

Supplementary Materials

Cell atlas of CCl₄-induced progressive liver fibrosis reveals stage-specific responses

Peng-Cheng Guo^{1,2,3,#}, Jing Zuo^{2,3,#}, Ke-Ke Huang⁴, Guang-Yao Lai^{2,3,5,6}, Xiao Zhang^{1,2,3}, Juan An^{2,3,5,7}, Jin-Xiu Li^{2,3,8}, Li Li⁵, Liang Wu⁵, Yi-Ting Lin⁵, Dong-Ye Wang⁵, Jiang-Shan Xu^{2,3}, Shi-Jie Hao^{2,3,8}, Yang Wang^{2,3}, Rong-Hai Li^{2,3}, Wen Ma^{2,3}, Yu-Mo Song^{2,3}, Chang Liu^{2,3}, Chuan-Yu Liu^{2,3}, Zhen Dai⁹, Yan Xu¹⁰, Amar Deep Sharma¹¹, Michael Ott¹¹, Qing Ou-Yang¹², Feng Huo¹², Rong Fan¹³, Yong-Yin Li¹³, Jin-Lin Hou¹³, Giacomo Volpe¹⁴, Long-Qi Liu^{2,3}, Miguel A. Esteban^{1,2,3,4,5,6,15,*}, Yi-Wei Lai^{2,3,*}

¹State Key Laboratory for Zoonotic Diseases, Key Laboratory for Zoonosis Research of Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, Jilin 130062, China

²BGI-Hangzhou, Hangzhou, Zhejiang 310012, China

³BGI-Shenzhen, Shenzhen, Guangdong 518103, China

⁴Key Laboratory of Biological Targeting Diagnosis, Therapy and Rehabilitation of Guangdong Higher Education Institutes, Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510799, China

⁵Laboratory of Integrative Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong 510530, China

⁶Joint School of Life Sciences, Guangzhou Institutes of Biomedicine and Health and Guangzhou Medical University, Guangzhou, Guangdong 510530, China

⁷School of Life Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui 230026, China

⁸College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

⁹Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong 510530, China

¹⁰Biotherapy Centre, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510630, China

¹¹Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover 30625, Germany

¹²Department of Hepatobiliary Surgery and Liver Transplant Center, General Hospital of Southern Theater Command, Guangzhou, Guangdong 510010, China

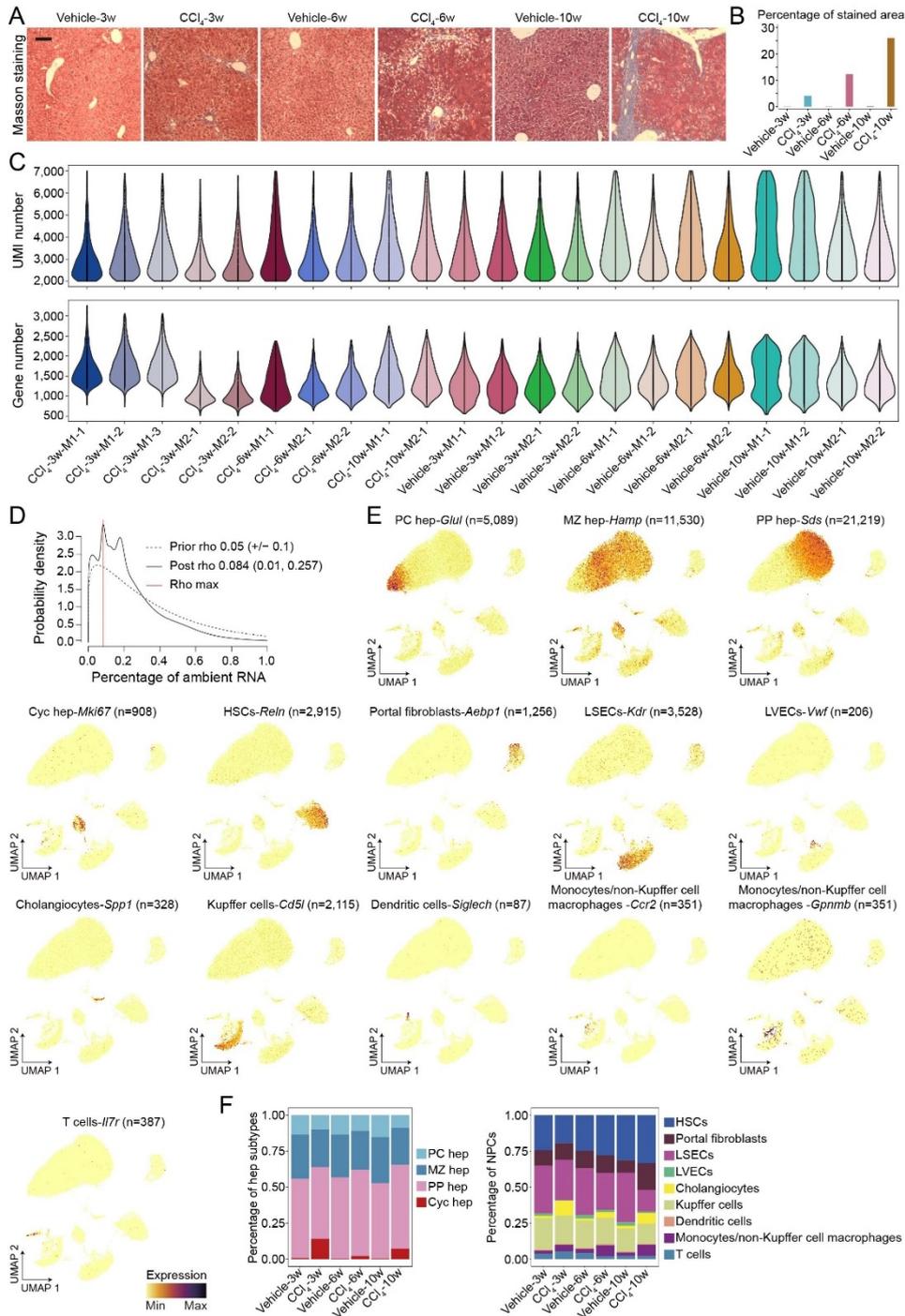
¹³Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Guangzhou, Guangdong 510515, China

