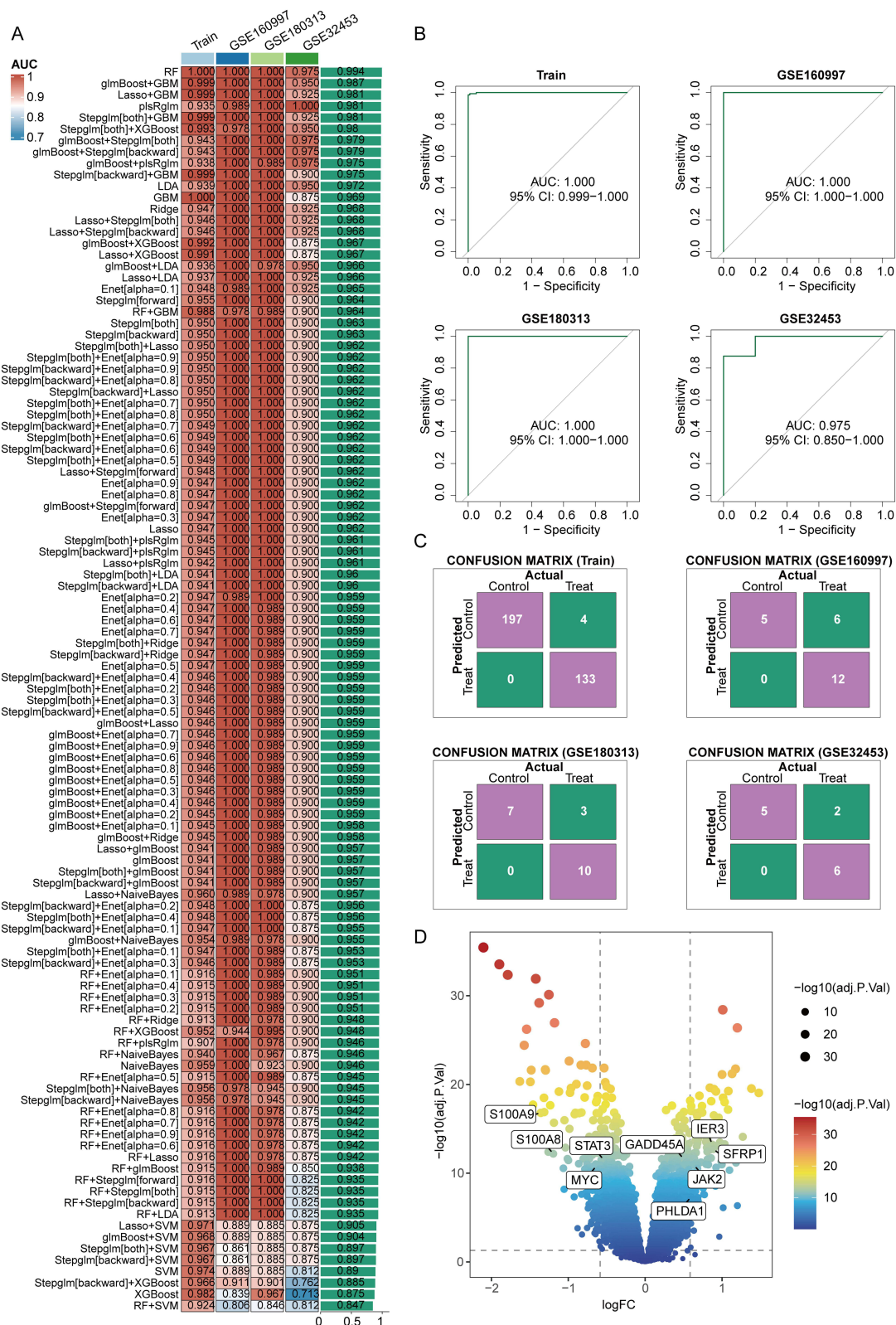


Figure 2 Machine learning-based identification of PANoptosis-related diagnostic markers in HCM. **(A)** Heatmap of AUC values for 101 machine learning model combinations evaluated in the training and three validation cohorts (GSE160997, GSE180313, and GSE32453). **(B)** ROC curves demonstrating model performance in training and validation sets. **(C)** Confusion matrices showing prediction accuracy for control and HCM samples across all cohorts. **(D)** Volcano plot highlighting key differentially expressed PANoptosis-related genes in HCM.

potential dataset heterogeneity, these diagnostic metrics should be interpreted as hypothesis-generating rather than definitive.

Two PANoptosis-Related Subtypes Identified in HCM Patients

To characterize molecular heterogeneity among HCM patients, unsupervised consensus clustering was performed based on the expression profiles of PANoptosis-related diagnostic genes. The consensus index showed stable fluctuations within the range of 0.2–0.6 ([Supplementary Figure 1A](#) and [B](#)). As the number of clusters (k) varied from 2 to 9, the area under the CDF curve indicated significant differences between clusters (k and $k-1$) ([Supplementary Figure 1C](#)). Notably, only the two-cluster solution ($k = 2$) yielded high intra-cluster consensus scores exceeding 0.9, as shown in [Supplementary Figure 1D](#). PCA analysis confirmed transcriptional heterogeneity between the two clusters, with C2 representing the larger subgroup ([Supplementary Figure 1E](#)). Further comparative analysis between the two clusters revealed marked transcriptomic differences. As shown in the heatmap ([Supplementary Figure 1F](#)), cluster-specific gene expression patterns were observed, with distinct modules enriched in each subgroup. Several inflammation- and stress-related genes, including CD44, STAT1, SERPINA3, and IL1R2, were highly expressed in C2, while metabolic and cell cycle-associated genes such as CENPA, BIRC7, and GADD45G were enriched in C1. Moreover, key PANoptosis-related diagnostic genes—including S100A9, GADD45A, IER3, STAT3, PHLDA1, MYC, and S100A8—were significantly upregulated in C2 compared to C1 ([Supplementary Figure 1G](#)), suggesting a more activated pro-inflammatory and PANoptotic phenotype. These subtypes delineate distinct immunometabolic states that may relate to clinical heterogeneity; however, prospective clinical correlation is required before translational deployment.

To further explore the functional heterogeneity between the two PANoptosis-related subtypes, GSVA was performed. As shown in the GO enrichment plot ([Supplementary Figure 2A](#)), subtype C2 was significantly enriched in immune- and inflammation-related pathways such as MHC class II protein complex binding, positive regulation of oligodendrocyte differentiation, and parasympathetic nervous system development. In contrast, C1 displayed increased activity in apoptotic regulation, macrophage differentiation, and intestinal stem cell homeostasis. KEGG-based GSVA analysis further revealed that C2 exhibited upregulation of pathways associated with type I diabetes mellitus, graft-versus-host disease, viral myocarditis, and allograft rejection, reflecting heightened immune activation ([Supplementary Figure 2B](#)). Conversely, metabolic and biosynthetic pathways such as phenylalanine metabolism, steroid hormone biosynthesis, and pantothenate and CoA biosynthesis were enriched in subtype C1. These results highlight divergent immune–metabolic programs across subtypes, providing a rationale for future risk stratification studies.

