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chenxuan li

The First Affiliated Hospital of Kunming Medical University

dedong yuan

The First Affiliated Hospital of Kunming Medical University

qinglei chen

The First Affiliated Hospital of Kunming Medical University

Jiyuan Shi

The First Affiliated Hospital of Kunming Medical University

Li Jin

The First Affiliated Hospital of Kunming Medical University

Zongrui Jin

The First Affiliated Hospital of Kunming Medical University

Deling Wei

The First Affiliated Hospital of Kunming Medical University

Zhong Zeng

The First Affiliated Hospital of Kunming Medical University

Zhuo Cheng

The First Affiliated Hospital of Kunming Medical University

Hanfei Huang

huanghanfei@kmmu.edu.cn

The First Affiliated Hospital of Kunming Medical University

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**Single-cell regulatory genomics reveals coordinated myeloid and T-cell
reprogramming in liver cirrhosis**

Chenxuan Li^{1†}, Dedong Yuan^{1†}, Qinglei Chen¹, Jiyuan Shi¹, Li Jin¹, Zongrui Jin¹,
Deling Wei¹, Zhong Zeng¹, Zhuo Cheng^{1,*}, Hanfei Huang^{1,*}

¹Organ Transplantation Center, First Affiliated Hospital of Kunming Medical
University, 295 Xichang Road, Kunming 650032, Yunnan, China

[†] These authors have contributed equally to this work and share first authorship

*Corresponding author

Hanfei Huang (huanghanfei@kmmu.edu.cn);

Zhuo Cheng (zhuocheng1995@126.com)

Abstract

Liver cirrhosis involves immune dysfunction, yet how immune-cell states are coordinated with fibrosis remains incompletely resolved. We performed single-cell RNA-seq on liver tissues from three patients with cirrhosis and three histologically non-cirrhotic controls, and analyzed 43,745 high-quality cells using cell-state annotation, SCENIC regulon inference, pseudotime reconstruction, and CellChat ligand–receptor modeling. Cirrhotic livers showed depletion of Mono1 and expansion of inflammatory macrophages and regulatory T cells. SCENIC revealed dysregulated YY1 and ETV6 regulon activity in monocyte/macrophage states, altered EOMES/KLF6 programs in cytotoxic T cells, and increased EP300/SP3 in regulatory T cells. Pseudotime indicated disease-associated state-occupancy shifts, while CellChat suggested fibrosis-biased ligand–receptor signaling. In two mouse injury models, YY1 increased in F4/80+ macrophages and EOMES decreased in CD8+ T cells. These findings are hypothesis-generating due to limited donor numbers and will require donor-level and functional validation.

Keywords: Liver cirrhosis; Single-cell RNA sequencing; Regulatory genomics;

Macrophage; T cell; Transcription factor; Cell–cell communication.

Introduction

Liver cirrhosis is the end stage of chronic liver disease, resulting from sustained inflammation and progressive fibrogenesis driven by diverse etiologies including hepatitis B/C infection, alcohol abuse, and non-alcoholic steatohepatitis (NASH)[1, 2]. It is characterized by excessive extracellular matrix (ECM) deposition, regenerative nodule formation, and portal hypertension, ultimately leading to liver failure. Currently, liver transplantation remains the only curative treatment for decompensated cirrhosis, yet its applicability is limited by organ availability and post-transplant complications[3, 4]. A deeper understanding of the cellular and molecular mechanisms underlying cirrhosis pathogenesis is therefore urgently needed[5].

The progression of cirrhosis involves a complex interplay between hepatocyte injury, activation of fibrogenic hepatic stellate cells (HSCs), and a dysregulated immune response[6, 7]. Innate and adaptive immune cells are pivotal in this process. Monocytes and macrophages are central players, contributing to both the initiation and resolution of fibrosis through pro-inflammatory cytokine secretion, HSC activation, and ECM remodeling [8, 9]. Similarly, T cells can exert dual roles: CD8+ cytotoxic T cells and Th1 cells may promote inflammation and injury, while regulatory T cells (Tregs) and certain tissue-resident memory T cells can modulate immune responses and even facilitate fibrosis resolution[10-15]. However, the extensive heterogeneity within these populations and the precise transcriptional programs that dictate their functional states in human cirrhosis are not fully understood.

The advent of single-cell RNA sequencing (scRNA-seq) has revolutionized our ability to dissect cellular heterogeneity and characterize gene expression at unprecedented resolution [16-19]. Previous scRNA-seq studies in liver disease have provided invaluable insights, such as revealing zonation alterations in liver sinusoidal endothelial cells (LSECs) in cirrhosis, identifying a pro-fibrotic TREM2+CD9+ macrophage subpopulation [20-25], and delineating distinct immune ecosystems in

alcoholic versus HBV-related cirrhosis. However, a systematic and integrative analysis that simultaneously focuses on the transcription factor networks, developmental trajectories, and intercellular crosstalk of both monocytes/macrophages and T cells in a cohort of human cirrhotic livers is still lacking.

Here, we analyzed liver tissues from patients with cirrhosis and histologically non-cirrhotic controls. We focused on monocyte/macrophage and T-cell compartments, evaluated disease-associated regulon activity, reconstructed transcriptional trajectories, and modeled ligand–receptor communication. YY1 and EOMES patterns selected from the human dataset were then examined in two mechanistically distinct mouse models of liver injury and fibrosis.

