

The ACVR1C-GAS6 Antagonistic Axis Drives Metabolic Decompensation and Liver Fibrosis in MASLD: Mechanistic Insights from Multi-omics Analyses

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The ACVR1C-GAS6 Antagonistic Axis Drives Metabolic Decompensation and Liver Fibrosis in MASLD: Mechanistic Insights from Multi-omics Analyses

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Abstract

Background

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide, and liver fibrosis stage is the strongest determinant of long-term morbidity and mortality in affected patients. However, the molecular switches driving the transition from compensated steatosis to progressive fibrogenesis remain poorly characterized, limiting the development of early diagnostic biomarkers and targeted therapeutic interventions.

Methods

We integrated machine learning-based bulk transcriptome screening, single-cell RNA sequencing, spatial transcriptomic validation, and transcriptional regulatory network analysis to systematically dissect the functional roles and interplay of ACVR1C and GAS6 during MASLD progression.

Results

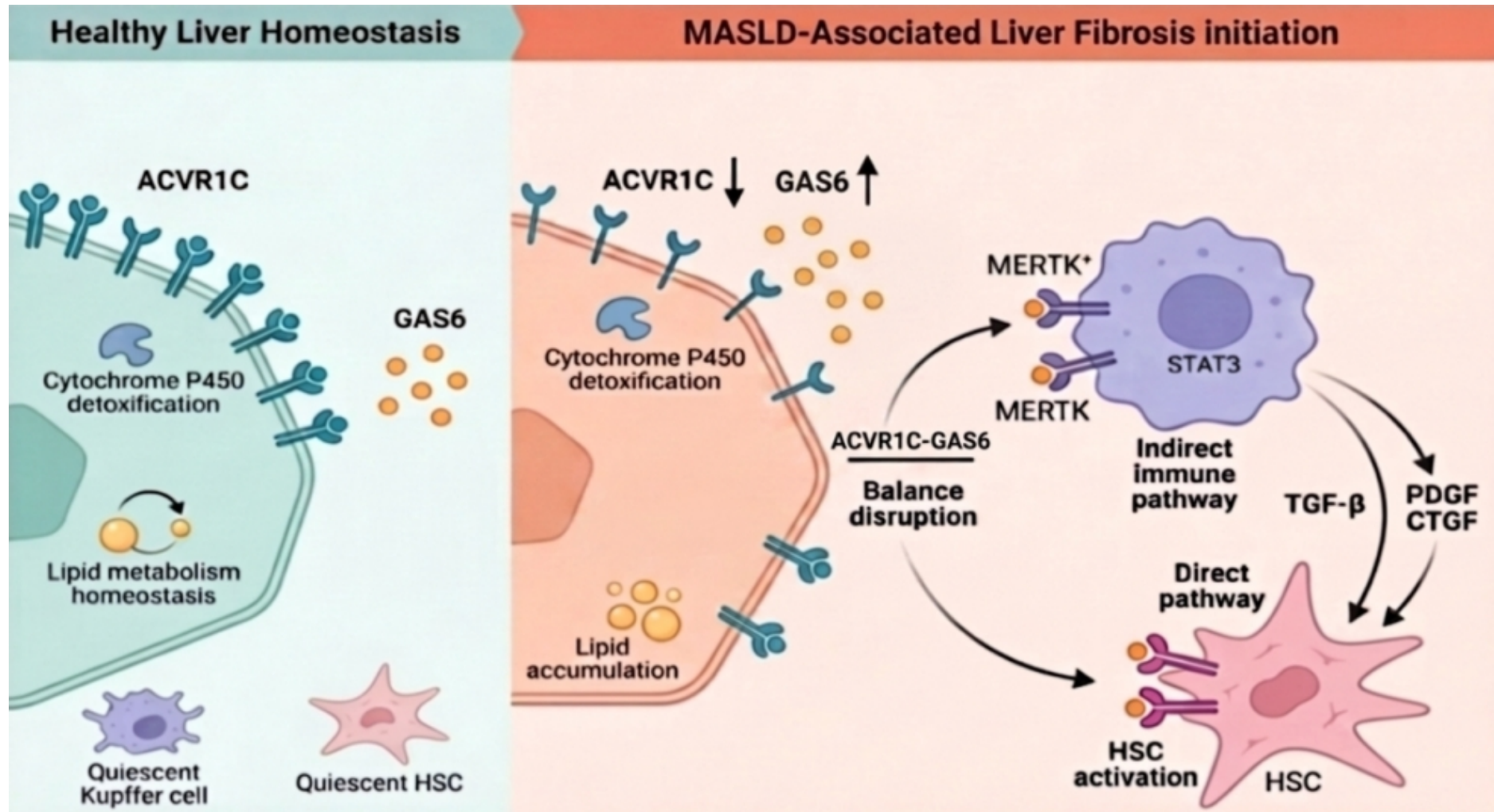
We identified the ACVR1C-GAS6 antagonistic axis as a central regulatory hub governing hepatocyte metabolic homeostasis and macrophage functional polarization. ACVR1C exerts a previously unrecognized homeostatic gatekeeper role in sustaining hepatocyte detoxification pathways, whereas GAS6 is progressively upregulated across fibrosis stages with a distinct expression threshold marking fibrogenesis onset. Mechanistically, GAS6 drives polarization of heme-related macrophages via the MERTK-STAT3 cascade, which synergizes with direct hepatic stellate cell activation to promote liver fibrosis through a dual pathway. Spatial transcriptomic analysis in a mouse MASLD model further confirmed enhanced in situ co-localization of Gas6 and Mertk in diseased liver tissue.

Conclusions

The ACVR1C-GAS6 axis provides a novel molecular framework for understanding MASLD progression, and represents promising candidate biomarkers for disease risk stratification and therapeutic targets for clinical translation.

Key words: MASLD; GAS6; ACVR1C; Liver fibrosis; Hepatocyte-macrophage crosstalk

Graphic abstract



Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly termed non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disease worldwide, affecting approximately 30% of the adult population. The disease spectrum ranges from simple steatosis and metabolic dysfunction-associated steatohepatitis (MASH) to liver fibrosis and cirrhosis, and may ultimately progress to hepatocellular carcinoma[1]. The pathogenesis of MASLD is multifactorial, involving insulin resistance, dysregulated lipid metabolism, mitochondrial dysfunction, and chronic inflammation[2]. The stage of liver fibrosis is a major determinant of long-term prognosis in patients with MASLD [3]; however, the molecular regulatory nodes that drive the transition from compensated steatosis to progressive fibrosis remain poorly defined, and there is an unmet clinical need for early diagnostic biomarkers and actionable therapeutic targets.

Sustained inflammatory responses driven by innate immune activation are widely recognized as a core driver of disease progression from simple steatosis to MASH and fibrosis. As the predominant innate immune cell population in the liver, macrophages play a central role in this process. For example, when hepatocytes undergo lipotoxic injury caused by insulin resistance and free fatty acid overload, damaged or dying hepatocytes release damage-associated molecular patterns (DAMPs). These DAMPs activate the NLRP3 inflammasome in macrophages, leading to the secretion of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). These pro-inflammatory mediators further exacerbate hepatocellular oxidative stress and activate hepatic stellate cells (HSCs), resulting in excessive extracellular matrix (ECM) deposition and accelerated liver fibrogenesis[4]. In addition, activated macrophages express Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), and TNF- α , which directly promote hepatocyte inflammation and apoptosis[5]. Despite the established role of hepatocyte-macrophage crosstalk in MASLD progression, the molecular mediators that orchestrate this intercellular communication and drive fibrosis initiation remain incompletely characterized.

Growth arrest-specific 6 (GAS6) is a common ligand for the TAM family of receptor tyrosine kinases (TYRO3, AXL, and MERTK), which regulates macrophage phagocytosis, inflammatory resolution, and tissue repair. TAM receptors are abundantly expressed on hepatic immune cells, particularly Kupffer cells, and aberrant GAS6/TAM signaling has been implicated in macrophage polarization and profibrotic mediator secretion, which may promote HSC activation and liver fibrosis[6,7]. Activin A receptor type 1C (ACVR1C, also termed ALK7) is a type I

receptor of the transforming growth factor- β (TGF- β) superfamily. It signals through SMAD2/3 phosphorylation to regulate lipid metabolism and cellular stress responses, and has been linked to hepatocyte lipotoxic injury and adipose tissue lipolysis [8,9]. While individual studies have suggested that GAS6 and ACVR1C are involved in MASLD pathogenesis [10,11], their cell-type-specific expression patterns in the human MASLD liver, their functional interaction, and their potential roles in regulating hepatocyte-macrophage crosstalk during fibrosis progression remain largely unknown.

In this study, we integrated machine learning-based bulk transcriptome screening, single-cell RNA sequencing (scRNA-seq) analysis, spatial transcriptomic validation, and transcriptional regulatory network analysis to systematically characterize the expression dynamics and functional roles of GAS6 and ACVR1C in MASLD progression. We aimed to identify the ACVR1C-GAS6 axis as a key regulatory node driving the transition from steatosis to liver fibrosis, and to explore its potential as a biomarker and therapeutic target for clinical translation.

Methods

Bulk Transcriptomic Analysis

Three independent human liver microarray datasets (GSE162694, GSE135251, and GSE126848) were retrieved from the Gene Expression Omnibus (GEO) database, all containing normal control and MASLD liver tissue samples. Raw expression matrices were $\log_2(x+1)$ transformed, and quality control was performed by removing infinite values (Inf) and missing values (NA). The GSE162694 dataset was designated as the training cohort, while GSE135251 and GSE126848 served as independent external validation cohorts.

To reduce data dimensionality and mitigate model overfitting, least absolute shrinkage and selection operator (LASSO) regression with 5-fold cross-validation was implemented. The lambda.min value corresponding to the minimum binomial deviance was selected to screen for key feature genes associated with MASLD.

Machine Learning Model Construction and Validation

Based on the feature genes screened by LASSO regression, seven conventional machine learning classifiers were established, including Elastic Net, Random Forest (RF), Extra Trees (ET), Extreme Gradient Boosting (XGBoost), support vector machine (SVM) with radial basis function kernel, K-nearest neighbors (KNN) with hyperparameter tuning, and Naive Bayes. In addition, an ensemble model was

constructed by averaging the predicted probabilities output by the seven aforementioned individual classifiers. All models were subjected to internal cross-validation during training to ensure robust generalization performance.

Model Performance Evaluation and Interpretability Analysis

To elucidate the biological relevance underlying model predictions, the SHapley Additive exPlanations (SHAP) algorithm[12] was applied to interpret the decision logic of the optimal performing model. The mean absolute SHAP value of each feature was calculated to quantify its global contribution to model prediction. Beeswarm plots, group-level average force plots, and partial dependence plots were generated to visualize the direction and marginal effect of core features on prediction outcomes.

To explore the synergistic effects of core driver genes in the MASLD pathological network, a linear regression model based on SHAP values was fitted to quantitatively evaluate the interaction between core genes and other key features. Furthermore, Spearman's rank correlation analysis was performed to assess the association between key gene expression levels and liver fibrosis stage, to validate their clinicopathological significance in MASLD progression.

Single-Cell RNA Sequencing Analysis

Dimensionality Reduction and Cell Clustering

A public human liver single-cell transcriptomic dataset (GSE330266) was included for mechanistic validation. Raw count matrices were first filtered to exclude low-quality cells and low-expressed genes, with thresholds set as $\text{min.cells} = 3$ and $\text{min.features} = 200$. Strict quality control was subsequently performed: cells with $\text{nCount_RNA} < 500$, $\text{nFeature_RNA} < 200$ or > 5000 , or mitochondrial gene percentage $> 25\%$ were discarded. DoubletFinder was applied to remove putative doublets.