Validation of PANoptosis-Related Diagnostic Genes

To evaluate the diagnostic potential of PANoptosis-related markers, a nomogram was constructed based on nine candidate genes (S100A9, GADD45A, IER3, STAT3, SFRP1, PHLDA1, JAK2, MYC, S100A8) identified through machine learning ([Figure 3A](#)). Calibration analysis demonstrated good agreement between predicted and observed outcomes ([Figure 3B](#)), while DCA confirmed favorable net clinical benefit across a wide range of thresholds ([Figure 3C](#)). External validation across three independent datasets demonstrated consistent diagnostic accuracy. In GSE160997, ROC curves showed high AUCs for S100A9 (0.958), GADD45A (0.933), and STAT3 (0.887), among others ([Figure 3D](#)). In GSE180313, most genes retained high diagnostic performance, including GADD45A (AUC = 0.934), STAT3 (AUC = 0.813), and SFRP1 (AUC = 0.791) ([Figure 3E](#)). Similarly, in GSE32453, AUCs for SFRP1 (0.875), GADD45A (0.775), and PHLDA1 (0.825) indicated sustained predictive value ([Figure 3F](#)). Corresponding violin plots of gene expression revealed significantly elevated levels of GADD45A, IER3, PHLDA1, and S100A8 in HCM patients across all datasets ([Figure 3G–I](#)). While these cross-cohort results support diagnostic promise, we acknowledge that clinical utility will require threshold optimization, prospective validation, and assessment against established markers.

Nine Core PANoptosis-Related Markers Were Validated in an HCM Mouse Model

To validate the bioinformatically predicted diagnostic markers *in vivo*, we established a murine HCM model via TAC. Histological staining confirmed typical pathological features: HE staining revealed cardiomyocyte hypertrophy and

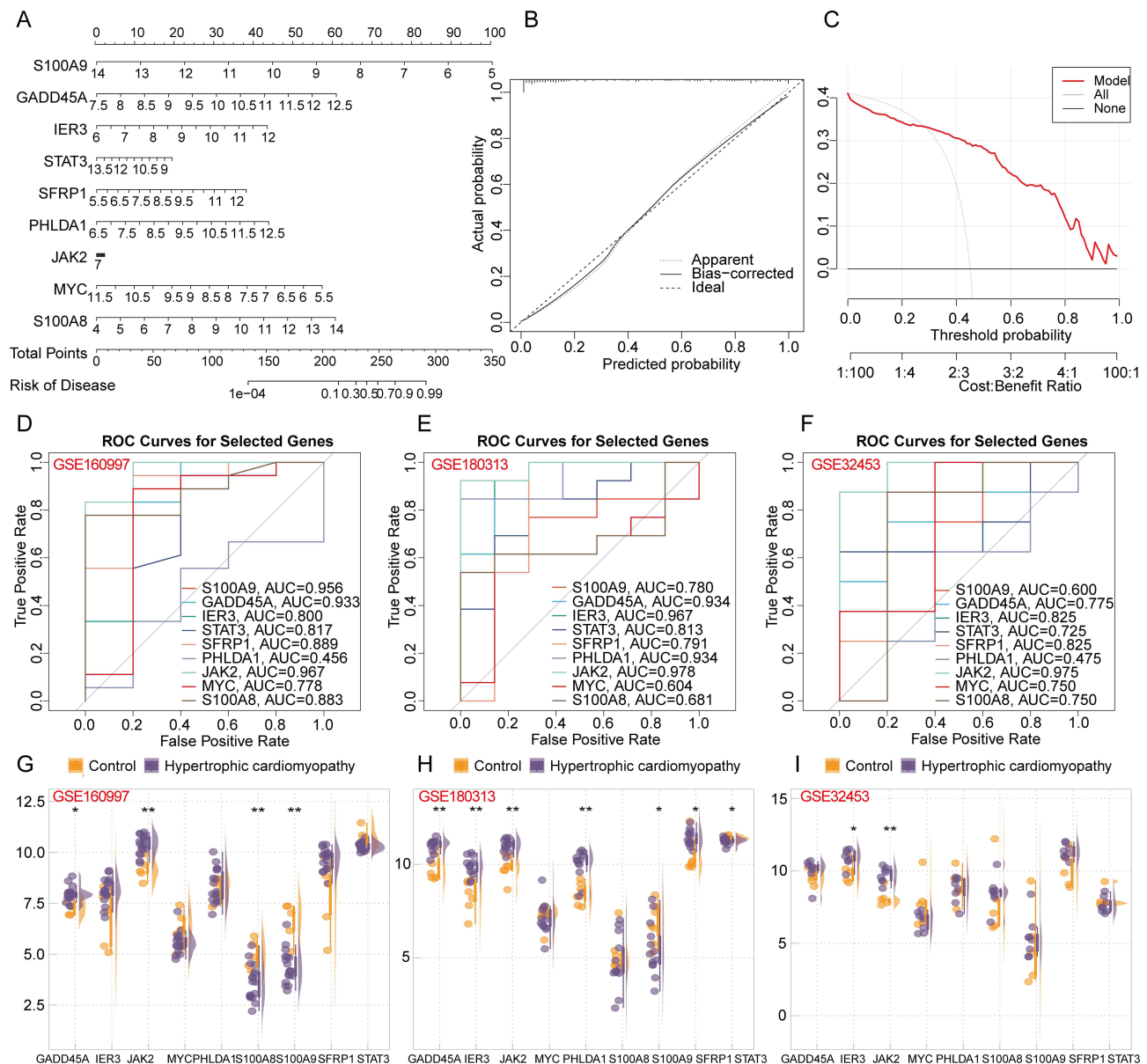


Figure 3 Construction and validation of a PANoptosis-related nomogram model for HCM diagnosis. (A) Nomogram model based on nine PANoptosis-related genes for HCM risk prediction. (B) Calibration curve demonstrating agreement between predicted and observed probabilities. (C) Decision curve analysis (DCA) showing clinical net benefit across varying thresholds. (D–F) ROC curves evaluating diagnostic performance of the selected genes in three external validation cohorts: GSE160997 (D), GSE180313 (E) and GSE32453 (F). (G–I) Violin plots comparing gene expression between HCM and control groups in GSE160997 (G), GSE180313 (H), and GSE32453 (I). * $p < 0.05$, ** $p < 0.01$.

structural disarray, while Masson's trichrome staining showed increased interstitial fibrosis in HCM hearts compared to the sham group (Figure 4A and B). These findings verified the successful induction of pathological hypertrophy and myocardial remodeling. Transthoracic echocardiography further demonstrated significant left ventricular dysfunction in the HCM group, with increased IVSd and IVSs, LV mass, and markedly reduced LVEF and LVFS (Figure 4C and D), confirming functional impairment. To assess transcriptional regulation, quantitative RT-PCR analysis was performed on left ventricular tissues. As shown in Figure 4E, PHLDA1, IER3, JAK2, GADD45A, and S100A8 were significantly upregulated in the HCM group, whereas S100A9, STAT3, MYC, and SFRP1 were markedly downregulated. These gene-level changes corroborate the computational predictions at the mRNA level; however, protein-level and functional confirmation remain priorities for future work.