Results

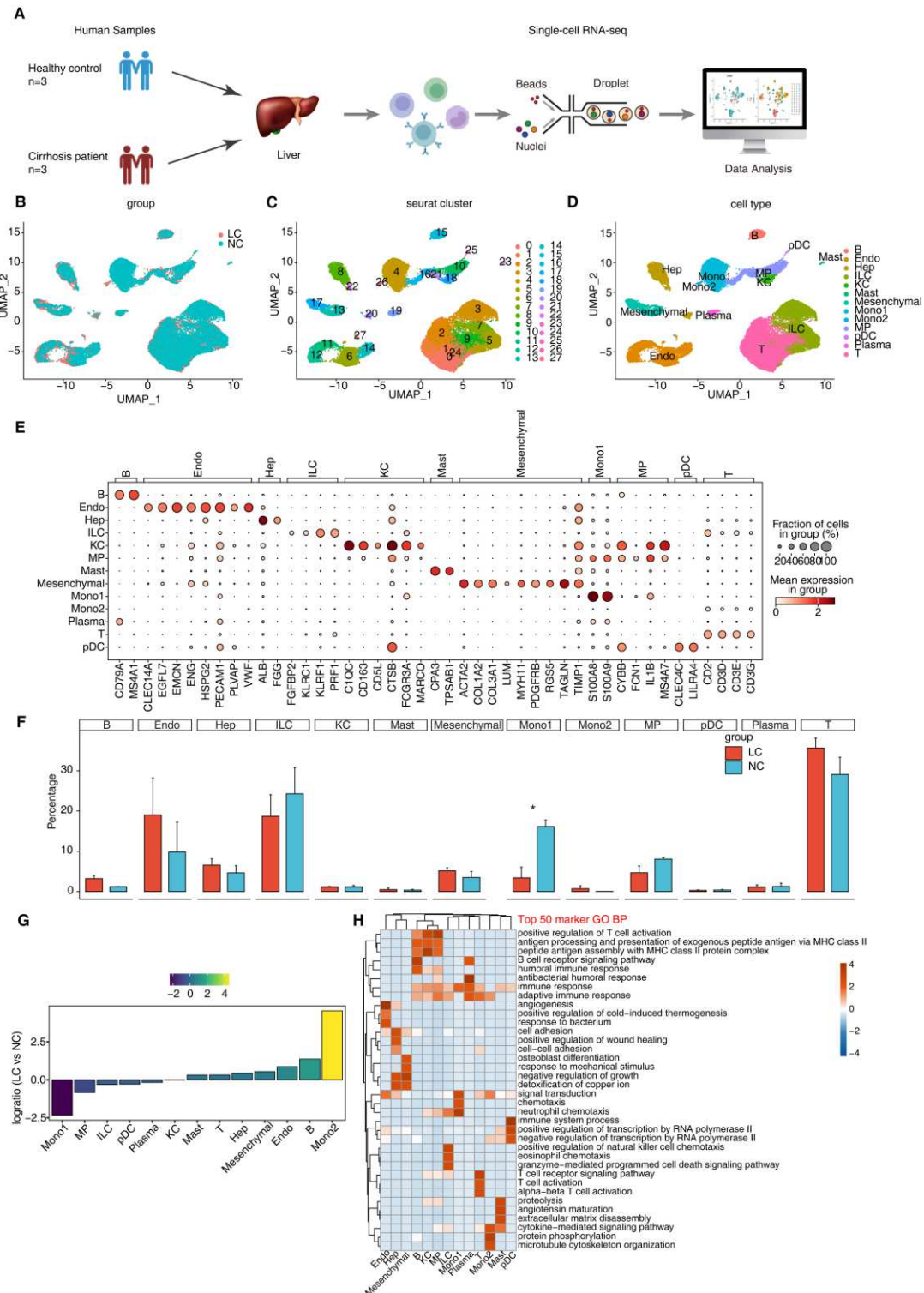
1. 3.1. A Single-Cell Atlas Identifies Disease-Associated Shifts in Liver Cell States

Cell Ranger identified 55,797 cell-containing barcodes across the six libraries, of which 43,745 cells from three cirrhotic and three histologically non-cirrhotic livers remained after downstream quality control (Fig. 1A, B). Unsupervised clustering resolved 28 clusters that were annotated as 13 major cell classes, including B cells, endothelial cells, hepatocytes, innate lymphoid cells, Kupffer cells, mast cells, mesenchymal cells, two monocyte states (Mono1 and Mono2), macrophages, plasmacytoid dendritic cells, plasma cells, and T cells (Fig. 1C, D).

Comparative analysis suggested shifts in cellular proportions. T cells constituted the predominant population in both groups[26-28]. In this cohort, the proportion of Mono1 was lower in cirrhosis, whereas Mono2, B cells, and endothelial cells were higher (Figure 1E-G)[8, 29, 30]. Other subsets also varied between groups, indicating remodeling of the cirrhotic liver microenvironment; these abundance patterns require confirmation in larger donor cohorts.

Gene Ontology (GO) enrichment of the top marker genes indicated functional specialization across cell types. Mono1 was associated with signal transduction and neutrophil chemotaxis, whereas Mono2 was associated with chemokine signaling and

91 cytoskeletal organization. Mesenchymal cells were linked to adhesion and
92 extracellular-matrix organization [31, 32]. Exploratory cell-type-specific
93 differential-expression analysis identified fibrosis-associated programs in
94 mesenchymal cells and altered innate-immune programs in monocyte populations
95 (Figure 1H and Figure S1). These findings describe cohort-associated transcriptional
96 patterns rather than population-level effects.

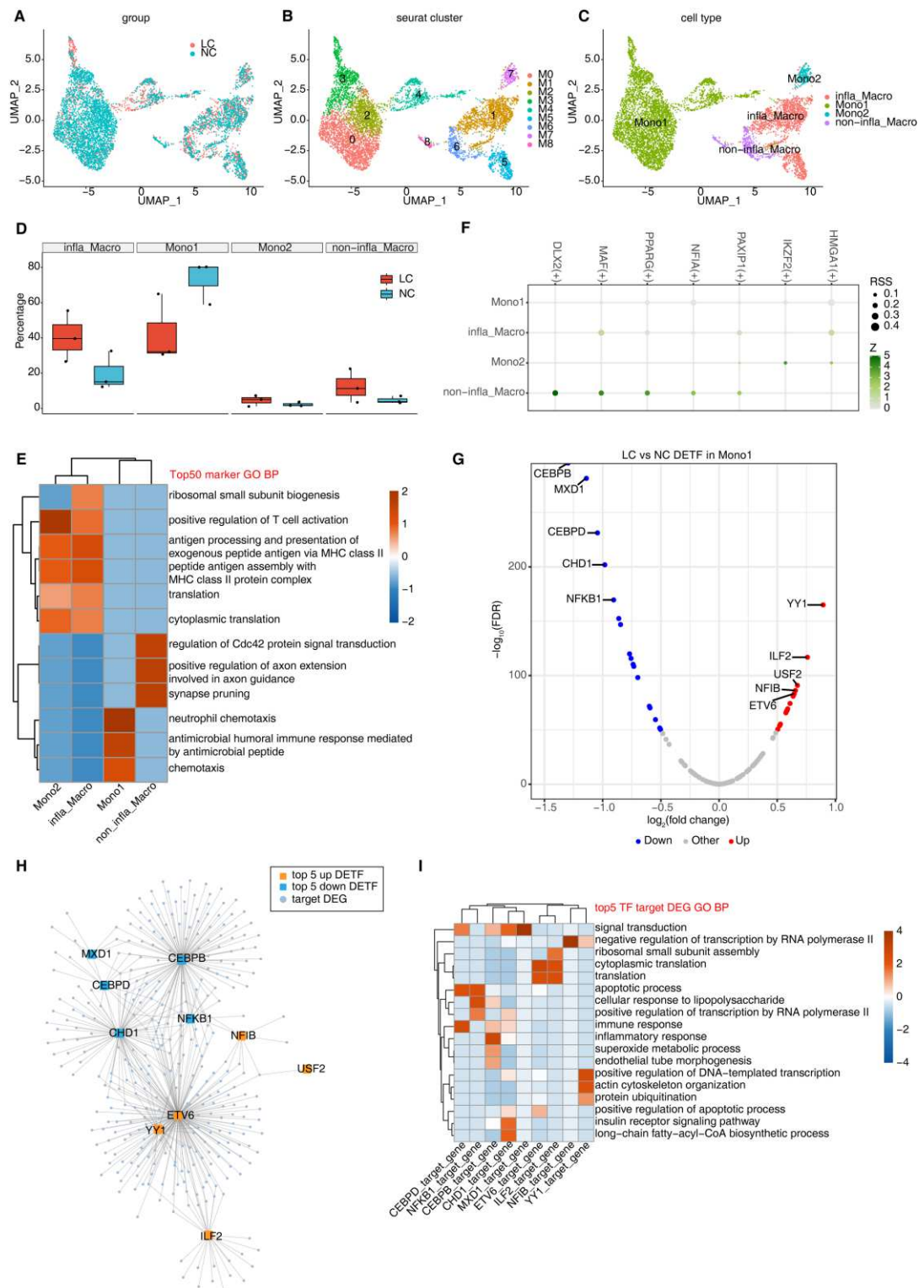


2. 3.2. YY1- and ETV6-Associated Regulons Characterize Dysregulated Myeloid States