After data normalization and identification of highly variable genes (HVGs), qualified cells were subjected to principal component analysis (PCA) for linear dimensionality reduction. Batch effects across samples were corrected using the Harmony algorithm [13]. Based on the corrected principal components, Uniform Manifold Approximation and Projection (UMAP) was performed for nonlinear dimensionality reduction and visualization, followed by shared nearest neighbor (SNN) graph-based clustering. The

optimal clustering resolution was determined using the Clustree tool.

Cell Type Annotation and Subset Refinement

Initial cell cluster annotation was performed manually based on the differential expression of canonical marker genes, and seven major cell types in the liver microenvironment were identified. The canonical markers for each cell type were as follows: macrophages (CD14, CSF1R, C1QA, MARCO, CD5L, VCAM1), hepatic stellate cells (PDGFRB, ACTA2, RGS5), B cells (CD19, CD79A, MS4A1), T cells (CD3D, CD3E, CD8A), hepatocytes (ALB, TTR, APOA1), cholangiocytes (KRT19, KRT7, CFTR), and endothelial cells (PECAM1, VWF, CDH5).

Fine-grained annotation of cell subsets was further conducted using a function-oriented stepwise workflow. For hepatocyte subsets, quantitative pathway activity analysis was first performed. Using the scMetabolism package[14] with the Kyoto Encyclopedia of Genes and Genomes (KEGG) and REACTOME databases, single-cell activity scores for hundreds of metabolic pathways were calculated as described below. Hierarchical clustering based on the average pathway activity matrix across subsets was then applied to identify coordinated metabolic modules. By integrating metabolic module profiles, differentially expressed genes (DEGs), and Gene Ontology (GO) biological process enrichment results, hepatocyte subsets were reannotated using a strategy of "functional phenotype combined with core metabolic module", to link subset identity to biological processes including liver homeostasis, lipid metabolism, inflammatory stress, and protein translation.

For macrophage subset annotation, a three-tier integrative framework combining module scoring, DEG analysis, and negative marker exclusion was established. Based on published liver macrophage atlases and MASLD-related studies[15–17], we constructed gene sets covering multiple functional states: homeostatic Kupffer cell module (TIMD4, VSIG4, CLEC4F, MARCO, CD5L, FOLR2); capsular and central vein-associated macrophage module (CX3CR1, ITGAX); monocyte-derived inflammatory macrophage module (CCR2, FCN1, S100A8, S100A9, LYZ, IL1B); lipid-associated macrophage (LAM) module (TREM2, GPNMB, SPP1, CD9, CD63); M1-like proinflammatory module (TNF, IL1B, IL6, CXCL10, IDO1, NOS2); M2-like reparative module (MRC1, CD163, IL10, TGFB1, MSR1, ARG1); heme/iron metabolism module (HMOX1, FTH1, FTL); and proliferation module (MKI67, TOP2A, STMN1, PCNA).

Module scores were calculated using the AddModuleScore function in the Seurat package and visualized via heatmaps. Macrophage subsets were finally defined

according to the principle of “dominant functional module + positive signature markers + exclusion of key negative markers”, with reference to the percentage of marker-positive cells, average expression of validation genes, and DEGs identified by the FindAllMarkers function (Wilcoxon rank-sum test, $\log_2$ fold change > 0.25 , adjusted $P < 0.05$ with Benjamini–Hochberg correction).

Ro/e Enrichment Analysis

To quantify the tissue enrichment preference of cell subsets between control and MASLD groups, the ratio of observed to expected (Ro/e) enrichment index was calculated using the calTissueDist function of the STARTRAC package[18], based on the chi-square test. This method effectively eliminates bias caused by variations in total cell count across samples, and reveals the enrichment characteristics of specific cell subsets in the liver microenvironment under different disease states, as well as their phenotypic associations.

Cross-Dataset Phenotype Mapping with Scissor

To bridge the single-cell transcriptomic atlas and the three independent bulk transcriptomic datasets, the Scissor algorithm was applied for phenotype reverse mapping[19]. This algorithm uses LASSO regression to model the association between single-cell expression profiles and bulk sample disease status, and identifies cells with high phenotypic correlation.

To ensure the robustness of the results, cells identified in at least two of the three datasets were defined as Scissor-positive (MASLD-associated) or Scissor-negative (control-associated). This analysis allowed extraction of core phenotype-associated cell subsets conserved across datasets, and validation of key feature gene expression at single-cell resolution.

Pseudotime Trajectory Inference

To elucidate dynamic cell state transitions during MASLD progression, Slingshot[20] was used to construct single-cell differentiation trajectories based on low-dimensional UMAP embeddings and cell cluster labels. Principal curves were fitted to the low-dimensional manifold, and the trajectory starting point was determined based on prior biological knowledge or set automatically by the algorithm, to calculate pseudotime values along the differentiation path.

For core target genes, locally weighted scatterplot smoothing (LOWESS) regression was applied to fit gene expression dynamics along the pseudotime axis. Potential

functional transition points were identified from inflection points of the expression curves, providing quantitative evidence for the dynamic regulatory roles of key genes in cell state transitions.

Metabolic Module Construction and Activity Quantification

To characterize metabolic reprogramming of hepatocyte subsets during MASLD progression, single-cell activity scores for hundreds of metabolic pathways from the KEGG and REACTOME databases were calculated using the scMetabolism package (based on single-sample gene set enrichment analysis) and gene set variation analysis (GSVA).

Ward.D2 hierarchical clustering was performed on the average pathway activity matrix to group metabolically correlated pathways into coordinated functional modules, and the elbow method was applied to determine the optimal number of modules. Spearman's rank correlation analysis was used to quantify the association between core gene expression and the activity of specific metabolic pathways. Module activity scores were projected onto UMAP embeddings to visualize their distribution patterns across cell subsets.

Cell–Cell Communication Analysis

To dissect the remodeling of cell–cell communication in the MASLD liver microenvironment, CellChat[21] was used to characterize and compare intercellular communication patterns between control and MASLD groups. Based on single-cell expression profiles and a curated ligand–receptor interaction database, cell communication networks were constructed for both groups, with a focus on signaling pathways relevant to MASLD pathogenesis. Differences in the number and strength of interactions between groups were calculated to identify MASLD-associated activated or suppressed signaling pathways.

For the GAS6-centered ligand–receptor signaling axis, we extracted the sender strength and receiver strength of each cell subset. Chord diagrams and bubble plots were generated to visualize the communication probability of GAS6-related ligand–receptor pairs and their source-target cell relationships.

To further explore the downstream regulatory mechanisms of GAS6 signaling, NicheNet was applied to predict ligand-target gene regulatory interactions. M2-like

Kupffer cells and heme-metabolizing macrophages were defined as receiver cells, and functional hepatocyte subsets were defined as potential sender cells. Based on DEGs in receiver cells between MASLD and control groups (adjusted $P < 0.05$, $\log_2$ fold change > 0.25) and a prior knowledge-based ligand-target regulatory matrix, ligand activity scores were calculated to identify key ligands driving macrophage transcriptional reprogramming. A GAS6-centered regulatory network, including ligands, receptors, downstream transcription factors, and target genes, was subsequently constructed.

PySCENIC-Based Regulon and Cooperative Transcriptional Module Analysis

To dissect the transcriptional regulatory network of macrophage subsets in MASLD, PySCENIC (v0.12.1) [22] was used to infer regulons from the single-cell expression profiles of macrophages. First, GRNBoost2 was applied to construct transcription factor (TF)-target gene co-expression networks based on the expression matrix. Next, cisTarget was used to perform motif enrichment analysis against the human hg19 motif database to identify direct target genes of TFs. Finally, area under the curve (AUC) scores for each regulon, representing regulon activity, were calculated in each cell using AUCell.

Regulon activity similarity was calculated based on Spearman's correlation between regulon activity profiles. Ward.D2 hierarchical clustering was then performed to group regulons with highly correlated activity into distinct cooperative transcriptional (CSI) modules. Average module activity scores were calculated across cell subsets and disease groups to reveal MASLD-associated differences in module activation. Regulon specificity scores (RSS) were computed to assess the specificity of TFs for each macrophage subset. Network diagrams were generated to visualize synergistic interactions between TFs within modules.

Spatial Transcriptomic Analysis

Data Source and Preprocessing

Spatial transcriptomic data were downloaded from the GEO database (accession number: GSE248077), which included 13 mouse liver tissue sections from 6 control and 7 MASLD samples. After data normalization, batch effects across tissue sections were corrected via Harmony integration.

Cell Type Deconvolution and Spatial Distribution Reconstruction

To delineate the spatial distribution patterns of cell subsets on tissue sections, deconvolution analysis was performed using the R package CARD[23]. This algorithm models the expression profile of each spot as a linear combination of cell type-specific expression profiles from a single-cell reference, and incorporates spatial coordinate constraints and spatial neighbor information to estimate the proportion of each cell type per spot.

Based on the deconvolution results, high-resolution spatial maps of cell subsets were reconstructed, and in situ differences in the proportions of hepatocytes and immune cells (especially macrophages) between control and MASLD groups were visualized.

Ligand–Receptor Spatial Colocalization Analysis

To validate the spatial functional interaction between ligand Gas6 and receptor Mertk in MASLD liver tissues, spatial colocalization analysis was performed using the R package SpaGene[24]. Based on a spatial proximity algorithm, normalized expression levels of the ligand and receptor within the local neighborhood of each spot were calculated to derive a ligand–receptor colocalization score. A higher colocalization score indicates concordant high expression of the ligand and receptor in the local microenvironment, suggesting spatial activation of the signaling axis.

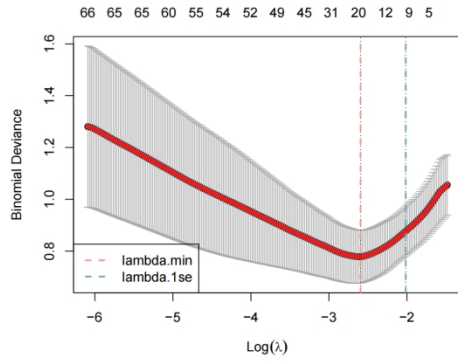
Empirical cumulative distribution function (ECDF) curves were used to compare the overall distribution of colocalization scores between control and MASLD groups. The Mann–Whitney U test was applied to evaluate the statistical difference in colocalization strength between groups, to demonstrate the spatial co-activation of the Gas6-Mertk signaling axis in the MASLD liver microenvironment.

Results

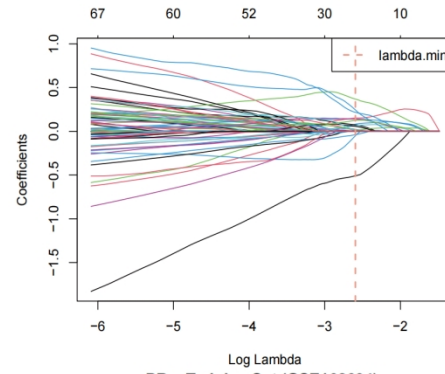
LASSO Feature Selection and Diagnostic Performance of Machine Learning Models

To screen core diagnostic biomarkers from high-dimensional transcriptomic data, least absolute shrinkage and selection operator (LASSO) regression was applied for feature dimensionality reduction in the training set. The optimal regularization parameter was determined by both the cross-validation error curve and coefficient shrinkage path, and 20 key feature genes were finally selected based on lambda.min (Figures 1A and 1B).