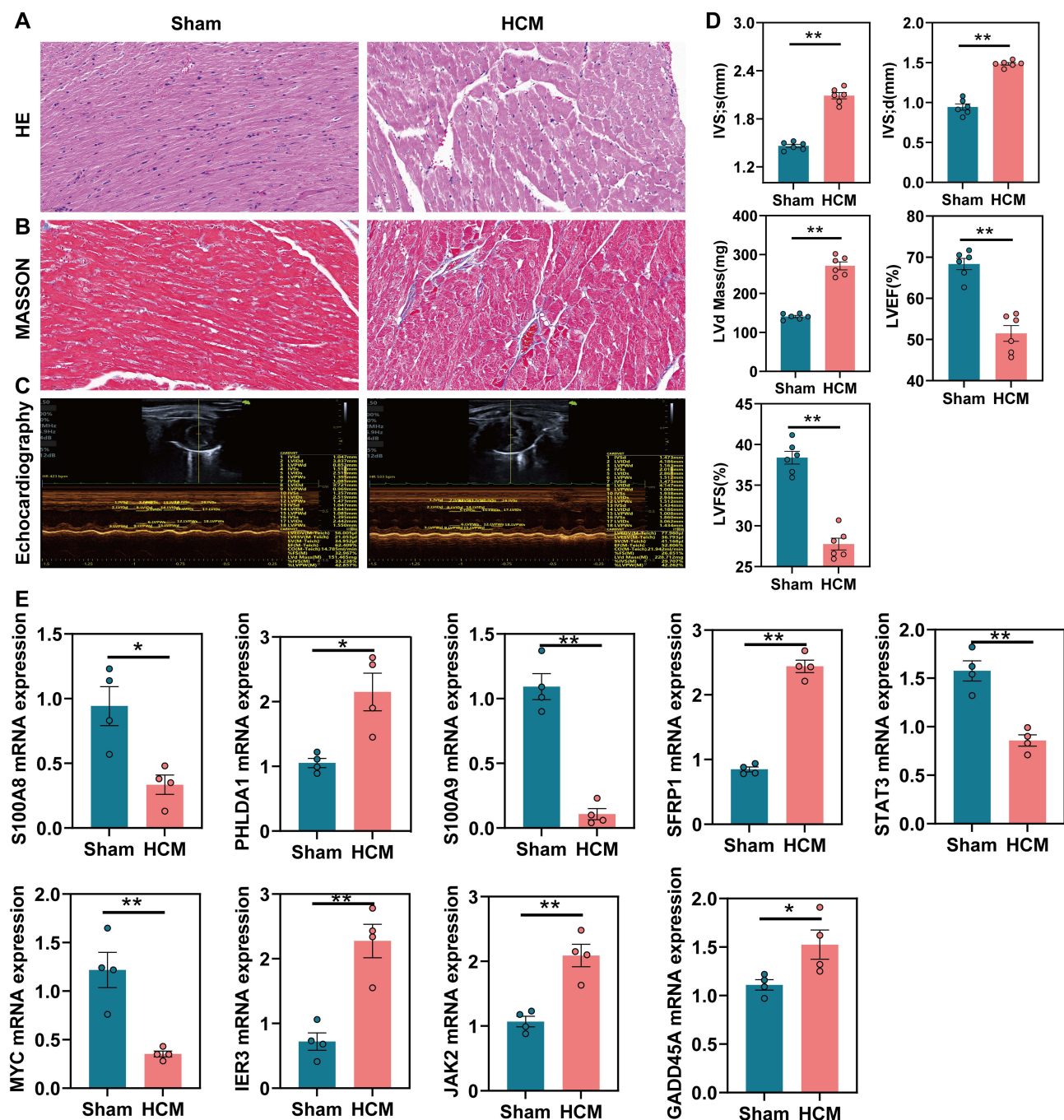


Figure 4 Histological, functional, and transcriptional validation of PANoptosis-related genes in HCM mouse model. (A) Representative HE staining showing cardiomyocyte hypertrophy and disorganization in HCM vs sham. (B) Masson's trichrome staining illustrating increased myocardial fibrosis in the HCM group. (C) Echocardiographic M-mode images of sham and HCM mice. (D) Quantification of echocardiographic parameters: IVSd, IVSs, LV mass, LVEF, and LVFS. (E) RT-qPCR analysis of nine PANoptosis-related diagnostic genes in myocardial tissue. Data are presented as mean $\pm$ SEM; * p < 0.05, ** p < 0.01.

Immune Correlates of PANoptosis-Related Genes in HCM

Immune correlation analysis revealed distinct immunological patterns among the nine PANoptosis-related diagnostic genes. As shown in the correlation matrix (Figure 5A), several genes—including IER3, GADD45A, JAK2, and MYC—exhibited notable associations with key immune populations. IER3 was positively correlated with $\gamma\delta$ T cells and T follicular helper cells, and negatively with macrophages M0 and resting NK cells (Figure 5B). GADD45A showed moderate positive correlations with activated dendritic cells and NK cells (Figure 5C). JAK2 was positively linked to