Reclustering monocytes and macrophages yielded nine clusters grouped into inflammatory macrophages, non-inflammatory macrophages, Mono1, and Mono2

(Fig. 2A–C). Cirrhotic samples showed a higher fraction of inflammatory macrophages and a lower fraction of Mono1 cells in this cohort (Fig. 2D). Functional enrichment linked Mono2 and inflammatory macrophage markers to antigen presentation and T-cell activation (Fig. 2E).

Regulon analysis identified state-associated factors, including MAF and PPARG in non-inflammatory macrophages [33-36] and HMGA1 in Mono2[36]. In Mono1 cells, YY1 and ETV6 activity was higher and NFkB1 activity was lower in cirrhosis (Figure 2F-G and Figure S2) [37-40]. Candidate regulon-target analysis linked NFkB1 to apoptosis and lipopolysaccharide-response programs[41] and CEBPB to inflammatory-response programs. YY1 and ETV6 were also increased in inflammatory macrophages. These associations nominate YY1 and ETV6 as candidate regulators but do not establish direct transcriptional causality.



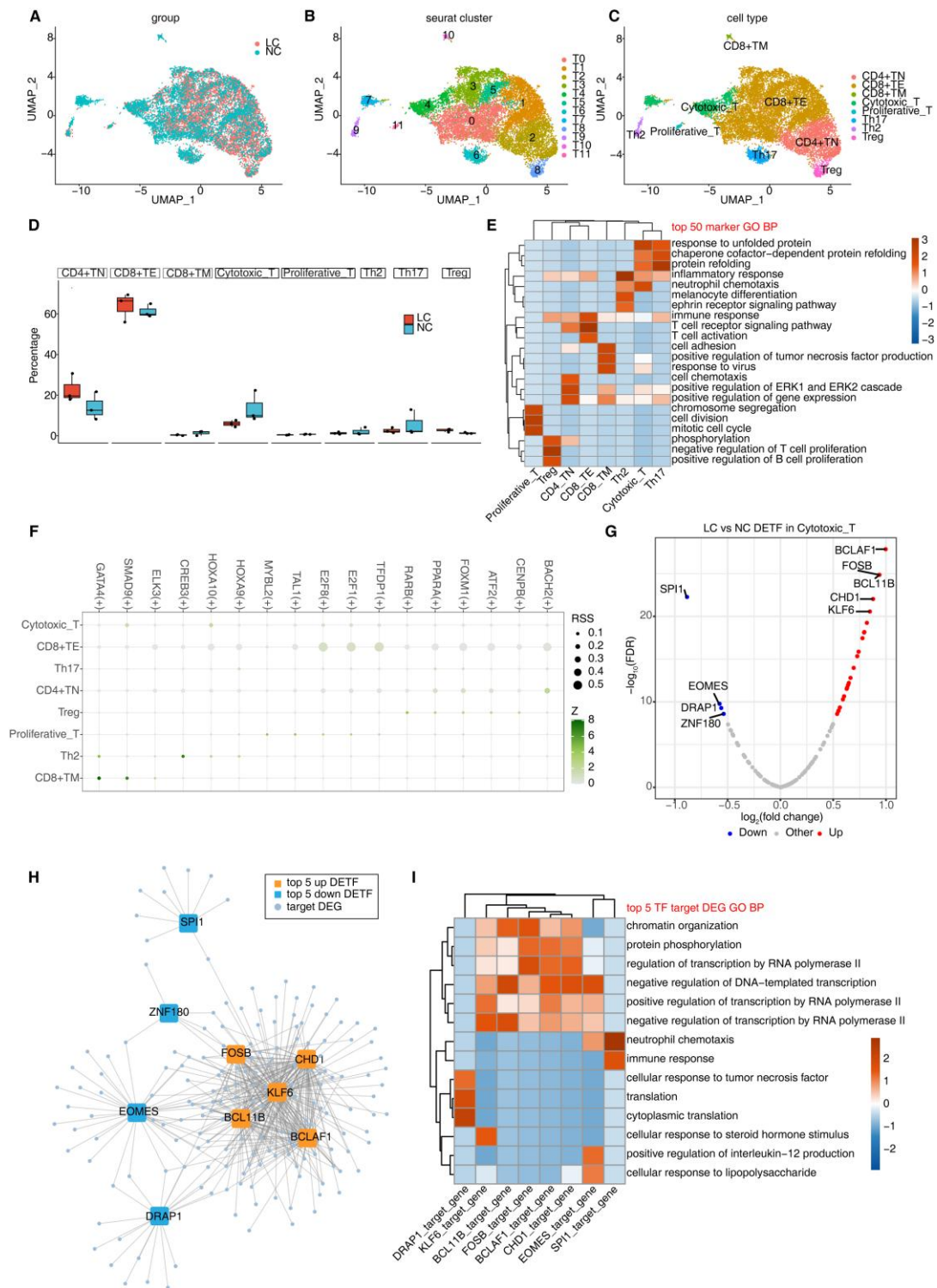
3.3.3. Cytotoxic and Regulatory T Cells Show Distinct Transcription-Factor Programs

T-cell reclustering identified 12 states, including naïve, effector, memory, cytotoxic, proliferating, T-helper, and regulatory populations (Fig. 3A–C and Fig. S3).

122 Cirrhotic samples showed higher fractions of CD4-naïve and regulatory T cells and
123 lower fractions of CD8-memory and cytotoxic T cells (Fig. 3D). Marker enrichment
124 was consistent with suppressive programs in regulatory T cells and inflammatory
125 programs in cytotoxic and Th17 populations (Fig. 3E).

126 Regulon analysis highlighted state-associated factors, including GATA4 in
127 CD8-memory T cells[42, 43], E2F1 in proliferating T cells [44], and FOXM1 in
128 regulatory T cells (Figure 3F and Figure S3) [45]. Cytotoxic T cells showed higher
129 KLF6 activity [46, 47]and lower EOMES activity in cirrhosis [48]. Regulatory T cells
130 showed higher EP300 and SP3 activity [49-51]. These patterns indicate
131 transcription-factor-associated T-cell remodeling; cytotoxic or suppressive function
132 was not measured directly.