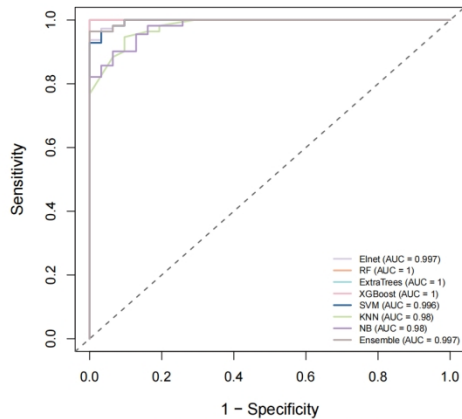
Based on this feature set, eight machine learning models were constructed, and their diagnostic performance was evaluated in the training set and two independent test sets. In the training set, the areas under the receiver operating characteristic (ROC) curve and precision-recall (PR) curve of most models approached 1.0 (Figures 1C and 1F). Among the external independent test sets, the Random Forest model exhibited the best overall cross-dataset generalization performance. Specifically, its areas under the ROC and PR curves reached 0.896 and 0.995 in the GSE135251 test set (Figures 1D and 1G), and 0.657 and 0.825 in the GSE126848 test set (Figures 1E and 1H). The average areas under the ROC and PR curves across the two test sets were 0.78 and 0.91, respectively, indicating reliable robustness of the model for MASLD classification and diagnosis.



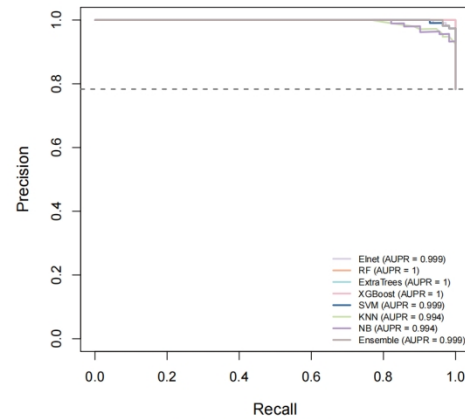
ROC - Training Set (GSE162694)



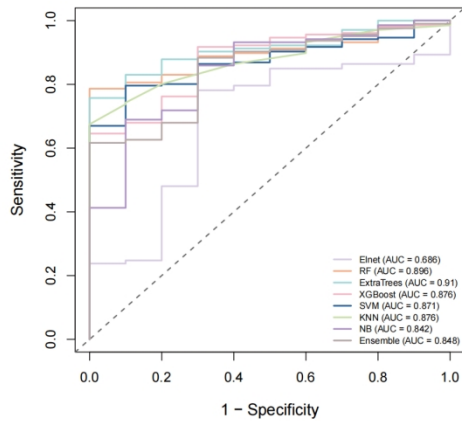
PR - Training Set (GSE162694)



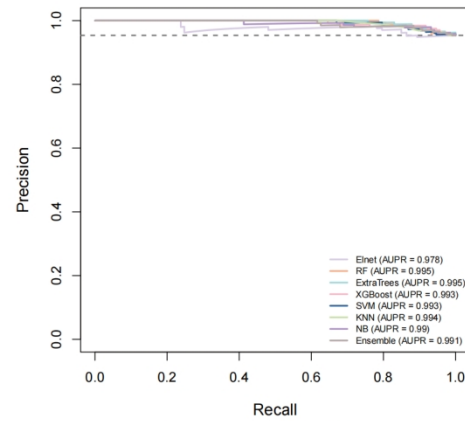
ROC - Test Set 1 (GSE135251)



PR - Test Set 1 (GSE135251)



ROC - Test Set 2 (GSE126848)



PR - Test Set 2 (GSE126848)

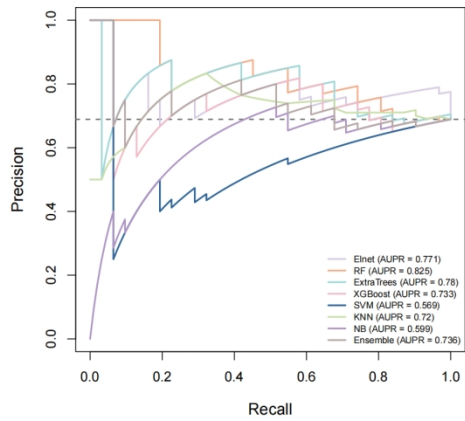
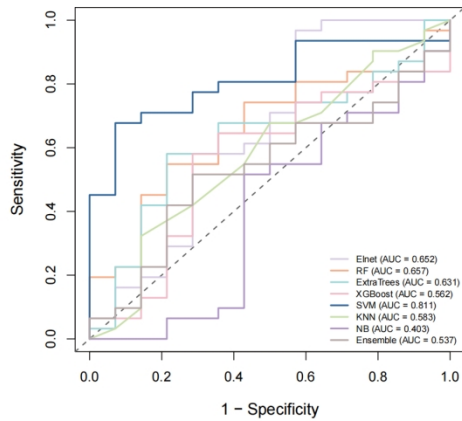


Figure 1. LASSO-based feature selection and diagnostic performance evaluation of machine learning models.

(A) Five- fold cross- validation error curve of LASSO regression.

(B) Feature coefficient path plot of LASSO regression. The plot shows the gradual shrinkage of the coefficients of selected feature genes to zero with increasing regularization penalty intensity, and the dashed line marks the lambda.min position for final model selection.

(C–E) ROC curves showing the classification performance of eight machine learning models in the training set GSE162694, test set GSE135251 and test set GSE126848, respectively. The diagonal dashed line represents the random classification baseline. Values in parentheses in the legend indicate the area under the curve of the corresponding evaluation metric.

(F–H) PR curves showing the classification performance of the corresponding models in the three aforementioned datasets, respectively. The horizontal dashed line represents the random prediction baseline for positive samples in the dataset. Values in parentheses in the legend indicate the area under the curve of the corresponding evaluation metric.

SHAP Interpretability of Core Predictive Genes and Their Association with Fibrosis Progression

To dissect the internal decision-making mechanism of the optimal machine learning model, the SHAP method was applied for interpretability analysis. SHAP beeswarm plots and feature importance ranking clearly revealed that GAS6, SFRP1, CST3, ACVR1C, and BLVRA ranked as the top five genes in global feature importance. High expression of GAS6 significantly drove an increase in the model's predicted probability, whereas low expression exerted a strong negative predictive effect (Figures 2A and 2B). Group-averaged SHAP force plots intuitively demonstrated diametrically opposite contribution directions of core features between the two groups (Figure 2C).

The cumulative contribution curve based on mean absolute SHAP values indicated that the total contribution of the top 15 key feature genes exactly reached the critical threshold of 80%, verifying the high efficiency of this streamlined feature set (Figure 2D). SHAP dependence plots were further generated for these 15 key genes, and the results revealed nonlinear dose-response or saturation-dependent relationships between the expression levels of genes including GAS6, CD72, and SFR1 and their corresponding SHAP values. Specifically, the SHAP dependence plot of GAS6 demonstrated a clear expression threshold effect: when GAS6 expression exceeded the critical threshold of approximately 8.0, the SHAP value rapidly shifted from negative contribution to positive contribution, marking its transition from suppressing model prediction to significantly promoting MASLD classification. With further elevation of expression, the SHAP value entered a stable plateau phase, suggesting that the pathological driving effect had reached saturation and its marginal contribution to the classification decision no longer increased (Figure 2E).

Gene interaction effect analysis showed that the interaction between GAS6 and ACVR1C was the most significant among all evaluated gene pairs (Figure 2F). Finally, in the two independent datasets GSE135251 and GSE162694, the expression patterns of core genes showed significant divergence with fibrosis progression. The expression level of GAS6 gradually increased as fibrosis stage advanced from Control to F4, whereas ACVR1C expression exhibited a clear downward trend with increasing fibrosis severity (Figures 2G and 2H). The disease-promoting threshold effect of GAS6 and the progressive downregulation of ACVR1C along disease progression are mutually consistent, suggesting that they may serve as an activation switch in the initiation stage of MASLD and a key negative feedback regulator in the disease progression stage, respectively.

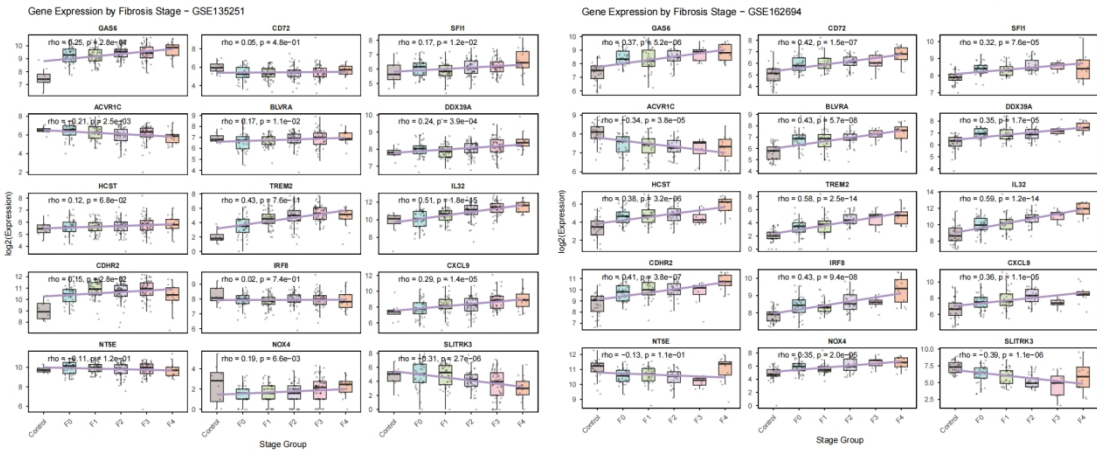
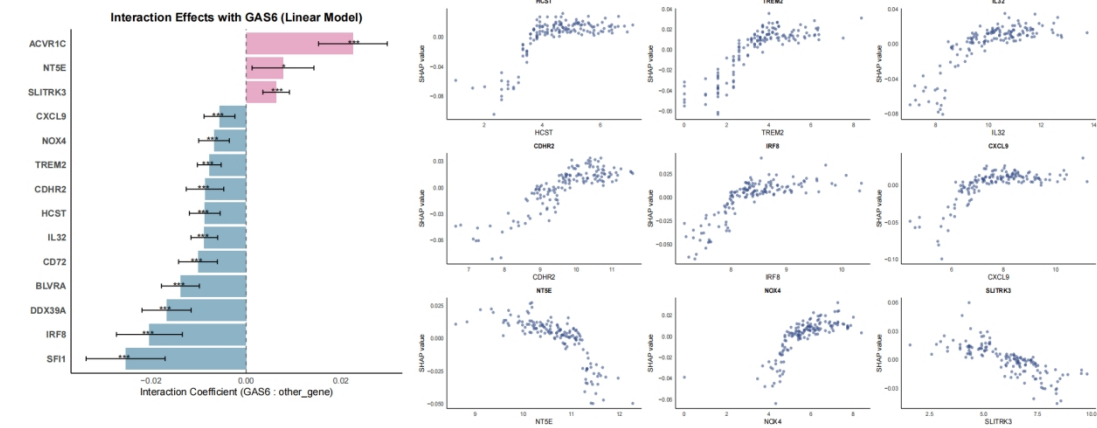
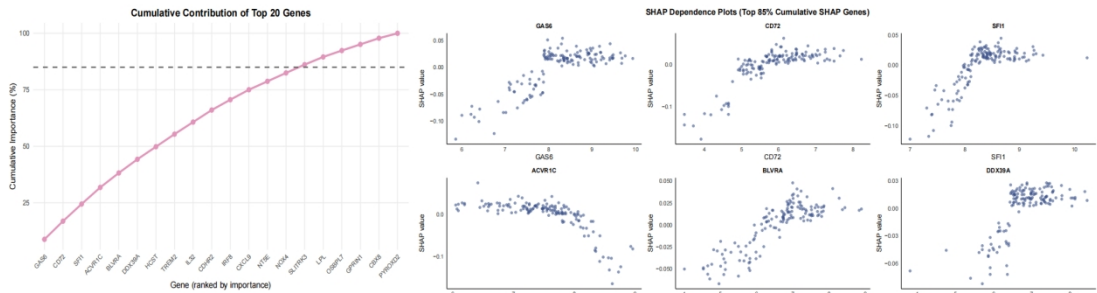
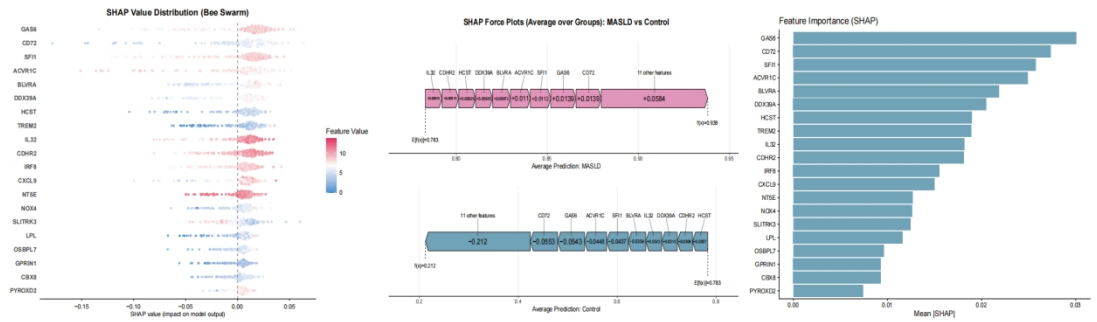