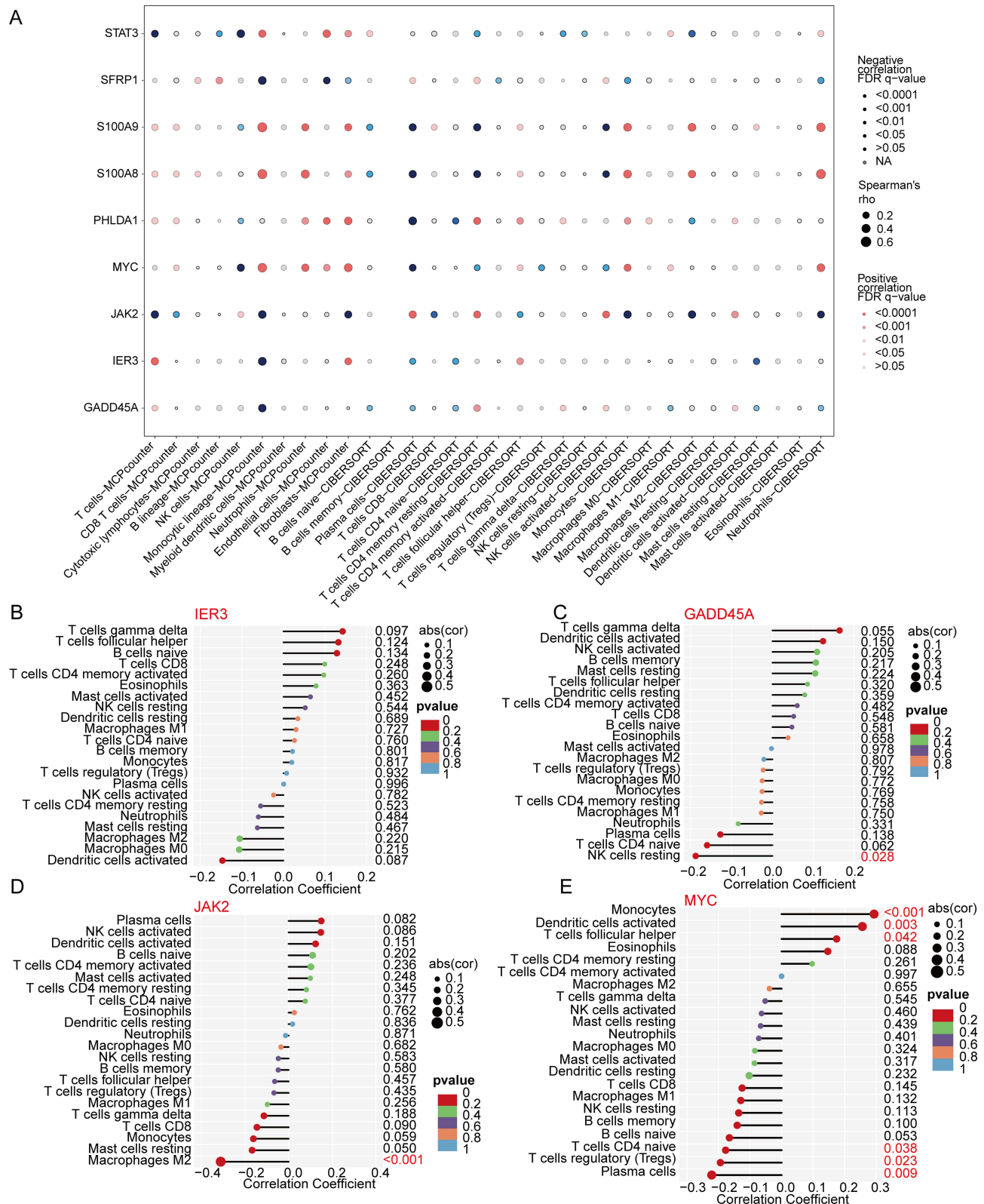


Figure 5 Correlation analysis between PANoptosis-related diagnostic genes and immune cell infiltration in HCM. **(A)** Bubble plot showing Spearman correlation between nine PANoptosis-related genes and immune cell types estimated by CIBERSORT, and MCPcounter. Dot size indicates correlation strength; color denotes direction and statistical significance (FDR-adjusted p-value). **(B–E)** Lollipop plots showing immune cell correlations for selected representative genes: IER3 (B), GADD45A (C), JAK2 (D), and MYC (E). The x-axis represents correlation coefficients; dot color represents p-value and dot size reflects absolute correlation strength.

plasma cells and memory CD4⁺ T cells, and inversely associated with monocytes and macrophages M0 (Figure 5D). MYC was associated with monocytes, activated dendritic cells, and T follicular helper cells, while negatively correlated with Tregs and plasma cells (Figure 5E). PHLDA1 was negatively correlated with dendritic cells, monocytes, and T follicular helper cells, but positively with plasma cells (Supplementary Figure 3A). S100A8 showed strong positive associations with M2 macrophages and monocytes, and negative correlations with CD8⁺ T cells and eosinophils (Supplementary Figure 3B). S100A9 was positively linked to monocytes and follicular helper T cells, and inversely to Tregs and dendritic cells (Supplementary Figure 3C). SFRP1 correlated negatively with naive B cells and NK cells, while STAT3 showed modest positive associations with CD4/CD8⁺ T cells and dendritic cells, but negative with $\gamma\delta$ T cells (Supplementary Figure 3D and E). These associations are consistent with an immuno-inflammatory milieu in HCM and support the plausibility of PANoptosis-related signaling; nevertheless, they are correlative and require mechanistic validation.

ceRNA Network Establishment

A ceRNA network containing 9 hub mRNAs, 61 miRNAs, and 181 lncRNAs was constructed (Supplementary Figure 4). In this ceRNA network, interactions supported by ≥ 2 databases were prioritized to enhance reliability, and 297 lncRNAs were predicted to competitively bind miRNAs targeting key mRNAs such as STAT3, JAK2, MYC, GADD45A, IER3, and S100A9. For example, H19, NEAT1, and XIST may function as molecular sponges for miRNAs including hsa-miR-342-3p, hsa-miR-18a-5p, and hsa-miR-1179, thereby modulating STAT3 expression. Specifically, 21 lncRNAs were identified to regulate GADD45A via miRNAs such as hsa-miR-502-5p, hsa-miR-362-5p, and hsa-miR-122. Similarly, MYC was targeted by 19 lncRNAs through miRNAs including hsa-miR-484 and hsa-miR-449c-5p, while 17 lncRNAs were associated with JAK2 regulation through hsa-let-7c-5p, hsa-miR-498-3p, and hsa-miR-888-5p. In addition, PHLDA1 was potentially modulated by 15 lncRNAs via miRNAs such as hsa-miR-181a-5p and hsa-miR-640, and SFRP1 was linked to 20 lncRNAs through hsa-miR-335-5p, hsa-miR-27b-3p. Notably, lncRNA H19 was predicted to simultaneously regulate STAT3, MYC, and GADD45A, suggesting its potential role as a central ceRNA hub within the network. These *in silico* interactions generate testable hypotheses for future perturbation studies.

Prediction of Targeted Drugs

To investigate the therapeutic potential of targeting PANoptosis-related diagnostic genes, molecular docking analysis was performed using folic acid and Tretinoin as a candidate compound. A compound–target interaction network was constructed, revealing that folic acid potentially interacts with all nine core diagnostic genes (S100A9, GADD45A, IER3, STAT3, SFRP1, PHLDA1, JAK2, MYC, and S100A8) (Figure 6A). To evaluate their binding potential, molecular docking analysis was performed with each target protein. Folic acid showed favorable binding poses with all proteins, particularly GADD45A, S100A9, and IER3, with docking energies of -60.4 , -34.4 , and -8.8 kcal/mol, respectively (Figure 6B and Supplementary Table 4). Tretinoin also exhibited stable interactions but with slightly weaker average binding affinities (Supplementary Figure 5). Docking predictions suggest tractability of selected targets but do not imply efficacy; experimental pharmacology will be required to establish therapeutic relevance.

Single-Nucleus Transcriptomic Profiling Reveals Aberrant Intercellular Signaling Patterns in HCM

To assess the distribution of PANoptosis-related genes in human cardiac tissue, we analyzed single-nucleus RNA-seq data from the SCP1303 dataset. t-SNE projection revealed clear transcriptional separation between HCM and NF myocardial nuclei (Figure 7A). Annotation identified seven major cell populations (Figure 7B), donor-specific mapping confirmed that cell distributions were consistent across samples and groups (Figure 7C). Z-score-scaled heatmaps of established cell-type markers validated cluster identity (Figure 7D). Feature plots demonstrated the spatial distribution of nine PANoptosis-related diagnostic genes across cell populations (Figure 7E). Notably, GADD45A, S100A9, and STAT3 were enriched in fibroblasts and macrophages, while IER3, PHLDA1, and S100A8 exhibited broader expression patterns.