133



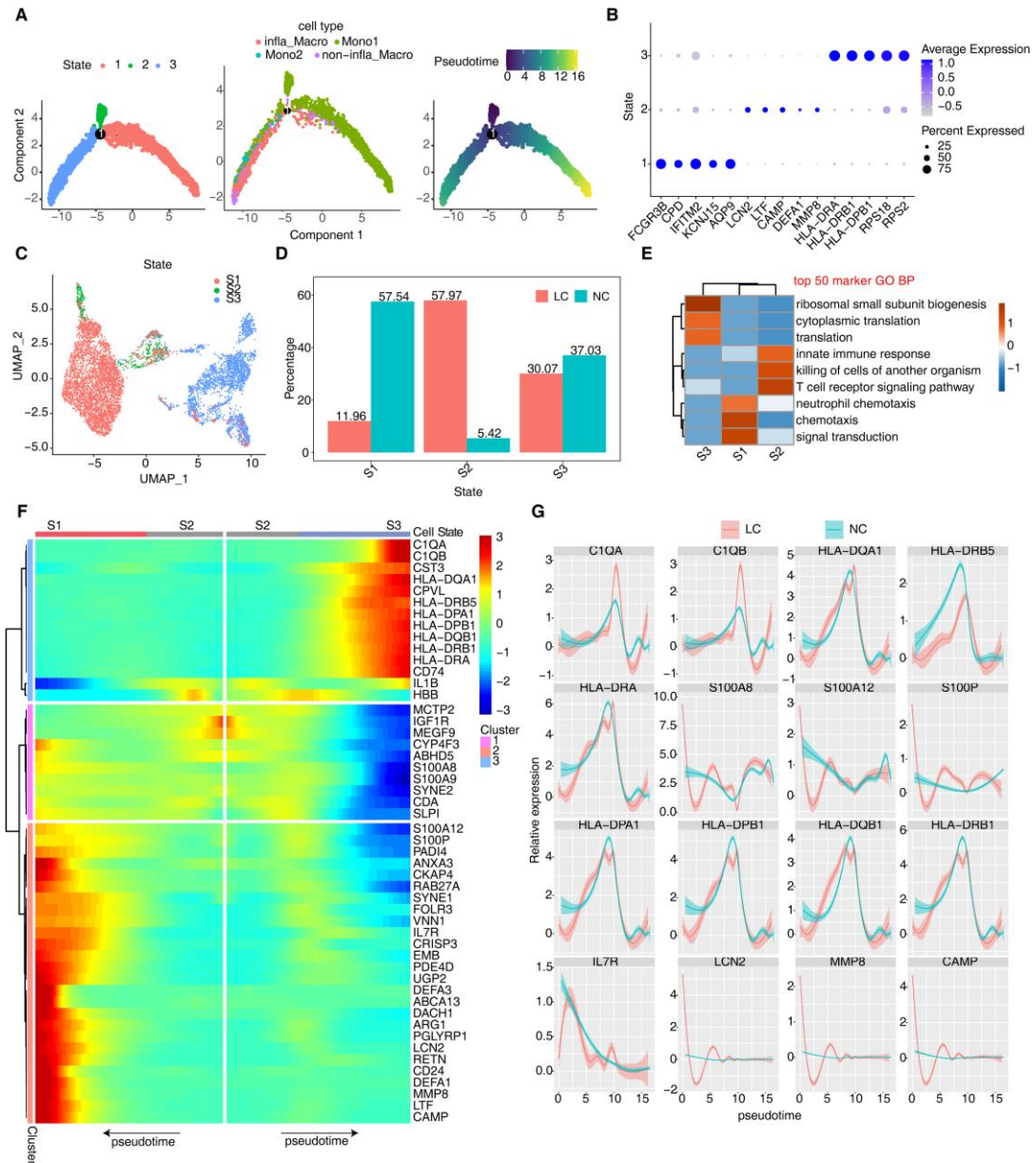
4.3.4. Cirrhosis Is Associated with Altered Occupancy of Immune Transcriptional Trajectories

Monocyte/macrophage trajectory reconstruction resolved three states (Fig. 4A–C). Cirrhotic samples were enriched in the Mono1-associated S2 state and showed lower occupancy of the S1 state (Fig. 4D). State and branch-associated genes included

141 IFITM2, AQP9, LCN2, CAMP, MMP8, complement genes, HLA genes, and
142 S100-family genes (Fig. 4E–G). These findings indicate altered state occupancy along
143 the inferred trajectory; they do not demonstrate a differentiation block because the
144 data are cross-sectional.

145 State-associated genes included IFITM2, AQP9, LCN2, and CAMP[52], together
146 with MMP8 (Figure 4E and Figure S4) [53-59]. Genes varying along the inferred
147 trajectory included complement genes, HLA genes, and S100-family members[60-62],
148 IL7R[63], LCN2[64], and MMP8[65]. Their expression patterns were associated with
149 altered trajectory-state occupancy[66-68]; pseudotime does not establish lineage
150 direction or a differentiation block.

151 T-cell pseudotime analysis identified five transcriptional states. Cirrhotic samples
152 showed higher occupancy of an early CD4-naive-associated state and lower
153 occupancy of later states. State-associated genes included HSPB1 and ZEB2 [69, 70].
154 Dynamic genes included NFKB1, LMNA, STAT4, KLRD1, CD160, and
155 KLRB1[71-78]. These findings support disease-associated remodeling of T-cell state
156 continua rather than impaired maturation proven by lineage tracing.