Figure 2. Interpretability analysis of the machine learning model and fibrosis-related characteristics of key genes.

(A) Beeswarm distribution plot of SHAP values for the top 20 most important feature genes. The x-axis represents SHAP values, and colors indicate the expression level of the corresponding gene (red for high expression, blue for low expression).

(B) Bar plot of feature importance, showing the mean absolute SHAP values of feature genes, with GAS6 exhibiting the highest global contribution.

(C) Group-averaged SHAP force plot, showing the average predicted probability and the contribution direction and magnitude of major features in the MASLD group and the control group, respectively.

(D) Cumulative importance contribution curve of key genes. The horizontal dashed line clearly marks that the cumulative contribution of the top 15 core genes exactly reaches the critical threshold of 80%.

(E) SHAP dependence plots of the top 15 core feature genes, revealing the nonlinear dependence between gene expression levels and their SHAP contribution values. The dependence plot of GAS6 demonstrates a distinct threshold effect: after expression exceeds approximately 8.0, the SHAP contribution rapidly shifts from negative to positive, accompanied by a saturation plateau of the driving effect.

(F) Bar plot of interaction effects between GAS6 and other key feature genes, showing the regression coefficients and confidence intervals of interaction terms. GAS6 and ACVR1C exhibit the strongest positive interaction.

(G–H) Box plots showing the changes in key gene expression levels with liver fibrosis stage (from Control to F4) in the GSE135251 (G) and GSE162694 (H) datasets, respectively. Spearman correlation coefficients and corresponding P values are annotated in the plots, and the superimposed trend lines reflect the linear change trend of gene expression along fibrosis progression, clearly demonstrating the upward trend of GAS6 and the downward trend of ACVR1C.

Single-Cell Transcriptomic Atlas Reveals Cellular Heterogeneity of the Liver Microenvironment

To dissect the cellular origin of core feature genes at the single-cell level, systematic clustering and annotation were performed on liver single-cell transcriptomic data. Seven major cell subsets were identified via UMAP dimensionality reduction, including hepatocytes, macrophages, hepatic stellate cells, endothelial cells, T cells, B cells, and cholangiocytes. The average expression levels of marker genes exhibited highly consistent subset specificity in the heatmap (Figures 3A and 3B).

Cell proportion donut plots and Ro/e enrichment analysis showed that under the MASLD condition, the relative abundance of hepatocytes decreased significantly, while the proportions of immune cells and other microenvironmental cell subsets showed an upward trend (Figures 3C and 3D). Cross-dataset phenotypic mapping based on the Scissor algorithm indicated that Scissor-positive cells were predominantly enriched in macrophages, with the remaining cells mainly distributed in hepatocyte subsets (Figures 3E and 3F). Count statistics of differentially expressed genes (DEGs) across cell subsets further revealed that hepatocytes underwent the most dramatic transcriptomic alterations, with the number of DEGs ranking first among all cell types (Figures 3G and 3H).

Expression distribution analysis of core predictive genes showed that GAS6 expression was significantly higher in MASLD samples than in control samples, while ACVR1C expression showed the opposite trend. Spatial expression feature plots further demonstrated that GAS6 presented a ubiquitous pan-expression pattern across all cell subsets, whereas ACVR1C expression was highly restricted to hepatocyte subsets. These specific expression patterns at the single-cell level are mutually consistent with the interaction effects and fibrosis progression trends revealed by the machine learning model (Figures 3I to 3K).

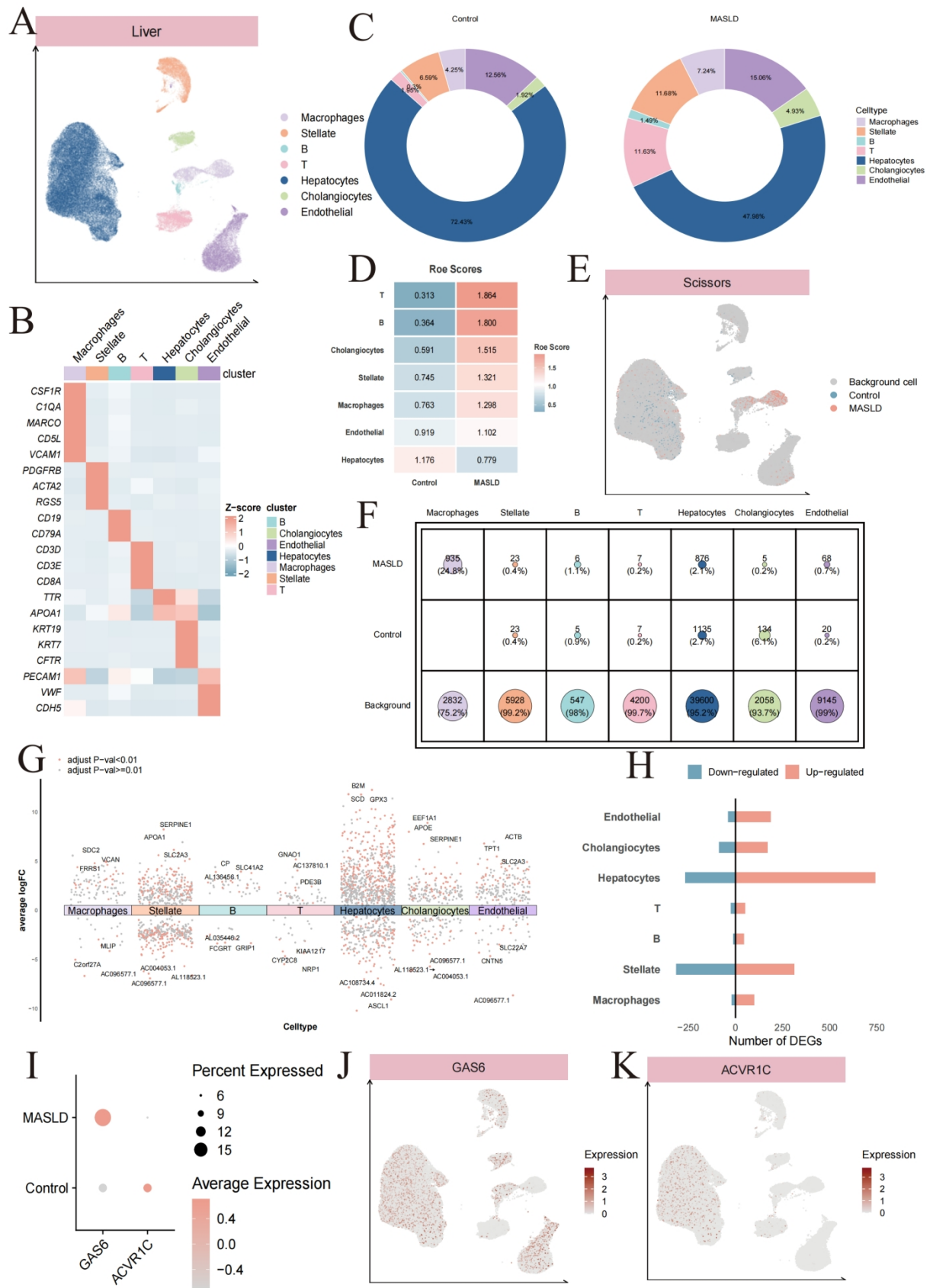


Figure 3. Construction of single-cell transcriptomic atlas and cell-specific expression characteristics of core genes.

(A) UMAP dimensionality reduction plot of liver single-cell transcriptomic data.

(B) Heatmap of average expression levels of canonical marker genes across cell subsets, with Z-score normalization showing subset-specific expression patterns.

(C) Donut plot of relative proportion distribution of cell subsets in the control and MASLD groups.

(D) Heatmap of Ro/e enrichment scores of cell subsets between the two groups, with red indicating higher enrichment in the corresponding group.

(E) Mapping distribution of MASLD-associated cells (pink) and control-associated cells (blue) screened by the Scissor algorithm integrating three bulk datasets, projected on the single-cell UMAP.

(F) Bubble plot of the distribution of Scissor-identified MASLD-associated, control-associated and background cells across cell subsets, with absolute counts and percentages annotated.

(G) Volcano plots of differentially expressed genes between MASLD and control groups in each cell subset, showing the magnitude of transcriptomic changes across different cell types.

(H) Bar plot of the numbers of significantly upregulated and downregulated differentially expressed genes in each cell subset.

(I) Bubble plot of average expression of core feature genes GAS6 and ACVR1C in the MASLD and control groups.

(J–K) Expression feature plots of GAS6 (J) and ACVR1C (K) on the single-cell UMAP dimensionality reduction plot, with color intensity of red representing gene expression levels.

Metabolic Reprogramming of Hepatocyte Subsets and Pseudotime Evolution Dynamics Reveal Mechanisms of Compensatory Imbalance in MASLD

To dissect the evolutionary pattern of hepatocyte functional states during MASLD progression, we performed re-clustering combined with functional pathway activity scoring and Gene Ontology (GO) enrichment analysis, and identified nine hepatocyte subsets with distinct metabolic phenotypes (Figures 4A to 4C).

Under disease conditions, the hepatocyte population exhibited a characteristic shift from metabolic homeostasis to injury stress. The C3, C4, and C6 subsets, which represent high mitochondrial metabolism and stemness-associated repair function, were significantly reduced; the proportion of the C6 subset dropped from 12.6% to 2.95%, indicating a compensatory collapse of mitochondrial oxidative phosphorylation function. Conversely, the C7 and C8 subsets, which represent stress-induced inflammation and translational burst, increased from less than 2% to over 26%, directly driving the progression from MASLD to MASH and fibrosis (Figure 4D).