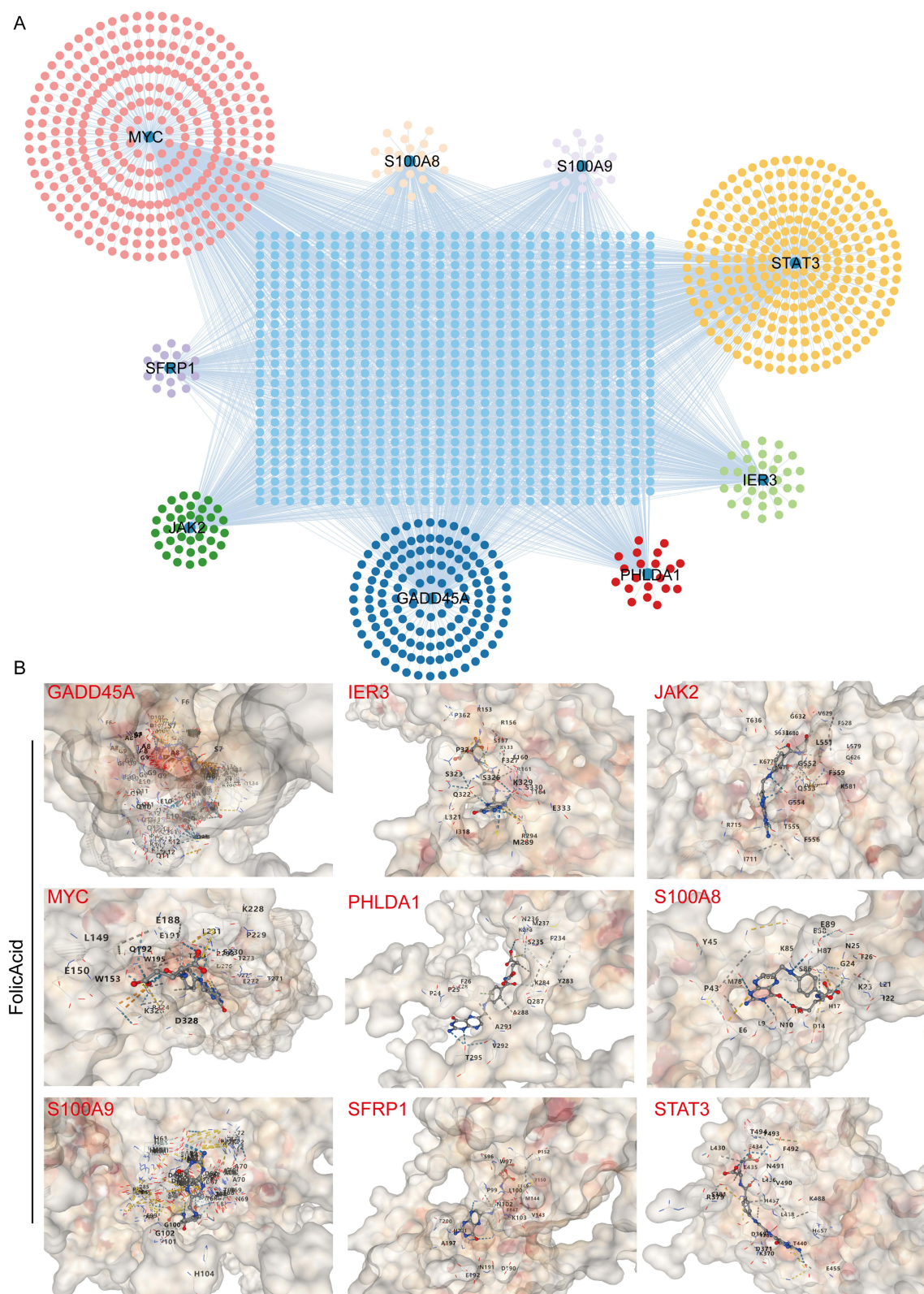


Figure 6 Drug-gene interaction network and molecular docking analysis of Folic Acid with core target proteins in HCM. **(A)** Drug-gene interaction network for nine core genes (GADD45A, SFRP1, S100A9, S100A8, IER3, STAT3, PHLDA1, JAK2, MYC). **(B)** Molecular docking models showing the binding interactions between Folic Acid and the core proteins GADD45A, SFRP1, S100A9, S100A8, IER3, STAT3, PHLDA1, JAK2, MYC.

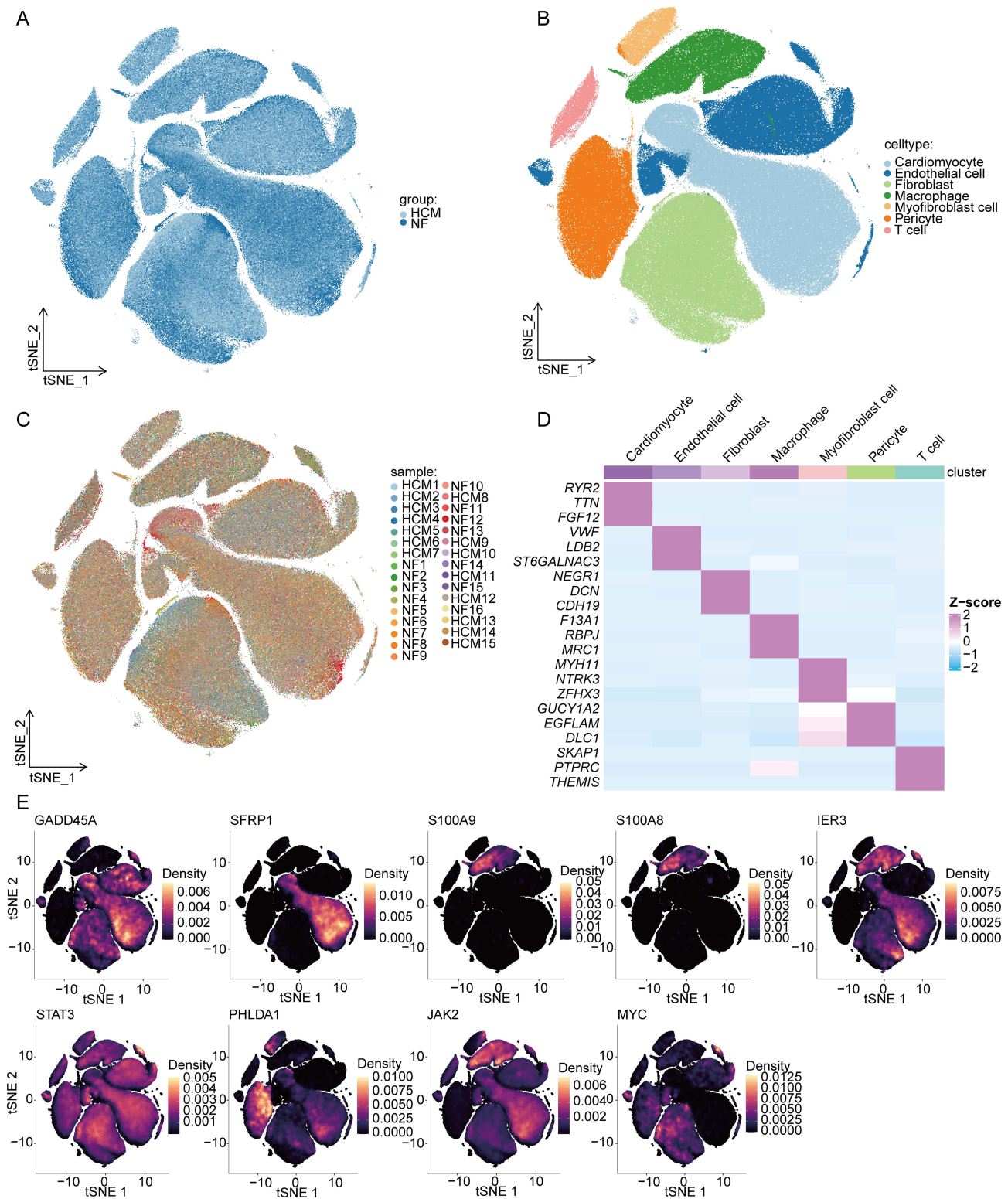


Figure 7 Cell type annotation and distribution of PANoptosis-related diagnostic genes in single-nucleus RNA-seq data from HCM and normal human hearts. **(A)** t-SNE plot showing myocardial nuclei colored by disease condition (HCM vs NF). **(B)** Cell type identification of all nuclei, including cardiomyocytes, fibroblasts, endothelial cells, macrophages, myofibroblast-like cells, pericytes, and T cells. **(C)** Sample-level distribution of cells across donors and conditions. **(D)** Z-score-scaled heatmap of canonical marker genes used to validate cell type annotations, with representative markers for each cluster. **(E)** Feature plots showing the spatial distribution and expression density of nine PANoptosis-related genes (GADD45A, SFRP1, S100A9, S100A8, IER3, STAT3, PHLDA1, JAK2, MYC) across all nuclei.

SFRP1, JAK2, and MYC showed heterogeneous but cell-type-specific localization. These distributions implicate non-myocyte cell types as potential hubs for PANoptosis-linked signaling in HCM.