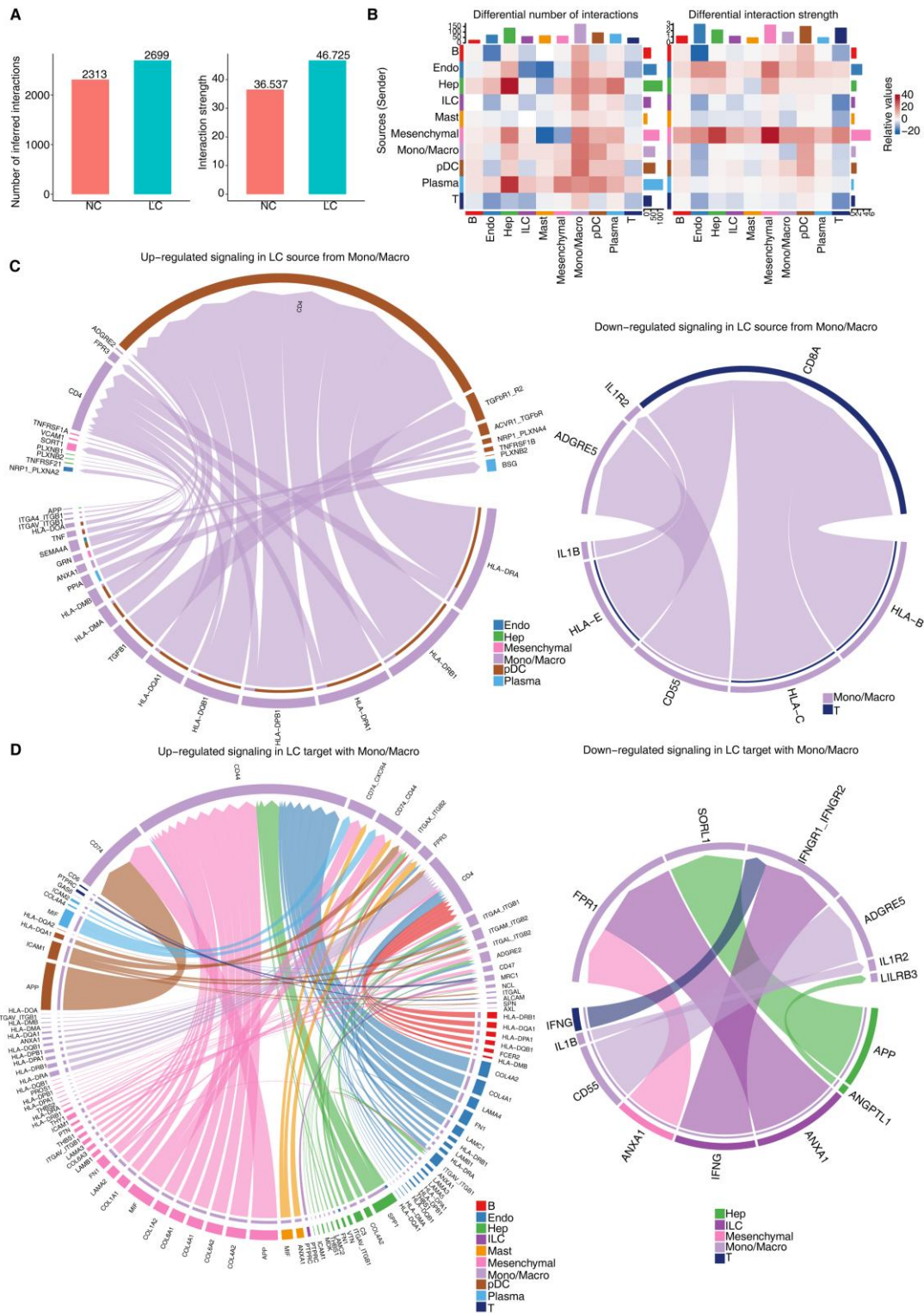


5. 3.5. Inferred Communication Shifts toward Fibrosis-Associated Signaling

CellChat predicted a greater number and aggregate strength of ligand–receptor interactions in cirrhotic samples (Fig. 5A, B and Fig. S5). Myeloid sender networks showed increased fibrosis-associated pathways, including TGF- β and matrix-related signaling toward mesenchymal cells, while several immune-activation signals involving T cells were reduced (Fig. 5C, D). Monocyte/macrophage and mesenchymal compartments contributed prominently to the inferred fibrosis-associated network. These results define computationally predicted communication changes that require spatial or perturbational validation.

CellChat predicted altered ligand-receptor programs involving monocytes/macrophages and T cells. Myeloid sender networks showed stronger fibrosis-associated pathways, including TGF- β and matrix-related signaling toward mesenchymal cells, while several immune-activation signals involving T cells were reduced (Figure 5C-D and Figure S5) [79-84]. These are computationally inferred communication changes and require spatial or perturbational validation.

These results define computationally predicted communication changes rather than directly measured biochemical interactions and therefore require spatial or perturbational validation.

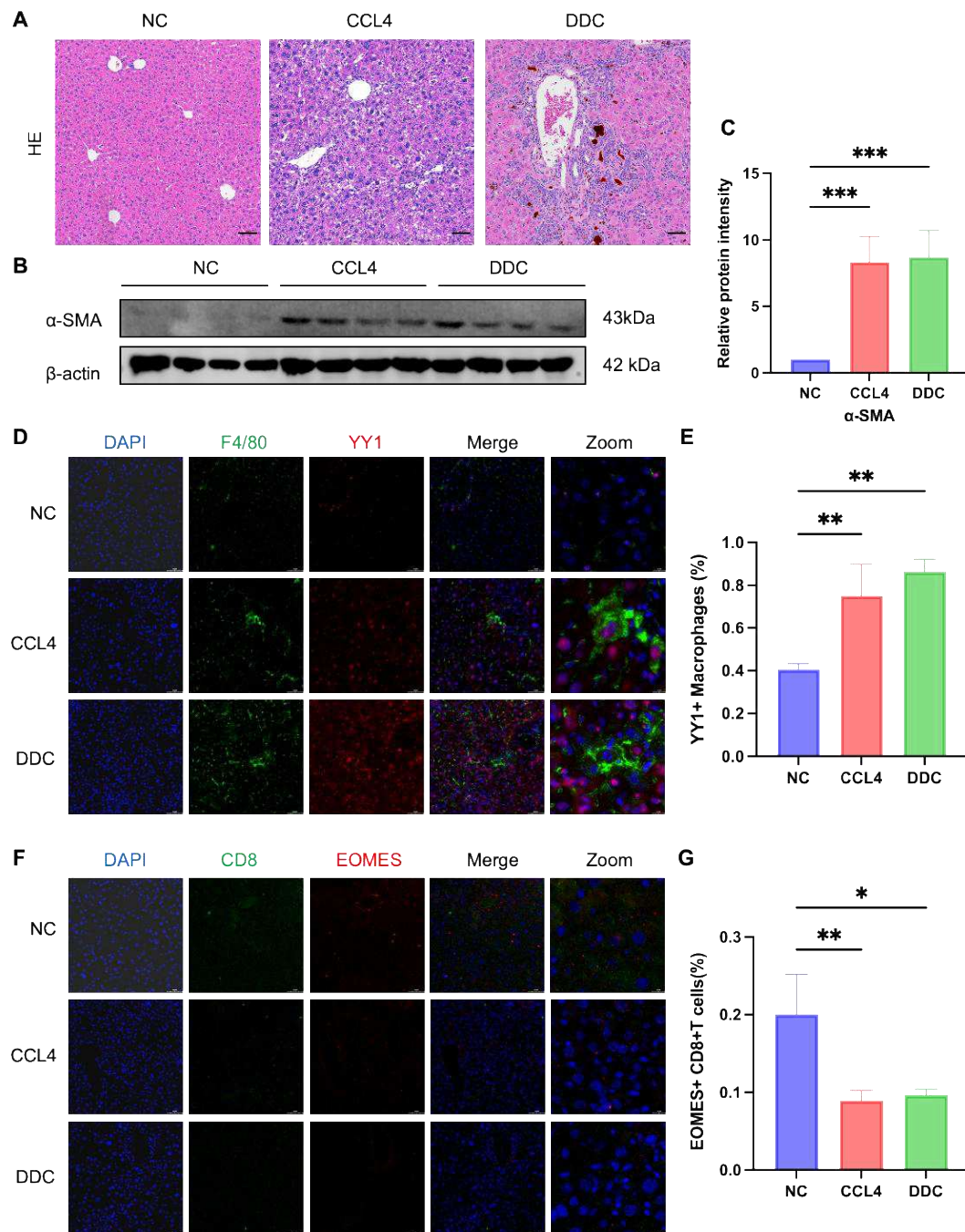


3.6. Two Mouse Fibrosis Models Reproduce YY1 and EOMES Expression Patterns

Hematoxylin and eosin staining showed liver injury and inflammatory infiltration after DDC or CCl₄ exposure, and α -SMA abundance was increased by immunoblotting (Fig. 6A–C). YY1 immunoreactivity was increased in F4/80-positive