Further integration of the spatial expression distribution of GAS6 and ACVR1C revealed that GAS6 presented a ubiquitous expression pattern across all hepatocyte subsets, whereas ACVR1C expression was highly restricted and markedly absent in the Hepatocyte_Stress and Hepatocyte_Translation subsets (Figures 4E and 4F). To dynamically track molecular regulatory events during the aforementioned metabolic transition, pseudotime analysis was performed. Three hepatocyte pseudotime evolutionary trajectories reflecting distinct cell fate directions were constructed (Figures 4G to 4I). In trajectory 1 and trajectory 3, cells underwent a typical metabolic decompensation process: GAS6 expression was significantly upregulated along pseudotime, while ACVR1C was progressively downregulated. At specific pseudotime nodes, their expression levels changed markedly and crossed the threshold, marking the complete transition of hepatocytes from homeostatic metabolism to an inflammatory stress state.

In sharp contrast, cells in trajectory 2 exhibited features of successful metabolic compensation. GAS6 and ACVR1C maintained intertwined expression patterns in dynamic equilibrium throughout the entire pseudotime evolution process, without evident dominant threshold crossing. This finding suggests that cells in this branch successfully resisted excessive metabolic stress via coordinated molecular networks.

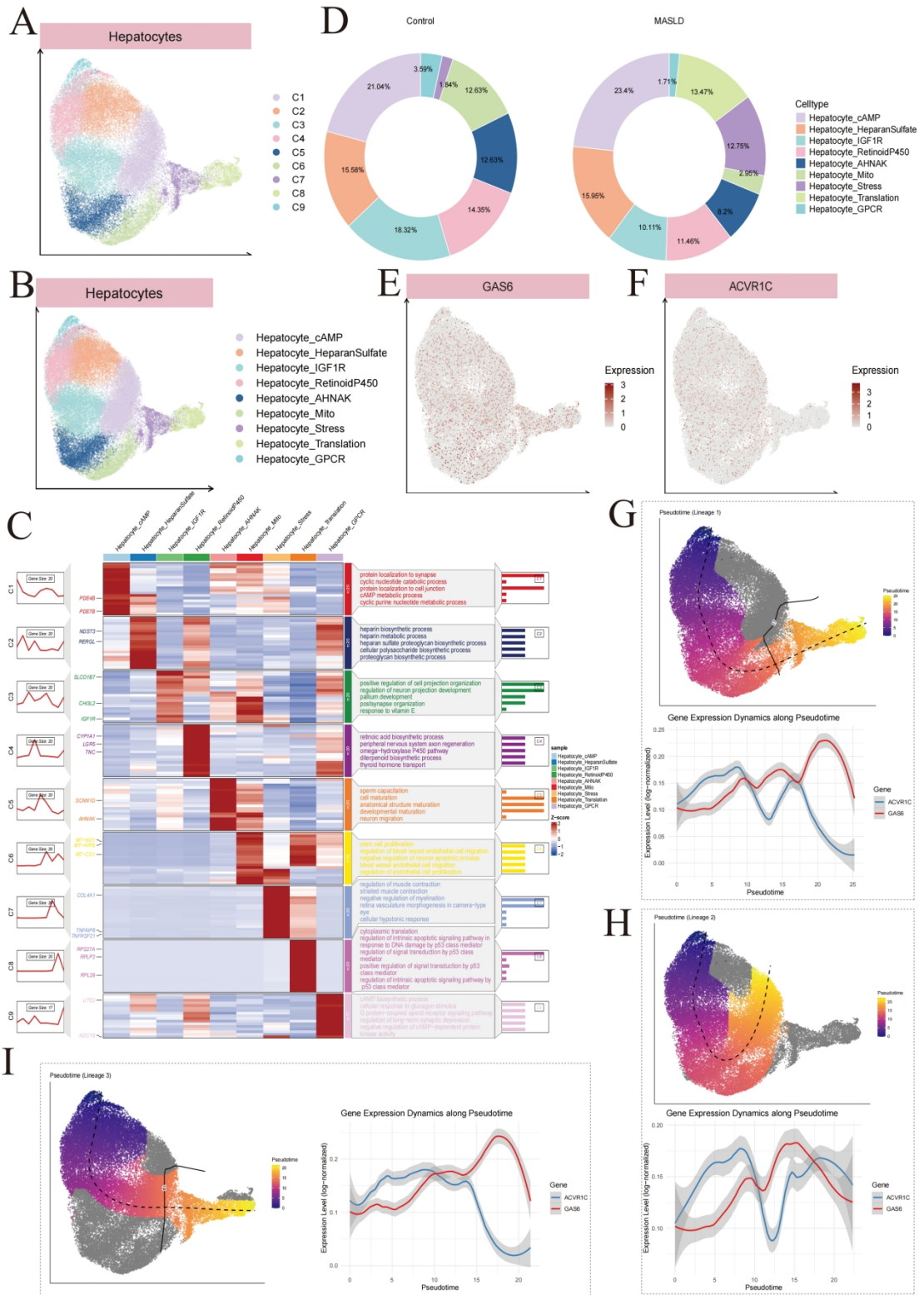


Figure 4. Functional heterogeneity of hepatocyte subsets and pseudotime metabolic evolution dynamics of core genes.

(A–B) UMAP dimensionality reduction plots of hepatocyte subsets, showing nine hepatocyte subsets identified by original unsupervised clustering (C1–C9) and annotated based on functional pathway modules and GO enrichment, respectively.

(C) Combined plot of signature gene expression patterns, transcriptional trends and corresponding GO biological process enrichment across hepatocyte subsets, demonstrating the differential functional preferences of each subset in metabolism, stress response and signal transduction.

(D) Donut plot of relative proportion distribution of nine hepatocyte subsets in the control and MASLD groups, revealing MASLD-induced niche remodeling of the hepatocyte population from high mitochondrial metabolism and stemness reserve to stress-induced inflammation and translational burst.

(E–F) Expression distribution of core feature genes GAS6 and ACVR1C on hepatocyte UMAP dimensionality reduction plots, with red indicating relatively high expression.

(G–I) Three pseudotime trajectory branches of hepatocyte subsets constructed by Slingshot, reflecting distinct cell fate directions. The line graphs below show the locally weighted regression expression dynamics of GAS6 (red) and ACVR1C (blue) along pseudotime. Trajectory 1 and trajectory 3 represent "metabolic compensation failure", where GAS6 crosses the threshold and exceeds ACVR1C at specific pseudotime nodes, driving cells toward inflammation/stress. Trajectory 2 represents "metabolic compensation success", where the two genes maintain an intertwined state of dynamic equilibrium throughout the entire evolution process.

Hepatocyte Metabolic Module Reprogramming Reveals Key Antagonistic Regulation of GAS6 and ACVR1C on Detoxification Function

To uncover the intrinsic mechanism of hepatocyte metabolic remodeling during MASLD progression, eight coordinated metabolic modules were constructed based on KEGG and REACTOME pathways. Among them, M1 to M5 covered basal metabolic functions including amino acid catabolism, glycolipid metabolism, and nucleotide synthesis; M6 was defined as the stress-degradation and repair module; while M7 and M8 exhibited generally low and relatively marginalized activity (Figure 5A).

Direct comparison of metabolic profiles between the Hepatocyte_Stress and Hepatocyte_Mito subsets revealed that M6, M2, and M5 were significantly upregulated under stress conditions, whereas M4, M1, and M3 were highly enriched in the mitochondrial high-metabolism subset. This feature clearly delineates the metabolic reprogramming trajectory of hepatocytes shifting from mitochondrial oxidative phosphorylation-driven energy supply to injury repair and stress response (Figure 5B).

Spatial distribution patterns and pathway activity heatmaps further confirmed that M4, as a homeostatic detoxification module centered on cytochrome P450-mediated detoxification, steroid and bile acid metabolism, exhibited widespread high activity in healthy hepatocytes, while M6 was specifically activated in corresponding stress regions (Figures 5C to 5E and 5G).

Correlation analysis revealed a strong antagonistic regulatory relationship between core driver genes and the aforementioned metabolic modules: GAS6 expression level was strongly negatively correlated with the vast majority of detoxification and bile acid metabolism pathways in M4, and positively correlated with stress repair pathways in M6. Conversely, ACVR1C was strongly positively correlated with M4 pathways and negatively correlated with M6 pathways (Figures 5F and 5H).

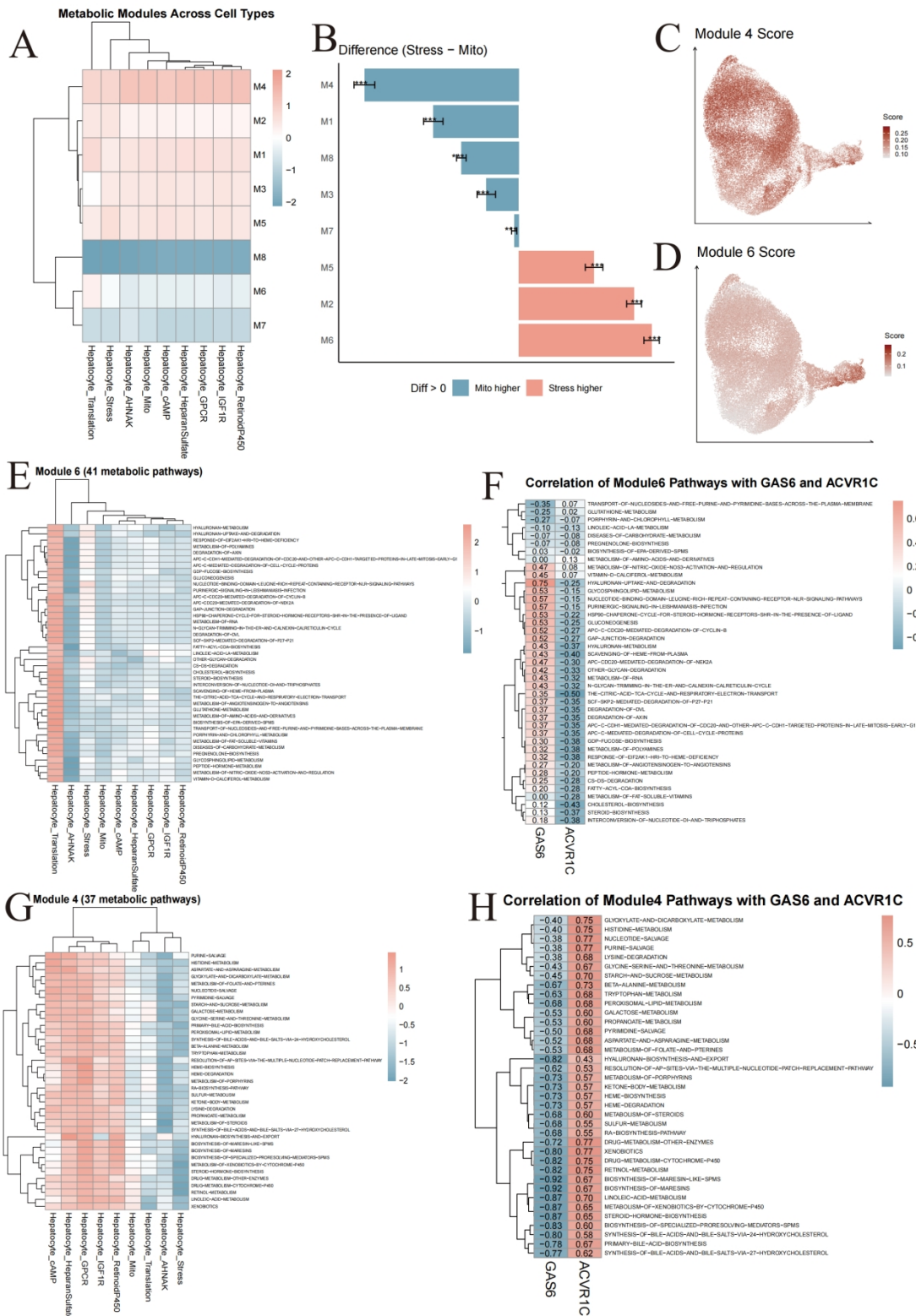


Figure 5. Division of hepatocyte metabolic modules and their functional association with GAS6 and ACVR1C.