The global communication network (Figure 8A) indicates that cardiomyocytes act as the primary signal senders, delivering PDGF signals to multiple cell populations including fibroblasts, endothelial cells, and macrophages. Further analysis revealed that fibroblasts are the predominant recipients of PDGF signaling, integrating input from cardiomyocytes, macrophages, and other cell types (Figure 8B). At the pathway level, bubble plots highlight PDGFC-PDGFR α as the most prominent ligand-receptor pair, predominantly enriched along the cardiomyocyte-to-fibroblast axis (Figure 8C). Chord diagram analysis clearly demonstrates that cardiomyocytes activate PDGFR α receptors in fibroblasts via PDGFC, forming a canonical cardiomyocyte-fibroblast communication axis (Figure 8D). Communication heatmaps further confirmed the strong signal-receiving capacity of fibroblasts within the PDGF pathway (Figure 8E). Finally, expression profiling indicates that PDGFC is mainly expressed in cardiomyocytes, PDGFR α is highly expressed in fibroblasts, while PDGFD and PDGFR β are also expressed in subsets of immune and perivascular cells (Figure 8F), suggesting that the PDGF pathway may contribute to fibrotic remodeling in HCM through multiple ligand-receptor pairings. Collectively, these findings highlight that cardiomyocyte-derived PDGFC-PDGFR α signaling may serve as a critical mediator of fibroblast activation in HCM. Given that several PANoptosis-related genes (eg, STAT3, S100A8, GADD45A) are co-expressed within these interacting cell types, it is plausible that PANoptosis-driven inflammatory signaling contributes to the activation of the PDGF pathway and subsequent fibrotic remodeling in HCM. These observations align with the proposed PANoptosis-fibrosis link but require functional perturbation to define causality.

Discussion

Although pyroptosis, apoptosis, and necroptosis have each been implicated in various forms of cardiovascular injury, the integrated activity of PANoptosis in HCM has remained uncharacterized. Unlike traditional single-pathway models of cell death, PANoptosis represents a coordinated signaling interface that integrates pyroptotic, apoptotic, and necroptotic cascades through shared molecular hubs. Our study provides the first comprehensive and multi-modal evidence that PANoptosis is transcriptionally active and biologically relevant in HCM. Through bulk transcriptomic profiling of two independent cohorts (GSE141910 and GSE36961), we identified 30 PANoptosis-related genes differentially expressed in HCM. To refine clinically relevant targets, we applied multiple machine learning algorithms—to derive a robust 9-gene diagnostic signature (S100A9, GADD45A, IER3, STAT3, SFRP1, PHLDA1, JAK2, MYC, and S100A8). These genes are not only involved in inflammatory and cell death regulation but also demonstrated consistent expression trends across three external validation cohorts and were experimentally validated in TAC-induced murine HCM models. This integrative approach reveals that PANoptosis is not a theoretical construct in HCM but a transcriptionally embedded and immunologically connected mechanism, potentially contributing to disease progression and tissue remodeling. Rather than defining single-gene specificity, our work emphasizes the coordinated activation of an interlinked cell-death-immune-fibrosis network, wherein PANoptosis serves as a mechanistic hub bridging inflammatory stress and maladaptive remodeling.

Mechanistically, several of the identified PANoptosis-related genes serve as convergence nodes linking apoptosis, pyroptosis, and necroptosis. STAT3 and JAK2 regulate the transcription of caspase-3 and gasdermin family members, bridging apoptotic and pyroptotic cascades. GADD45A modulates DNA-damage-induced apoptosis through p38 MAPK inhibition, while IER3 and MYC coordinate mitochondrial stress and necroptotic signaling. The S100A8/A9 complex functions as a DAMP that amplifies NLRP3 inflammasome activation, further promoting pyroptosis-associated inflammation. SFRP1 acts upstream by modulating Wnt signaling, which intersects with apoptosis and fibrotic remodeling. These molecular interconnections support the concept that PANoptosis acts as a unifying regulatory platform integrating multiple programmed death pathways in hypertrophic myocardium.^{31,32}

In vivo validation using a TAC-induced murine model of HCM confirmed the transcriptional alterations of the nine PANoptosis-related diagnostic genes. Quantitative RT-PCR analysis revealed that PHLDA1, IER3, JAK2, GADD45A, and S100A8 were significantly upregulated, while S100A9, STAT3, MYC, and SFRP1 were markedly downregulated in diseased myocardium, consistent with a dynamic and gene-specific PANoptotic response under pressure overload-induced hypertrophic stress. These results provide biological plausibility for the computational predictions and highlight transcriptional reprogramming of cell-death and inflammatory pathways during HCM progression. S100A9 and S100A8, which form the calprotectin complex, act as potent DAMPs and are elevated in various cardiovascular diseases, contributing to inflammatory infiltration and myocardial