macrophages in both models (Fig. 6D, E). Conversely, the fraction of EOMES-positive cells among CD8-positive T cells was reduced (Fig. 6F, G). The concordant direction across a cholestatic dietary model and a toxic injury model supports robustness of the expression pattern, but these experiments do not test whether YY1 or EOMES causes fibrosis.

YY1 immunoreactivity was increased in F4/80-positive macrophages in both models, whereas the proportion of EOMES-positive cells among CD8-positive T cells was reduced (Figure 6D-G). Concordance across the cholestatic DDC model and toxic CCl₄ model supports robustness of the expression pattern but does not establish that YY1 or EOMES causes fibrosis.



Discussion

This study connects four layers of single-cell evidence—cellular composition, regulon activity, transcriptional trajectories, and inferred intercellular communication—to describe coordinated myeloid and T-cell remodeling in human cirrhosis. The central pattern comprises YY1/ETV6-high myeloid states, an EOMES-low cytotoxic T-cell state, EP300/SP3-high regulatory T cells, altered trajectory-state occupancy, and a fibrosis-biased communication network. Tissue-level

validation in DDC and CCl₄ models supports the reproducibility of selected expression changes across species and injury contexts.

The observed immune-cell proportions suggest altered hepatic immune homeostasis. Mono1 cells were reduced in this cohort [8, 29, 30], whereas Mono2 and inflammatory macrophage states were expanded and associated with antigen-presentation programs[26-28]. The T-cell compartment also showed more regulatory T cells and fewer cytotoxic and memory CD8-positive T cells. These cohort-level patterns are compatible with cirrhosis-associated immune dysfunction but require replication in larger, clinically annotated cohorts.

Regulon analysis identified candidate factors associated with dysregulated immune states. YY1 activity was increased in monocyte/macrophage subsets. YY1 has previously been linked to hepatic fibrogenic programs[37, 38], and the present analysis extends this observation to macrophage-associated states. ETV6 was co-upregulated[39], supporting a candidate stress-responsive myeloid program. EOMES activity was reduced in cytotoxic T cells[11, 48]. EOMES participates in cytotoxic and memory programs, while CD8 tissue-resident memory cells have been implicated in fibrosis resolution. EP300 activity was increased in regulatory T cells and has been linked to fibrotic transcriptional programs[49, 50]. These associations require cell-type-specific perturbation before any causal interpretation.

The use of both DDC and CCl₄ models is a strength because the models represent distinct injury contexts. Nevertheless, mouse experiments validated localization and direction of protein-expression changes only. They did not establish conservation of the full human cell state, therapeutic efficacy, or causality. Macrophage-specific YY1 perturbation and T-cell-specific EOMES manipulation, ideally combined with fibrosis phenotyping and spatial profiling, would directly test the proposed regulatory axis.

Pseudotime analysis provided a representation of disease-associated state distributions. Cirrhotic monocyte/macrophage and T-cell populations showed higher occupancy of earlier inferred states. Because the data are cross-sectional, this pattern is interpreted as altered trajectory-state occupancy rather than arrested differentiation.

State-associated genes included LCN2[55, 56] and MMP8 [59] in myeloid cells, together with STAT4 and NFKB1[72, 74] in T cells. These genes prioritize hypotheses for future donor-aware and functional analyses rather than established therapeutic targets.

CellChat integrated the single-cell data into a predicted tissue-level communication network. The analysis suggested stronger fibrosis-associated TGF- β , collagen, and laminin programs alongside weaker immune-activation programs, including CD8A-associated signaling[85] and HLA class I-associated signaling[86]. CellChat infers compatible ligand-receptor relationships but cannot confirm ligand availability, receptor activation, spatial proximity, or signaling direction in tissue.

Several limitations constrain interpretation. First, only three donors per group were included, and the controls were histologically non-cirrhotic peri-tumoral tissues rather than livers from healthy volunteers. Second, etiology was heterogeneous, clinical metadata were incomplete in the current manuscript, and residual confounding cannot be excluded. Third, cell-level differential tests can underestimate variance when donor structure is ignored; donor-level pseudobulk or mixed-effects validation is needed. Fourth, trajectory and communication results are computational inferences. Finally, the animal cohort was small and lacked targeted perturbation. The study should therefore be viewed as a focused discovery dataset that generates a testable immune-regulatory model rather than definitive evidence of mechanism or a therapeutic target.

Conclusion

Single-cell regulatory analysis of human cirrhotic liver identified coordinated remodeling of myeloid and T-cell states. YY1/ETV6-associated myeloid programs, reduced EOMES in cytotoxic T cells, increased EP300/SP3 in regulatory T cells, altered trajectory-state occupancy, and fibrosis-associated inferred communication formed a coherent disease-linked pattern. Concordant YY1 and EOMES expression changes in two mouse models prioritize this axis for donor-aware replication and functional perturbation.

Materials and Methods

1. 1. Human Liver Tissue Collection

Liver tissue was obtained from three patients with cirrhosis of mixed etiology (post-hepatic or alcohol-associated) during transjugular intrahepatic portosystemic shunt procedures. Comparator tissue (n = 3) was sampled from histologically non-cirrhotic peri-tumoral liver of patients undergoing resection for benign liver tumors or metastatic cancer. These samples are therefore referred to as histologically non-cirrhotic controls rather than healthy controls. Normal histology was confirmed locally. None of the participants had received immunosuppressive therapy before tissue collection. The Ethics Committee of the First Affiliated Hospital of Kunming Medical University approved the study (2023-Lunshen-L-211), and all participants provided written informed consent. Procedures conformed to the Declaration of Helsinki.

2. Single-Cell Suspension Preparation and Sequencing

Fresh tissues were minced and digested in Gey's Balanced Salt Solution containing Liberase TM (Roche) and DNase I (Sigma-Aldrich) at 37 °C for 45 min with gentle agitation. Suspensions were passed through 70-µm and 40-µm strainers, erythrocytes were lysed, and nonviable cells were depleted with the Dead Cell Removal Kit (Miltenyi Biotec). Suspensions with viability >85% were processed with the Chromium Next GEM Single Cell 3' Kit v3.1 (10x Genomics). Libraries were sequenced on an Illumina NovaSeq 6000 platform. The observed mean sequencing depth ranged from 32,745 to 51,738 reads per called cell across the six libraries. All processed single-cell gene expression matrices generated in this study were deposited in the NCBI Gene Expression Omnibus (GEO) repository under accession number GSE348509.