(A) Heatmap of relative activity of eight metabolic modules divided by hierarchical clustering of KEGG and REACTOME metabolic pathways across nine hepatocyte subsets. M1 to M5 cover basal metabolism, and M6 represents stress-degradation and repair function.

(B) Bar plot of activity differences of each metabolic module between the Hepatocyte_Stress and Hepatocyte_Mito subsets. Red and blue indicate relatively higher module activity in the stress subset and mitochondrial subset, respectively.

(C–D) Spatial distribution patterns of the activity of metabolic module 4 and module 6 on hepatocyte UMAP dimensionality reduction plots, with redder color indicating higher module activity.

(E–F) Heatmaps of activity of 41 metabolic pathways in module 6 and 37 metabolic pathways in module 4 across nine hepatocyte subsets, showing the differential distribution patterns of stress repair pathways and homeostatic detoxification pathways.

(G–H) Spearman correlation heatmaps between metabolic pathway activity in module 6 and module 4 and core genes GAS6 and ACVR1C, respectively. Values in the plots represent correlation coefficients, and color maps the magnitude and direction of correlation.

Heterogeneity Landscape of Hepatic Macrophages

To systematically dissect the heterogeneity and functional remodeling of hepatic macrophages during MASLD progression, we accurately isolated the macrophage population from liver single-cell data and performed re-clustering, identifying 8 macrophage subsets with distinct functional phenotypes (Figure 6A).

Bubble plots and module score heatmaps validated the differential characteristics of each subset across functional modules, including Kupffer cell (TIMD4, VSIG4, CLEC4F, MARCO, CD5L), capsular and central vein (CX3CR1, ITGAX), monocyte-derived (CCR2, FCN1, S100A8, S100A9), lipid-associated (TREM2, GPNMB, SPP1, CD9), heme metabolism (HMOX1, FTH1, FTL), M1-like pro-inflammatory, M2-like reparative, and proliferation modules (Figures 6B and 6C).

Cell proportion donut plots and Ro/e enrichment analysis revealed significant niche remodeling of the intrahepatic macrophage population from homeostatic residence to disease-specific infiltration. The proportion of the Kupffer_M2_like homeostatic subset characterized by TIMD4 and VSIG4 was significantly decreased in MASLD, while heme-related macrophages were markedly expanded and showed prominent enrichment under disease conditions (Figures 6E and 6F).

GAS6 and its downstream TAM receptor tyrosine kinase family members AXL and MERTK were ubiquitously expressed in multiple macrophage subsets, while AXL and MERTK exhibited stronger region-specific enrichment in particular subsets (Figures 6G to 6J).

To further track cell transformation dynamics during the aforementioned population remodeling, Slingshot pseudotime trajectories were constructed. Trajectory 5 explicitly represented the transition path from homeostatic Kupffer_M2_like cells to heme-related macrophages. The expression level of MERTK was sharply upregulated at the late pseudotime stage, which was highly consistent with the specific activation of TAM receptors in the heme-related macrophage subset (Figures 6K and 6L).

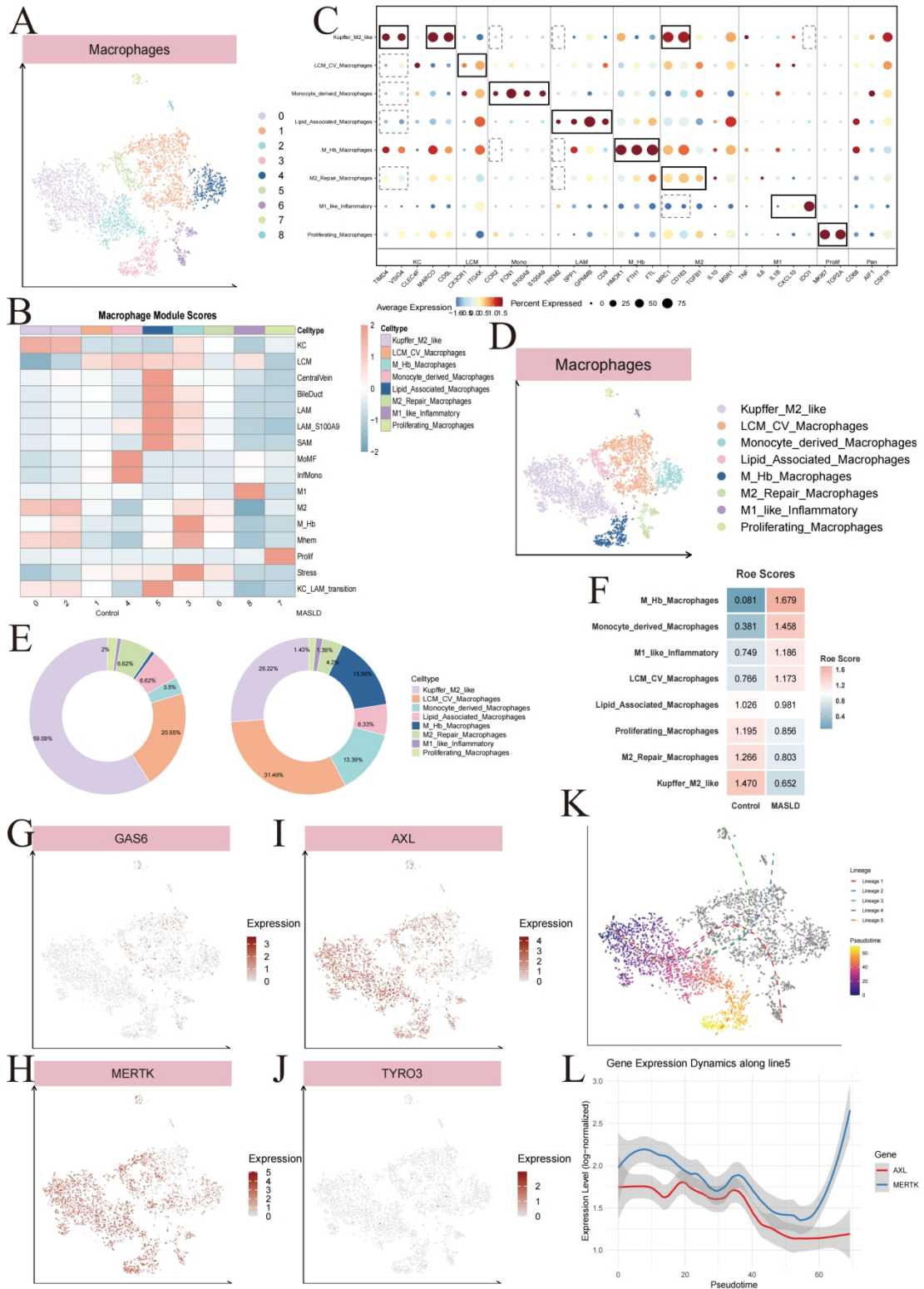


Figure 6. Identification of hepatic macrophage subsets and their niche remodeling and pseudotime dynamics in MASLD.

- (A) UMAP dimensionality reduction plot of original unsupervised clustering of macrophages.
- (B) Bubble plot of representative functional modules and marker genes across macrophage subsets. Solid lines and solid boxes indicate positive marker sets, while dashed lines and dashed boxes indicate negative marker sets.
- (C) Standardized heatmap of activity scores of eight macrophage functional modules in the corresponding annotated subsets.
- (D) UMAP dimensionality reduction plot of eight annotated macrophage subsets based on the "combined module scoring and marker gene" strategy.
- (E) Donut plot of relative proportion distribution of eight macrophage subsets in the control and MASLD groups.
- (F) Heatmap of Ro/e tissue enrichment index of macrophage subsets between the two groups. Color shift toward one side indicates higher enrichment of the subset in the corresponding group.
- (G–J) Expression distribution of core driver gene GAS6 and TAM receptor family members AXL, MERTK, and TYRO3 on macrophage UMAP dimensionality reduction plots, with red indicating relatively high expression.
- (K) Pseudotime trajectory branches of macrophage subsets constructed by the Slingshot algorithm, with color representing the pseudotime evolution process.
- (L) Locally weighted regression expression dynamics curves of AXL and MERTK along the specific differentiation trajectory (trajectory 5), showing marked upregulation of MERTK at the trajectory end during the transition from Kupffer_M2_like to heme-related macrophages.

Enhanced GAS6-Driven Hepatocyte-Macrophage Communication and Remodeling of Downstream Regulatory Networks

To dissect aberrant changes in cell-cell communication in the MASLD microenvironment, systematic cell-cell interaction analysis was performed between the control and MASLD groups. The overall communication strength and number of interactions were increased in MASLD (Figure 7A). Differential interaction heatmaps between cell subsets showed that the number and strength of interactions of hepatocyte subsets (particularly Hepatocyte_Stress and Hepatocyte_Translation) were upregulated in MASLD (Figure 7B).

Comparative analysis of relative information flow across signaling pathways revealed that the GAS signaling pathway was an exclusive pathway in the MASLD group (Figure 7C). Further dissection of specific ligand-receptor pairs in the GAS pathway showed that the communication probabilities of GAS6 with MERTK and AXL were higher in the MASLD group, indicating that GAS6 exerts signaling effects mainly by activating MERTK and AXL receptors on the macrophage surface (Figure 7D). Chord diagrams of the GAS signaling pathway intuitively demonstrated that hepatocyte subsets (Hepatocyte_Stress, Hepatocyte_IGF1R, and Hepatocyte_cAMP) served as major senders and broadly transmitted signals to multiple macrophage subsets (Figure 7E).

NicheNet-based target gene prediction further constructed the downstream signaling regulatory network of GAS6. This network clearly delineated a cascade of molecular events: upon binding to its receptors AXL and MERTK, GAS6 activates key transcription factors including STAT5B, STAT3, LMO4, GRB2, and PIK3R1, which in turn regulate a panel of inflammation- and stress-related target genes (Figure 7F). Taken together, these results closely link the macroscopic enhancement of cell-cell communication with the microscopic activation of the GAS6-AXL/MERTK signaling axis, and systematically demonstrate the pivotal driving role of GAS6 as a core ligand in the MASLD-associated hepatocyte-macrophage communication axis.