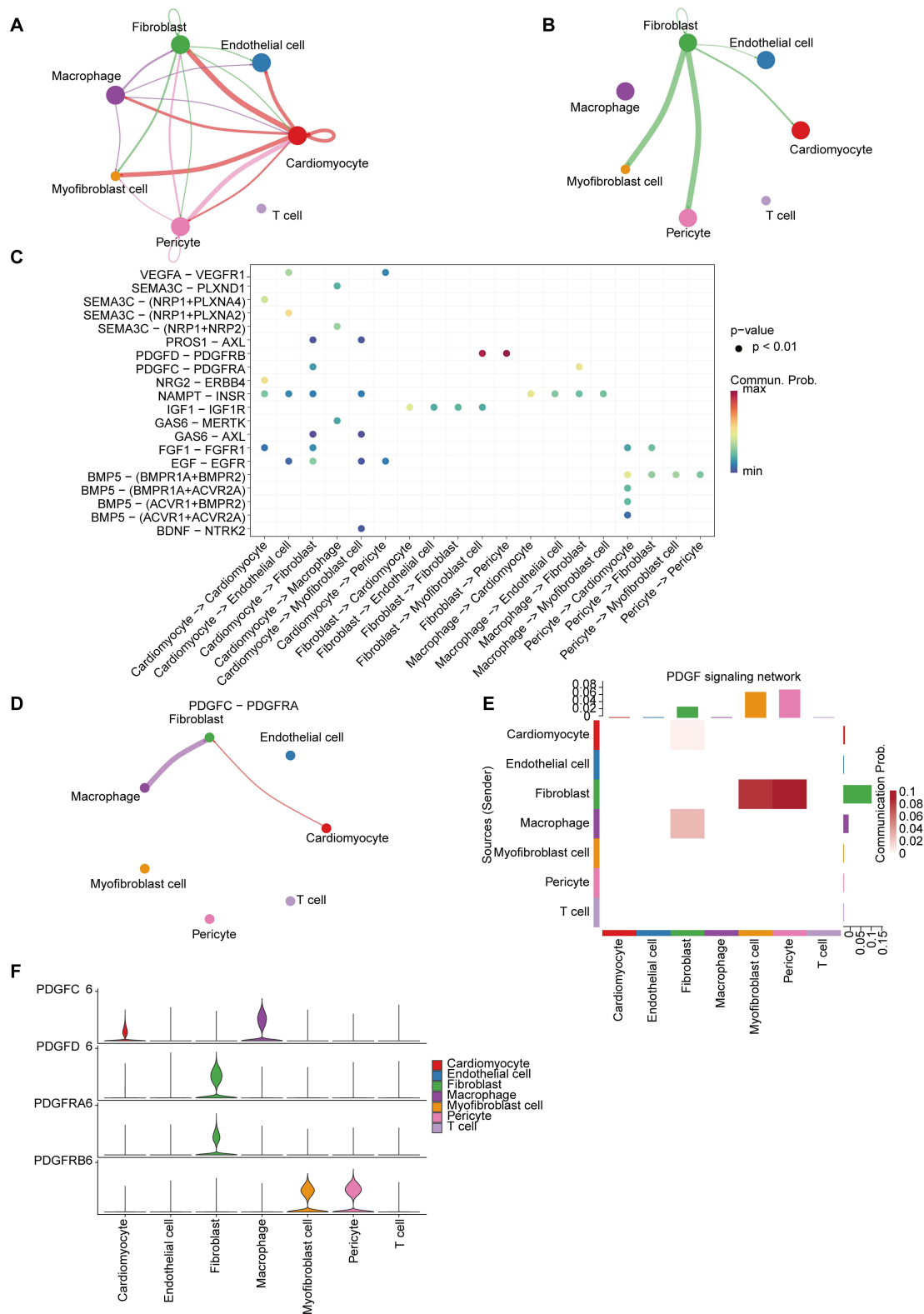


Figure 8 Fibroblast-driven intercellular communication and PDGF signaling in HCM myocardium. **(A)** Circle plot showing global intercellular communication among myocardial cell types. **(B)** Interaction network highlighting fibroblast-centered connectivity. **(C)** Bubble plot of significant ligand-receptor pairs among cell types; dot size indicates p-value, and color reflects communication probability. **(D)** Visualization of the PDGFC-PDGFR signaling axis across cell populations. **(E)** Heatmap of PDGF signaling probability between sender and receiver cells. **(F)** Violin plots showing cell-type-specific expression of PDGF signaling components (PDGFC, PDGFD, PDGFRA, PDGFRB).

fibrosis.^{33,34} GADD45A is a stress-responsive gene that modulates DNA repair and apoptosis, and has been associated with cardioprotective responses via inhibition of the p38 MAPK pathway.³⁵ IER3, an immediate early gene, is involved in regulating apoptosis and mitochondrial stability, particularly under oxidative stress conditions relevant to ischemia-reperfusion injury.³⁶ STAT3 and JAK2, as central components of the JAK-STAT pathway, exhibit context-dependent roles in cardiac remodeling.³⁷ Transient STAT3 activation may confer cardioprotection, whereas chronic activation promotes fibrosis and hypertrophy.³⁸ JAK2 is also implicated in erythropoietin and leptin signaling in cardiac tissues, suggesting a broader role in inflammatory and metabolic processes in HCM.^{39,40} SFRP1, an antagonist of Wnt signaling, is known to be upregulated in failing myocardium and modulates extracellular matrix remodeling.^{41,42} PHLDA1 has been identified as a potential biomarker in heart failure and is closely associated with immune cell infiltration, particularly involving macrophages and mast cells, suggesting a regulatory role at the interface of inflammation and myocardial remodeling.⁴³ Moreover, PHLDA1 is a transcriptional target of mineralocorticoid receptor signaling and is modulated via CK1-dependent phosphorylation mechanisms, which enhance aldosterone-MR nuclear trafficking and genomic activation.⁴⁴ These findings collectively implicate PHLDA1 as a stress-responsive effector involved in both immune-metabolic crosstalk and hormone-mediated transcriptional regulation during cardiac pathology. MYC, a master transcription factor, is critical for cell proliferation and metabolic reprogramming; while essential during development, its reactivation in the adult heart promotes pathological hypertrophy.^{45,46} In aggregate, these gene-level observations are consistent with PANoptosis-adjacent signaling, while underscoring the need for protein-level and functional perturbation studies to define causal roles.

Unsupervised clustering of PANoptosis-related gene expression identified two distinct molecular subtypes of HCM, one characterized by high immune activity and PANoptosis signaling, and the other by lower inflammatory signatures and enhanced metabolic pathways. This molecular stratification corresponds with clinical heterogeneity observed in HCM and may inform precision medicine approaches. Immune cell infiltration analysis demonstrated strong correlations between core PANoptosis genes and immune cell subsets including $\gamma\delta$ T cells, follicular helper T cells, dendritic cells, and macrophages, suggesting a mechanistic link between PANoptosis and immune remodeling in HCM. Functional enrichment by GSVA further revealed that the PANoptosis-high subtype exhibited enhanced immune signaling pathways, including viral myocarditis and graft-versus-host disease, whereas the PANoptosis-low subtype was enriched in metabolic and biosynthetic processes such as steroid biosynthesis and CoA metabolism. These subtype-specific immunometabolic signatures provide a rationale for future studies to test associations with fibrosis burden, arrhythmic risk, and clinical outcomes, and to evaluate whether PANoptosis-based grouping adds value beyond sarcomeric genotype and conventional risk scores.

In addition to bulk transcriptome-based findings, single-nucleus RNA sequencing further delineated the cellular distribution of diagnostic PANoptosis genes. Key genes such as GADD45A, IER3, and S100A9 were predominantly localized in cardiomyocytes, fibroblasts, and macrophage-like immune populations, suggesting spatially defined stress and inflammatory signaling hubs within the HCM myocardium. Moreover, CellChat analysis revealed an enriched cardiomyocyte-fibroblast communication axis, with PDGF signaling emerging as a dominant interaction pathway. This observation aligns with recent spatial transcriptomics findings demonstrating disrupted PDGF signaling and fibroblast enrichment in histologically disarrayed regions of HCM myocardium.⁴⁷ These results support the hypothesis that myocyte-derived PDGF ligands may activate PANoptosis-related remodeling in fibroblasts, thereby promoting extracellular matrix deposition and regional fibrosis. Mechanistically, such signaling interactions are consistent with prior *in vivo* studies in TAC-induced HCM models, where PDGF and downstream inflammatory mediators were shown to promote cardiac inflammation, oxidative stress, apoptosis, and fibrotic remodeling, all of which are closely tied to PANoptosis pathways.⁴⁸ The convergence of PDGF-mediated communication, PANoptosis activation, and fibroblast reprogramming may thus represent a pathophysiologically relevant axis in HCM. Together, our findings integrate transcriptional, spatial, and cell-cell interaction data to provide novel insights into the cell-type-specific and paracrine mechanisms underlying PANoptosis-mediated myocardial remodeling in HCM. Moreover, the potential link between PANoptosis and myocardial fibrosis can be conceptually explained by the dual role of PANoptotic cell death in amplifying inflammatory signaling and releasing pro-fibrotic mediators. During PANoptosis, simultaneous activation of pyroptosis, apoptosis, and necroptosis leads to massive cytokine and DAMP release, including IL-1 β , IL-18, TNF- α , and S100A8/A9, which can activate fibroblasts through paracrine signaling. Recent studies have demonstrated that PANoptosis-related inflammasome activation promotes TGF- β /Smad and PDGF-PDGFR signaling cascades, driving myofibroblast differentiation and extracellular matrix deposition in the heart and vasculature.^{31,49} Our single-nucleus transcriptomic and CellChat analyses further support this mechanism by showing that

PANoptosis-related genes are enriched in both cardiomyocytes and fibroblasts and that cardiomyocyte-derived PDGF ligands interact with fibroblast PDGFRA receptors. Taken together, these findings suggest that PANoptosis may not only contribute to inflammatory injury but also indirectly accelerate cardiac fibrosis through cytokine-driven fibroblast activation and maladaptive remodeling. Beyond PDGF-mediated communication, protein-level cross-talk among PANoptosis regulators provides additional mechanistic insight into HCM pathogenesis. For instance, the STAT3–JAK2 axis integrates cytokine and stress responses, coordinating apoptotic and inflammatory transcriptional programs; S100A8/A9 amplifies inflammasome activation via NLRP3–caspase-1 interactions; and MYC–GADD45A signaling couples metabolic reprogramming to cell-death sensitivity. This multi-dimensional interplay underscores PANoptosis as an integrated signaling network rather than discrete cell-death pathways. Clinically, the identified nine-gene panel may facilitate risk stratification and therapeutic targeting by identifying patients with inflammation-dominant or PANoptosis-high phenotypes. Such molecular profiling could complement conventional imaging and genetic diagnostics to guide anti-inflammatory or anti-fibrotic therapies in HCM.