3. scRNA-seq Data Processing and Quality Control

The six libraries were processed independently with Cell Ranger v7.0.0 using the Single Cell 3' v3 chemistry and a human reference built from GENCODE v45. Intronic reads were included during counting. Cell Ranger identified 55,797 cell-containing barcodes before downstream quality control. Across libraries, valid

barcodes ranged from 96.5% to 98.3%, sequencing saturation from 57.1% to 85.2%, confidently transcriptome-mapped reads from 59.8% to 79.3%, and reads assigned to cells from 71.2% to 93.1%. Downstream analysis was performed in R v4.1.0 using Seurat v4.1.1. Cells with <200 or >6,000 detected genes or >15% mitochondrial reads were removed, leaving 43,745 cells. Counts were normalized with LogNormalize; 2,000 highly variable genes were selected with the vst method. Principal-component analysis, graph-based clustering, and UMAP visualization were performed. Cell identities were assigned using SingleR v1.8.1 with BlueprintEncodeData and were reviewed against canonical markers.

4. Dimensionality Reduction, Clustering, and Cell Annotation

Counts were normalized using LogNormalize, and 2,000 highly variable genes were selected with the vst method. Principal-component analysis, graph-based clustering, and UMAP visualization were performed. Cell identities were assigned using SingleR v1.8.1 with BlueprintEncodeData and reviewed against canonical marker genes.

5. Differential Expression and Functional Enrichment

Cell-level exploratory differential-expression analyses between cirrhosis and control groups used Seurat FindMarkers with MAST ($|\log_2 \text{ fold change}| > 0.25$ and Benjamini–Hochberg-adjusted $P < 0.05$). Gene Ontology enrichment was performed with clusterProfiler v4.0.5. Because cells from the same donor are not independent biological replicates, cell-level P values were used to prioritize patterns and not to estimate population-level effects. The donor was treated as the biological replicate when interpreting group-level findings.

6. Transcription Factor Regulon Analysis

SCENIC v1.2.4 was used to infer transcription-factor regulons. Co-expression networks were generated with GRNBoost2, candidate targets were filtered by hg38 motif enrichment, and regulon activity was quantified by AUCell. Differential activity was explored with Wilcoxon rank-sum tests. These analyses identify candidate regulatory associations and do not demonstrate direct transcriptional control.

7. Pseudotime Trajectory Analysis

Monocle2 v2.22.0 with DDRTree was used to order cells along transcriptional

trajectories, and BEAM identified branch-associated genes. Pseudotime was interpreted as computational ordering rather than direct lineage tracing.

8. Cell–Cell Communication Analysis

CellChat v1.1.3 was used to infer ligand–receptor communication probabilities and compare signaling patterns between groups. Inferred interactions were not interpreted as direct biochemical measurements.

9. Animal Models of Liver Injury and Fibrosis

Male C57BL/6 mice (6–8 weeks, 20–22 g) were purchased from SpePharm (Beijing) Biotechnology Co., Ltd. (License No. SCXK (Jing) 2024-0001) and maintained in an SPF facility. Mice were allocated to control, carbon tetrachloride (CCl₄; 0.5 μL/g i.p., twice weekly for 8 weeks), or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC; 0.1% diet for 4 weeks) groups (n = 4/group). At endpoints, mice were deeply anaesthetised with isoflurane (3%–5% in oxygen). Following the loss of the pedal withdrawal reflex, animals were promptly euthanized via cervical dislocation to achieve instantaneous, painless death. Death was confirmed by permanent cessation of respiration and heartbeat, pupillary dilation, and absent reflexes prior to blood and liver tissue harvesting. Carcasses were disposed of as biohazardous waste by the animal center. All procedures complied with GB/T 35892-2018 and ARRIVE guidelines, and were approved by the Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of Kunming Medical University (Protocol No. 2025DF013).

10. Western Blotting

For immunoblotting, 30 μg liver protein was separated by SDS–PAGE, transferred to PVDF membrane, and probed with antibodies against α-SMA and β-actin (Cell Signaling Technology). Cell Signaling Technology secondary antibodies were used. Signals were detected by enhanced chemiluminescence and quantified in ImageJ; α-SMA was normalized to β-actin and expressed relative to control.

11. Immunofluorescence Staining

Paraffin sections (4 μ m) underwent antigen retrieval and were incubated overnight at 4 °C with antibodies against F4/80 (Thermo Fisher Scientific, 14-4801-82; 1:200), YY1 (Proteintech, 22156-1-AP; 1:200), CD8 α (Thermo Fisher Scientific, 14-0081-82; 1:200), or EOMES (Abcam, ab319166; 1:100), followed by fluorophore-conjugated secondary antibodies (Cell Signaling Technology). Nuclei were counterstained with DAPI. Images were acquired by confocal microscopy, and five randomly selected fields per section were quantified. Fields were averaged within each animal; the animal, not the microscopic field, was the experimental unit.

12. Statistical Analysis

Analyses were conducted in R v4.1.0 and GraphPad Prism v9.0. Animal-level data are shown as individual points with mean $\pm$ SD. Three-group comparisons used one-way ANOVA followed by Tukey adjustment when assumptions were met. Exact two-sided P values should accompany significance symbols in the source data. For the human single-cell dataset, the donor was the biological replicate; analyses performed at cell level are explicitly described as exploratory. No prospective sample-size calculation was reported.

List of abbreviations

LC: Liver cirrhosis
NC: Histologically non-cirrhotic control
TF: Transcription factor
DEG: Differentially expressed gene
GO: Gene Ontology
ECM: Extracellular matrix
HSC: Hepatic stellate cell
Treg: Regulatory T cell
Mono1: Monocyte subset 1
Mono2: Monocyte subset 2
MP: Macrophage

KC: Kupffer cell
Endo: Endothelial cell
Hep: Hepatocyte
ILC: Innate lymphoid cell
pDC: Plasmacytoid dendritic cell
CAID: Cirrhosis-associated immune dysfunction
UMAP: Uniform Manifold Approximation and Projection
PCA: Principal component analysis
SCENIC: Single-cell regulatory network inference and clustering

Declarations

Ethics approval and consent to participate

Human study: Approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University (Approval No. 2023-Lunshen-L-211); written informed consent was obtained from all donors. **Animal study:** Approved by the Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of Kunming Medical University (Protocol No. 2025DF013).

Consent for publication

Not applicable.