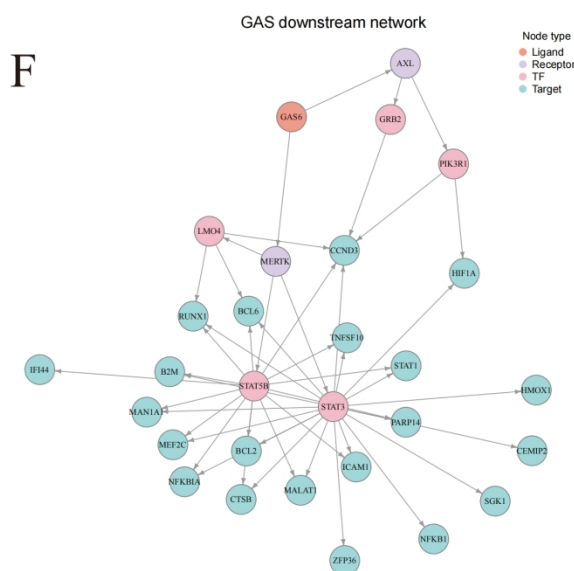
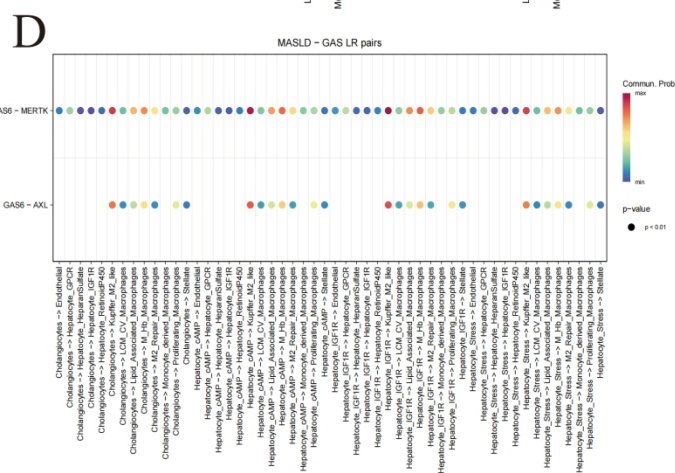
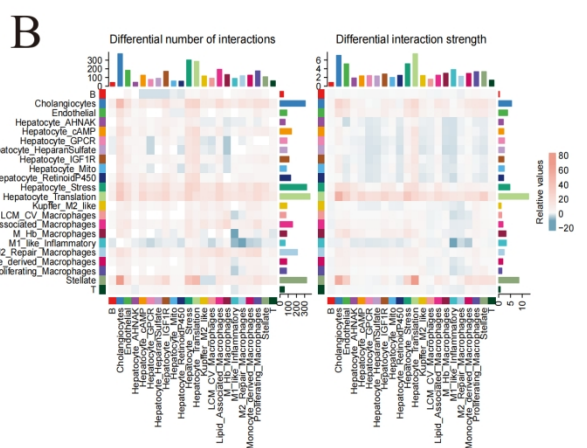


Figure 7. Enhancement of cell-cell communication network and GAS6-driven downstream regulatory mechanisms in MASLD

(A) Bar plots comparing the total number of cell-cell interactions (right) and total communication strength (left) between the control and MASLD groups. Blue represents the control group, and red represents the MASLD group.

(B) Heatmaps showing the differential number of interactions (left) and differential interaction strength (right) between cell subsets in the MASLD group compared with the control group. Red indicates upregulated interactions, and blue indicates downregulated interactions.

(C) Stacked bar plot of relative information flow of each signaling pathway between the two groups. Blue and red represent the relative contribution proportion of the pathway in the control and MASLD groups, respectively. The dashed box highlights the GAS signaling pathway.

(D) Bubble plot of communication probabilities of ligand-receptor pairs in the GAS pathway in the MASLD group. The x-axis represents each target cell type, and both color and point size reflect the level of communication probability.

(E) Chord diagram network of the GAS signaling pathway in the MASLD group, showing the signal transmission relationship from hepatocyte subsets as the major senders to macrophages and other microenvironmental cells.

(F) Network diagram of GAS6 downstream signaling regulation constructed based on NicheNet. Different node types represent ligands, receptors, transcription factors, and target genes, respectively, and arrows indicate the direction of cascade signal transduction.

Cooperative Remodeling of Macrophage Transcriptional Regulatory Modules and Validation of GAS6 Downstream Target Gene Network

To systematically dissect the upstream transcriptional regulatory mechanisms underlying MASLD-driven macrophage phenotypic transition, we constructed a regulon activity atlas of macrophages based on PySCENIC. Clustering heatmap of regulon similarity indices identified seven highly cooperative regulon modules. The transcriptional regulatory characteristics of different modules were systematically demonstrated via bubble plots of the top 3 transcription factor (TF) activity per module and heatmaps of module activity across cell subsets (Figure 8A).

Differential activity heatmap of modules across disease groups showed that the M1 module exhibited the largest activity difference between the MASLD and control groups, and its activity in the M_Hb_Macrophages subset was significantly higher than in all other cell subsets, indicating that this module is the core cooperative unit driving transcriptional reprogramming of heme-related macrophages.

Bubble plots of regulon specificity scores revealed the most characteristic transcription factors for each subset. Specifically, HNF4A showed the most prominent enrichment in the M2_Repair_Macrophages subset; RFX5 was most significantly enriched in M1-like inflammatory subsets and LCM_CV_Macrophages; MAF was predominantly enriched in the Kupffer_M2_like subset; E2F2 exhibited the highest enrichment in proliferative macrophages; VDR was most enriched in monocyte-derived macrophages; STAT3 showed the most significant enrichment in M_Hb_Macrophages; and ZBTB33 was most prominently enriched in lipid-associated macrophages (Figure 8B).

Line plots of regulon specificity scores for the M_Hb_Macrophages subset further confirmed that STAT3, BHLHE40, JUND, JUNB, and KLF5 ranked the top in this subset (Figure 8C). For the M1 transcriptional module, network diagrams showed that CEBPB, JUNB, FOSL2, BHLHE40, STAT3, HIF1A, and NFkB1 constituted the core transcriptional hubs with the highest internal connectivity within the module. These key transcription factors exhibited dense positive co-expression correlations and were tightly associated with the core stress response transcriptional regulation of the M_Hb_Macrophages subset, further reinforcing the core role of this module in driving macrophage stress and metabolic adaptation phenotypes (Figure 8D).

By further filtering the GAS6 downstream network with active regulons identified by SCENIC, we precisely constructed the GAS6-MERTK-STAT3-target gene cascade regulatory network, which revealed the complete molecular signaling axis through which GAS6 activates the MERTK receptor on the macrophage surface, thereby triggering the STAT3 transcription factor and driving the expression of downstream inflammation and stress response target genes (Figure 8E).

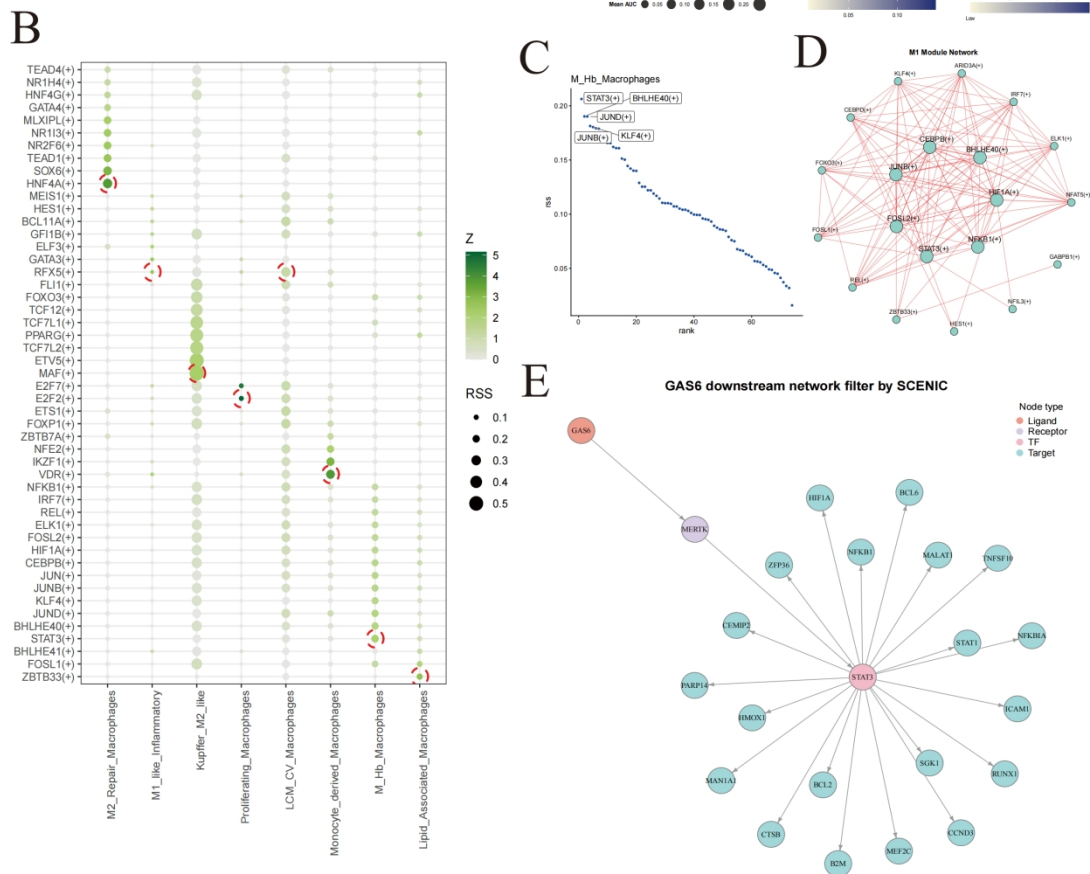


Figure 8. Cooperative remodeling of macrophage transcriptional regulatory modules and validation of GAS6 downstream target gene network

(A) Clustering heatmap of macrophage regulon similarity indices, showing the division of seven cooperative regulatory modules. To its right are the activity bubble plots of the top three transcription factors per module, the activity heatmap of modules across cell subsets, and the activity difference heatmap between the MASLD and control groups, in sequence.

(B) Bubble plot of regulon specificity scores. Z-score magnitude and color represent the specific enrichment degree of transcription factors in each cell subset, and red dashed boxes highlight the most characteristic core transcription factors of each subset.

(C) Line plot of regulon specificity score ranking in the M_Hb_Macrophages subset.

(D) Network diagram of co-expression correlations between transcription factors within the M1 regulatory module, with red edges representing positive correlations.

(E) Network diagram of GAS6 downstream signaling regulation filtered by SCENIC active regulons. Different node types represent ligands, receptors, transcription factors, and target genes, respectively, and arrows indicate the direction of cascade signal transduction.

Spatial Transcriptomics Reveals Enhanced Macrophage Infiltration and Significant Co-localization of Gas6-MERTK in MASLD Liver

To dissect cell population changes and spatial activation of key signaling axes induced by MASLD in situ, we performed cell composition deconvolution analysis on spatial transcriptomic sections. Spatial cell type pie charts showed that liver sections in the control group were mainly composed of hepatocytes, while in the MASLD group, the proportion of hepatocytes was relatively decreased, accompanied by obvious infiltration of immune cells dominated by macrophages in the tissue (Figure 9A).

Spatial macrophage proportion plots and stacked bar plots across samples further confirmed that the proportion of macrophages in each sample of the MASLD group was significantly higher than that in the control group, verifying the massive expansion and spatial enrichment of macrophages in the liver microenvironment under disease conditions (Figures 9B and 9C).

To explore the spatial association between the infiltrating macrophages and the core disease signaling pathway, we performed spatial co-localization analysis of GAS6 and its receptor MERTK. Spatial distribution maps of co-localization showed that Gas6-Mertk co-localization scores were generally at low levels in the control group, but presented prominent localized high-signal clusters in the MASLD group (Figures 9D and 9E). Empirical cumulative distribution function (ECDF) analysis further showed that the co-localization score distribution curve of the MASLD group was shifted rightward as a whole, exhibiting a broader distribution at higher score ranges compared with the control group (Figure 9F).

Box plots from Wilcoxon rank-sum test also consistently confirmed that the Gas6-MERTK co-localization score in the MASLD group was significantly higher than that in the control group, indicating significant spatial cooperative activation of the GAS6-MERTK signaling axis in the MASLD liver microenvironment (Figure 9G).