To explore upstream regulatory mechanisms, we constructed a comprehensive ceRNA network comprising 71 miRNAs and 108 lncRNAs. Notably, lncRNAs such as H19, XIST, and NEAT1 emerged as potential regulatory hubs, consistent with their previously reported roles in cardiac hypertrophy and fibrosis. For instance, H19 modulates TGF- β signaling via miR-675, a pathway intricately involved in fibrotic remodeling and immune activation.⁵⁰ Molecular docking simulations revealed that folic acid exhibits favorable binding affinities toward all nine PANoptosis-related proteins, particularly GADD45A, IER3, JAK2, and MYC. These interactions were characterized by stable hydrogen bonding and hydrophobic interactions within conserved binding domains. These interactions suggest their potential as multi-target modulators in HCM. Clinically, folic acid has been shown to reduce stroke risk and improve cardiovascular outcomes in large meta-analyses.⁵¹ Mechanistically, it attenuates vascular calcification by preserving BH4 bioavailability and mitigating oxidative stress.⁵² In hypertensive populations, folic acid combined with enalapril is both effective and cost-efficient for stroke prevention.⁵³ Tretinoin, meanwhile, enhances cardiomyocyte differentiation and maturation by promoting sarcomere organization and mitochondrial function.⁵⁴ These observations should be regarded as exploratory leads for subsequent preclinical evaluation in HCM models, further studies are warranted to evaluate their efficacy in HCM.

Although this study systematically identified PANoptosis-related signatures in HCM and established a robust diagnostic model validated across multiple external cohorts, while integrating single-nucleus transcriptomic analysis, immune landscape profiling, cell–cell communication mapping, and murine model validation to elucidate the functional implications of core PANoptotic genes, several limitations should be acknowledged. First, the analyses relied on publicly available GEO datasets, which may introduce heterogeneity in sample acquisition, processing, and clinical metadata. Despite rigorous batch correction using the ComBat algorithm, residual variability and incomplete clinical information (eg, medication use, fibrosis grade, and long-term outcomes) could influence model generalizability. Second, the study employed a TAC-induced hypertrophy model for in vivo validation, which reflects pressure-overload-driven remodeling rather than the sarcomeric mutations underlying genetic HCM. Thus, the animal experiments were intended only to confirm gene expression trends under hypertrophic stress, not to reproduce the full genetic or histopathological spectrum of human HCM. Future studies using patient-derived or gene-edited models will be essential to establish causal relevance. Third, the computational and cross-sectional design limits causal inference; although qPCR validation confirmed consistent mRNA expression trends in TAC-induced mouse models, corresponding protein-level and functional perturbation studies remain essential to establish mechanistic relevance. Fourth, the single-nucleus RNA-seq dataset had a limited sample size and lacked spatial resolution, which may affect the accuracy of inferred intercellular signaling, particularly PDGF-mediated cardiomyocyte–fibroblast crosstalk. Fifth, the machine-learning framework—despite repeated cross-validation, external testing, and stability analysis—remains susceptible to potential overfitting and sampling bias. Finally, the ceRNA and molecular docking analyses are exploratory and require experimental validation to confirm biological and therapeutic relevance. Beyond these findings, the emerging development of RNA-based therapeutics (eg, siRNA or antisense oligonucleotide approaches) provides a promising avenue to selectively modulate PANoptosis-related genes, offering future translational potential for precise intervention in HCM. In summary, this study provides integrative and multi-dimensional evidence implicating PANoptosis in the pathogenesis of HCM. While further proteomic, functional, and clinical validation is warranted, our findings lay a foundation for future mechanistic and translational investigations into PANoptotic signaling as a potential diagnostic and therapeutic axis in HCM.

Conclusion

This study provides integrative and multi-dimensional evidence linking PANoptosis to the pathogenesis of HCM. By combining bulk transcriptomics, multi-algorithm machine learning, and external validation, we identified a nine-gene PANoptosis-related candidate diagnostic panel that reflects coordinated activation of inflammatory and cell-death pathways rather than single-gene specificity. Single-nucleus transcriptomic and cell–cell communication analyses further revealed a potential cardiomyocyte-to-fibroblast PDGF signaling axis that may mediate PANoptotic crosstalk and contribute to myocardial remodeling. Experimental validation in a TAC-induced mouse model confirmed corresponding gene expression alterations, supporting the biological plausibility of the computational predictions. From a translational perspective, these findings provide a conceptual framework for incorporating PANoptosis-related biomarkers into HCM precision medicine—potentially aiding risk stratification, prognostic assessment, and therapeutic targeting in patients with inflammation- or fibrosis-dominant molecular phenotypes. However, given the multifunctional nature of the identified genes, these results should be regarded as hypothesis-generating. Limitations include reliance on heterogeneous public datasets, cross-sectional design, and mRNA-level validation without direct proteomic or longitudinal confirmation. Future studies integrating proteomic, spatial, and functional analyses, as well as prospective clinical validation, are warranted to establish causality and evaluate the clinical utility of PANoptosis-based interventions. Overall, rather than defining single-gene specificity, our work emphasizes the coordinated activation of an interlinked cell-death–immune–fibrosis network, wherein PANoptosis acts as a mechanistic hub bridging inflammatory stress, fibroblast activation, and maladaptive remodeling in HCM.

Abbreviations

AUC, area under the curve; CDF, cumulative distribution function; CeRNA, competing endogenous RNA; CTD, Comparative Toxicogenomics Database; DCA, Decision Curve Analysis; DEGs, Differentially Expressed Genes; DSP, Desmoplakin; GBM, Gradient Boosting Machine; GEO, Gene Expression Omnibus; GLM, Generalized Linear Model; GO, Gene Ontology; GSVA, gene set variation analysis; HCM, Hypertrophic cardiomyopathy; HE, hematoxylin and eosin; IVSD, Interventricular septal thickness during diastole; IVSS, Interventricular septal thickness during systole; KEGG, Kyoto Encyclopedia of Genes and Genomes; KNN, K-Nearest Neighbors; LASSO, Least Absolute Shrinkage and Selection Operator; LDA, Linear Discriminant Analysis; LVEF, Left ventricular ejection fraction; LVFS, Left ventricular fractional shortening; PCA, Principal Component Analysis; PDB, Protein Data Bank; qPCR, Quantitative Real-Time PCR; RF, Random Forest; SVM, Support Vector Machines; XGBoost, Extreme Gradient Boosting.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

The study was based on publicly available, de-identified datasets from the Gene Expression Omnibus (GEO) database, which had obtained ethical approval and informed consent in the original studies. This exemption was confirmed and approved by the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University (Approval No. KY2024057).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; 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.

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Disclosure

The authors declare that they have no competing interests.

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