Data Availability Statement

The processed single-cell RNA sequencing datasets (filtered feature-barcode matrices) generated and analysed during the current study have been deposited in the NCBI Gene Expression Omnibus (GEO) repository, an INSDC member repository, under accession number GSE348509 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE348509>). The bioinformatic analytical pipelines and custom scripts are comprehensively detailed within the "Materials and Methods" section and are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

Hanfei Huang and Zhong Zeng: Conceptualization, Supervision. Chenxuan Li: Methodology, Study design. Dedong Yuan, Qinglei Chen, Jiyuan Shi, Li Jin, Zongrui Jin, and Deling Wei: Investigation, Data curation, Formal analysis. Chenxuan Li and Dedong Yuan: Writing – original draft. Hanfei Huang, Zhong Zeng, and Zhuo Cheng: Writing – review & editing. All authors approved the submitted version.

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Figure Legends

Figure 1. Single-cell landscape of cirrhotic and histologically non-cirrhotic liver. (A) Study workflow. (B–D) UMAP plots by group, cluster, and annotated cell class. (E) Cell-class proportions. (F) Log-ratio of proportions. (G) Gene Ontology enrichment of marker genes. (H) Enrichment of exploratory cell-type-specific differentially expressed genes.

(A) Schematic of scRNA-seq workflow. (B-D) UMAP plots colored by group (B), cluster (C), and cell type (D). (E) Cell-type proportions in LC and NC. (F) Log-ratio of cell-type proportions. (G) GO enrichment heatmap of top 50 marker genes per cell type. (H) GO enrichment of upregulated DEGs per cell type (LC vs. NC).

Figure 2: Monocyte/macrophage subclustering and transcription factor analysis

(A-C) UMAP plots of monocytes/macrophages colored by group (A), cluster (B), and cell type (C). (D) Box plot of cell-type proportions. (E) GO enrichment of top 50 markers per cell type. (F) Dot plot of TF regulon activity per cell type. (G) Volcano plot of differentially expressed TFs in Mono1. (H) Network of top differentially expressed TFs and their target DEGs in Mono1. (I) GO enrichment of TF target DEGs in inflammatory macrophages.

Figure 3: T cell subclustering and transcription factor analysis

(A-C) UMAP plots of T cells colored by group (A), cluster (B), and cell type (C). (D) Box plot of cell-type proportions. (E) GO enrichment of top 50 markers per cell type. (F) Dot plot of TF regulon activity per cell type. (G) Volcano plot of differentially expressed TFs in cytotoxic T cells. (H) Expression and activity of key TFs across T-cell subsets. (I) GO enrichment of TF target DEGs in cytotoxic T cells.

Figure 4: Pseudotime trajectory analysis of monocyte/macrophage differentiation

(A) Pseudotime trajectories colored by state, cell type, and pseudotime value. (B) Dot plot of top 5 markers per state. (C) UMAP colored by cell state. (D) State proportions in LC versus NC. (E) GO enrichment of top 50 markers per state. (F) BEAM heatmap of differentially expressed genes along pseudotime. (G) Expression dynamics of representative genes along pseudotime.

Figure 5: Cell-cell communication analysis in cirrhotic and normal liver tissues

(A) Numbers and strengths of cell-cell interactions in LC and NC. (B) Heatmap of differential interactions. (C) Chord diagram of differentially regulated signaling

pathways sourced from monocytes/macrophages. (D) Chord diagram of differentially regulated pathways targeting monocytes/macrophages.

Figure 6. YY1 and EOMES expression in two mouse liver-injury models. (A) Hematoxylin and eosin staining; arrows indicate inflammatory infiltrates. Scale bar, 100 μ m. (B, C) α -SMA immunoblot and quantification. (D, E) F4/80 (green), YY1 (red), and DAPI (blue), with animal-level quantification of YY1-positive F4/80-positive cells. Scale bar, 20 μ m. (F, G) CD8 α (green), EOMES (red), and DAPI (blue), with animal-level quantification of EOMES-positive CD8 α -positive cells. Scale bar, 20 μ m. n = 4 animals per group; mean $\pm$ SD; one-way ANOVA with Tukey adjustment. *P < 0.05, **P < 0.01, ***P < 0.001.

(A) H&E staining of liver sections from NC, CCl₄, and DDC groups. Arrows indicate inflammatory infiltration. Scale bar, 100 μ m. (B) Western blot of α -SMA and β -actin. (C) Quantification of relative α -SMA expression (n = 4). (D) Immunofluorescence of F4/80 (green), YY1 (red), and DAPI (blue). Insets: zoomed merge. Arrows: F4/80⁺YY1⁺ cells. Scale bar, 20 μ m. (E) Percentage of YY1⁺F4/80⁺ macrophages (n = 4). (F) Immunofluorescence of CD8 (green), EOMES (red), and DAPI (blue). Insets: zoomed merge. Arrows: CD8⁺EOMES⁺ cells. Scale bar, 20 μ m. (G) Percentage of EOMES⁺CD8⁺ T cells (n = 4). Data are mean $\pm$ SD. One-way ANOVA with Tukey's test. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure S1

(A) Bar plot presents the number of differentially regulated genes (DEGs) with LC vs NC. (B) Heat map showing the up regulated DEGs in each cell type enrichment result of GO BP. (C) Heat map showing the down regulated DEGs in each cell type enrichment result of GO BP.

Figure S2

(A) Box plot presents the cell ratio in both group. (B) Dot plot of the top 3 marker genes in each cell cluster. (C) Volcano plot showing the differentially expressed

transcription factors (DETFs) in infla_Macro cell. (D) Network plot presents the interaction of top5 up DETFs and targeted DEGs in infla_Macro cell. (E) Heat map showing the targeted DEGs in (D) enrichment result of GO BP. (F) Violin plot presents the expression level and AUC cell activity of TFs.

Figure S3

(A) Box plot presents the cell ratio in both group. (B) Dot plot of the top 3 marker genes in each cell cluster. (C) Volcano plot showing the differentially expressed transcription factors (DETFs) in Treg cell. (D) Network plot presents the interaction of top5 up DETFs and DEGs in Treg cell. (E) Heat map showing the targeted DEGs in (D) enrichment result of GO BP. (F) Violin plot presents the expression level and AUC cell activity of TFs.

Figure S4

(A) Pseudotime plots of sub annotation in T cell. Color-coded according to cell state(left), cell type(middle), pseudo-time value(right). (B) Dot plot showing top 5 markers in each cell state. (C) UMAP plot of sub annotation in T cell. Colors indicate cell state. (D) Bar plot presents the cell ratio in both group. (E) Heat map presents the top50 marker genes in each cell state enrichment result of GO BP. (F) Beam plot showing the expression level of differential pseudo-time genes. (G) Line chart showing the expression level differential pseudo-time genes according to pseudo-time.

Figure S5

(A) Chord diagram showing the number and strength of differentially regulated signal pathway that sourced from T cell. (B) Chord diagram showing the number and strength of differentially regulated signal pathway that targeted with T cell. (C) Violin plot showing the expression level of ligands in signal pathway MHC-II, COLLAGEN, LAMININ, ICAM, FN1, ANNEXIN, MHC-I.

764 (D) Violin plot showing the expression level of ligands in signal pathway TGFb,
765 MHC-I, CLEC.
766

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