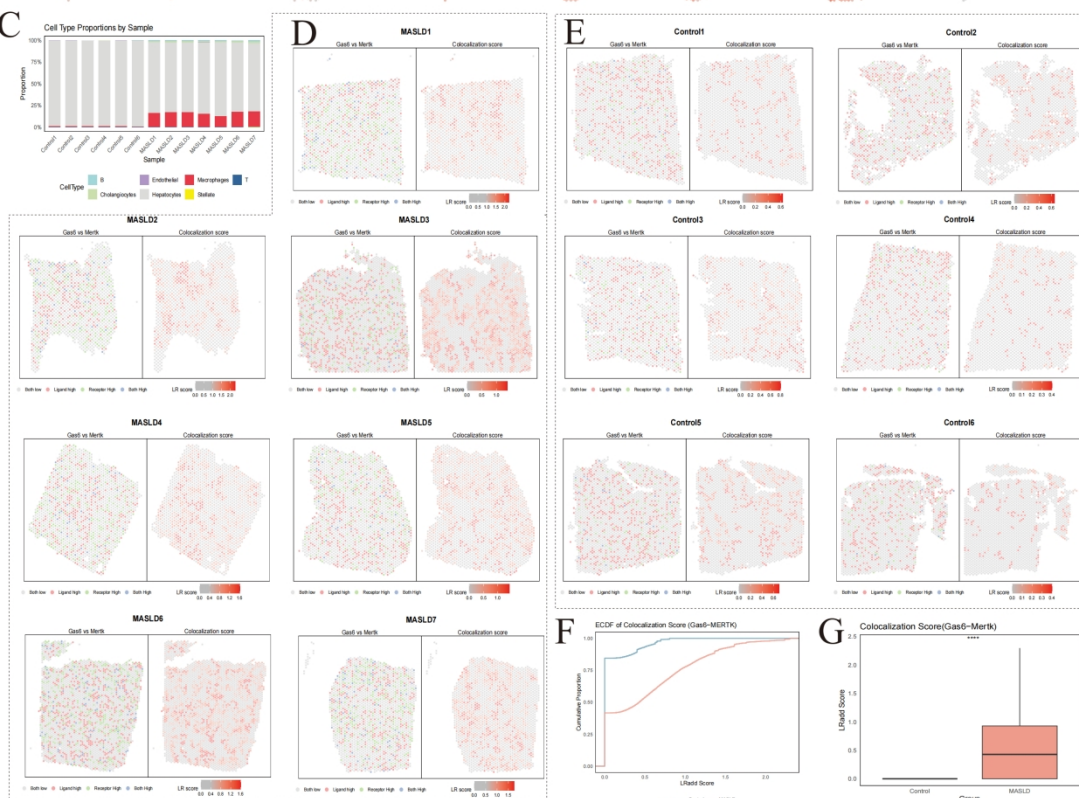
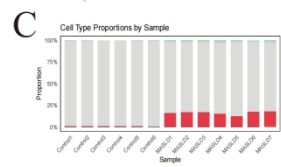
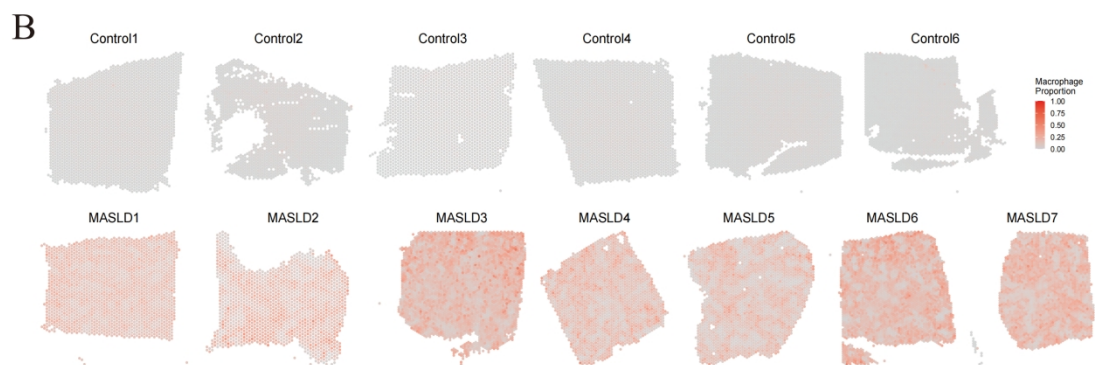


Figure 9. Spatial cell composition remodeling of MASLD liver and enhanced spatial co-localization of the GAS6-MERTK signaling axis.

(A) Pie charts of spatial cell type composition based on deconvolution by the CARD algorithm, showing the spatial distribution and proportion of each cell type in sections from 6 control samples (upper row) and 7 MASLD samples (lower row), respectively.

(B) Continuous distribution feature plot of macrophage proportion across spatial sections, with red intensity representing the level of macrophage proportion at each spatial point.

(B) Stacked bar plot of relative proportions of cell types in each sample, intuitively comparing the structural differences in immune cell and hepatocyte proportions between the control and MASLD groups.

(D–E) Spatial co-localization analysis results of GAS6 and MERTK in sections from the MASLD group (D) and control group (E). The left subplots show the spatial coverage of high-expression regions of the ligand and receptor, and the right subplots show the spatial distribution of co-localization scores in continuous color.

(F) Empirical cumulative distribution function curves of GAS6-MERTK co-localization scores. The blue curve represents the control group, and the red curve represents the MASLD group.

(G) Box plot of GAS6-MERTK co-localization scores between the control and MASLD groups.

Discussion

Liver fibrosis represents the primary determinant of long-term outcomes in patients with MASLD, yet the molecular switches that drive the transition from compensatory steatosis to progressive fibrogenesis remain poorly characterized. Building on the integration of machine learning-based transcriptome screening, single-cell atlas, spatial transcriptomics, and transcriptional regulatory network analysis, this study identifies the ACVR1C-GAS6 antagonistic axis as a critical regulatory hub governing hepatocyte metabolic homeostasis and macrophage functional polarization, and demonstrates that disruption of this axis is a key molecular event underlying MASLD progression from compensated steatosis to decompensated fibrosis.

As a type I TGF- β superfamily receptor, ACVR1C has been previously reported to regulate DNL in hepatocytes via the SREBP-1c/FAS/ACC axis[25], which was corroborated in our machine learning-based screening identifying ACVR1C as a key initiating factor of MASLD. Notably, single-cell metabolic module analysis revealed a previously unrecognized role of ACVR1C-GAS6 axis in maintaining hepatocyte detoxification homeostasis: ACVR1C was strongly positively correlated with the M4 detoxification module centered on cytochrome P450-mediated metabolism, steroid and bile acid turnover, and negatively correlated with the M6 stress response module. In parallel, GAS6 exhibited an opposite expression trajectory along fibrosis progression, with a clear expression threshold effect marking the onset of fibrogenesis. The inverse phase-specific dynamics of ACVR1C and GAS6 suggest that they operate as a functional antagonistic pair: ACVR1C serves as a homeostatic gatekeeper to constrain aberrant GAS6 elevation and suppress stress pathway activation

Beyond intrahepatocyte metabolic reprogramming, our study provides novel insights into hepatocyte-macrophage crosstalk in MASLD. Using Scissor-based cross-dataset phenotype mapping, we identified hepatocytes and macrophages as the two core cell populations involved in disease progression. Within the macrophage subset, we characterized eight functionally distinct subsets, and identified heme-related macrophages as a prominently expanded subset in MASLD. This subset was characterized by high expression of HMOX1 and the ferritin subunits FTH1/FTL, while retaining partial Kupffer cell markers (low-to-moderate TIMD4/VSIG4 positivity) and LAM markers (SPP1/GPNMB). It may represent Kupffer cells undergoing phenotypic transition in a stress microenvironment. Previous studies of hepatic macrophages in MASLD have largely focused on classical M1/M2 polarization and lipid-associated macrophages[17]; our identification of the heme-

related macrophage subset expands the landscape of macrophage functional heterogeneity in MASLD. We further demonstrated that GAS6 derived from stressed hepatocytes signals to macrophages via MERTK and AXL receptors, with the highest communication strength observed in heme-related macrophages. Spatial transcriptomic analysis in a mouse MASLD model further confirmed enhanced spatial co-localization of Gas6 and Mertk in diseased liver tissues, providing in situ evidence for this ligand-receptor interaction.

Mechanistically, we delineated the downstream transcriptional cascade mediating GAS6-driven macrophage polarization. Using ySCENIC analysis, we identified STAT3 as the core transcription factor downstream of MERTK activation in heme-related macrophages. STAT3 is a well-documented regulator of macrophage polarization and profibrotic mediator secretion, including TGF- β , PDGF, and CTGF, which in turn drive myofibroblast activation and ECM deposition[26,27]. Integrating these findings with the established role of GAS6 in directly activating HSCs via AXL, our results support a dual pathway model of GAS6-driven liver fibrosis: GAS6 not only directly stimulates HSC activation and ECM production, but also indirectly amplifies fibrogenesis by promoting a profibrotic macrophage phenotype via the MERTK-STAT3 axis. This dual mechanism complements the current understanding of cell-cell interaction networks in MASLD fibrosis, and highlights the central role of hepatocyte-derived GAS6 in orchestrating intrahepatic cellular crosstalk.

From a translational perspective, the findings of this study have several clinical implications aligned with unmet needs in MASLD management. First, the threshold effect of circulating GAS6 supports its development as a non-invasive blood-based biomarker for staging liver fibrosis and identifying patients at risk of progression, consistent with previous reports[28]. In particular, the ACVR1C/GAS6 expression ratio may serve as a more robust stratification biomarker than either gene alone, to predict the transition from compensated steatosis to progressive fibrosis. Second, the ACVR1C-GAS6 axis represents a promising therapeutic target. ACVR1C agonists may help preserve hepatocyte metabolic homeostasis and detoxification function in early-stage MASLD, while GAS6 neutralizing antibodies or MERTK inhibitors may disrupt the profibrotic signaling cascade in established disease. Combined targeting of both hepatocyte ACVR1C and macrophage MERTK may exert synergistic antifibrotic effects, representing a potential combinatorial therapeutic strategy.

In summary, this study proposes a model in which dysregulation of the ACVR1C-GAS6 antagonistic axis drives metabolic decompensation and liver fibrosis in MASLD. Under homeostatic conditions, ACVR1C maintains hepatocyte lipid

metabolism and detoxification function, and restrains aberrant GAS6 expression to prevent excessive stress responses. Under disease conditions, downregulated ACVR1C and upregulated GAS6 disrupt this balance, and promote fibrogenesis through both intra-hepatocyte metabolic reprogramming and hepatocyte-macrophage paracrine crosstalk. This model provides a new molecular framework for understanding MASLD progression, and identifies candidate biomarkers and therapeutic targets for clinical translation. Future studies should focus on validating the non-canonical pathway by which ACVR1C regulates hepatocyte detoxification, characterizing the functional role of heme-related macrophages in MASLD, and evaluating the clinical utility of the ACVR1C-GAS6 axis in prospective patient cohorts.

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Availability of data and materials

The datasets supporting the findings of this study are publicly available in the NCBI Gene Expression Omnibus (GEO) repository (accession numbers: GSE162694, GSE135251, GSE126848, GSE330266, GSE248077). No-new experimental sequencing data were generated for this study. Relevant analysis scripts can be obtained from the corresponding author upon reasonable request.

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Authors' contributions: Shu-Ming Chen and Zhi-Hao Zeng contributed equally to this work as co-first authors. Shu-Ming Chen was responsible for data curation, formal bioinformatic analysis, data processing, and figure visualization. Zhi-Hao Zeng was responsible for study conceptualization, result interpretation, manuscript drafting, and literature review. Min Zhang and Xiao Liu contributed equally to this work as co-corresponding authors. Min Zhang supervised the overall research project, provided critical intellectual guidance, and revised the manuscript thoroughly for important academic content. Xiao Liu participated in study design, validated the analytical results, and reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

All data analyzed in this study were obtained from publicly available Gene Expression Omnibus (GEO) datasets. All original datasets were published with completed ethical review and informed consent from participants. As this work constitutes secondary analysis of de-identified public data, no additional ethics approval or consent to participate was required.

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Competing interests

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