¹⁴Hematology and Cell Therapy Unit, IRCCS-Istituto Tumori 'Giovanni Paolo II', Bari 70124, Italy

¹⁵Institute of Experimental Hematology, Hannover Medical School, Hannover 30625, Germany

#Authors contributed equally to this work

*Corresponding authors, E-mail: miguelesteban@genomics.cn; laiyiwei@genomics.cn

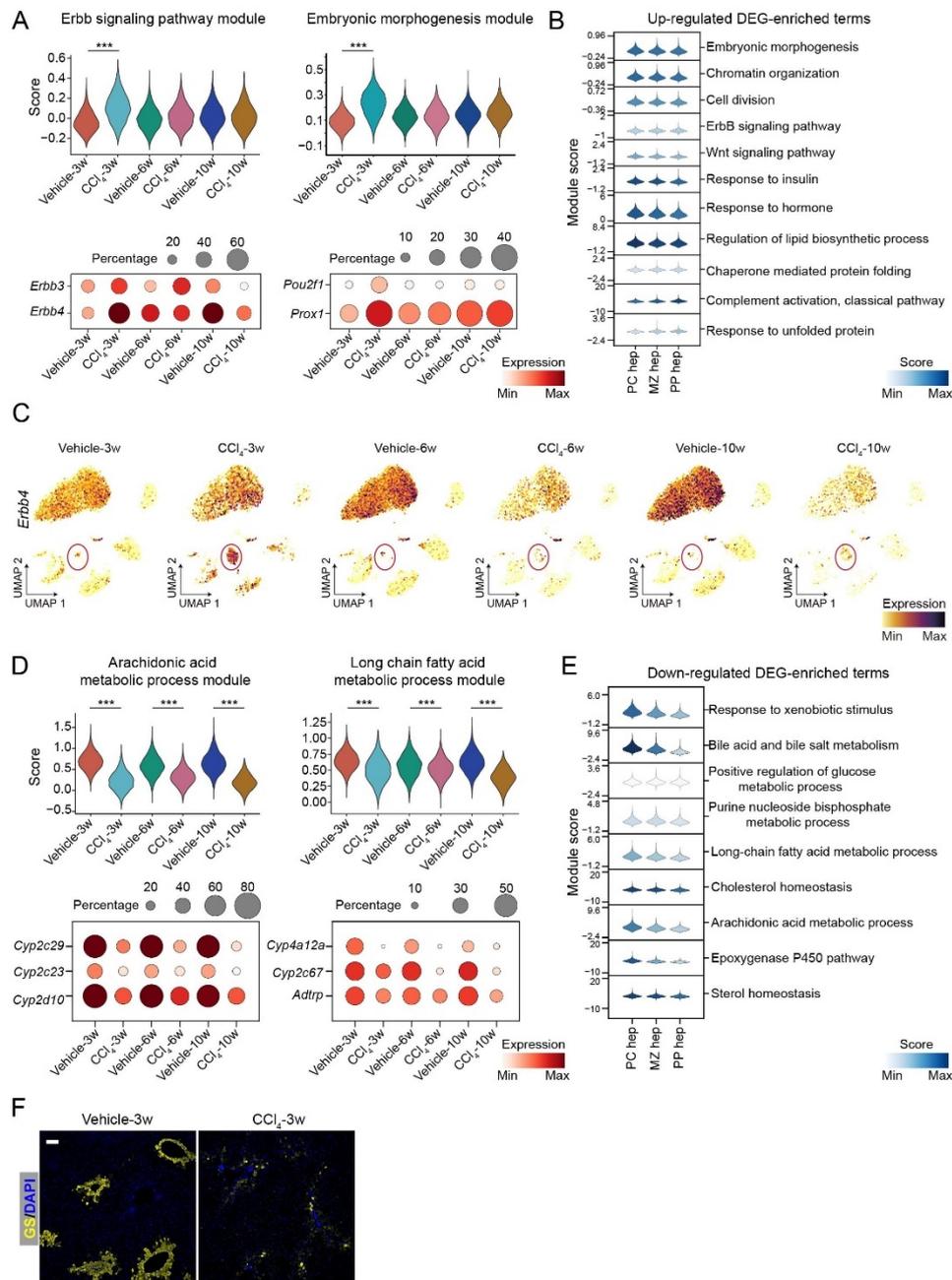


Supplementary Figure S1 Quality control of snRNA-seq data, related to Figure 1

- A.** Masson staining of liver sections from CCl₄-treated and vehicle control groups at different time points.
- B.** Quantification of stained area for corresponding sections.
- C.** Violin plots showing number of UMIs (top) and genes (bottom) after quality control filtering in different snRNA-seq libraries.
- D.** Estimation of ambient RNA contamination rate. Red line indicates median contamination rate.

E. UMAP visualization of expression of canonical marker genes used for cell type annotation, numbers of nuclei for each cell type are labeled on top.

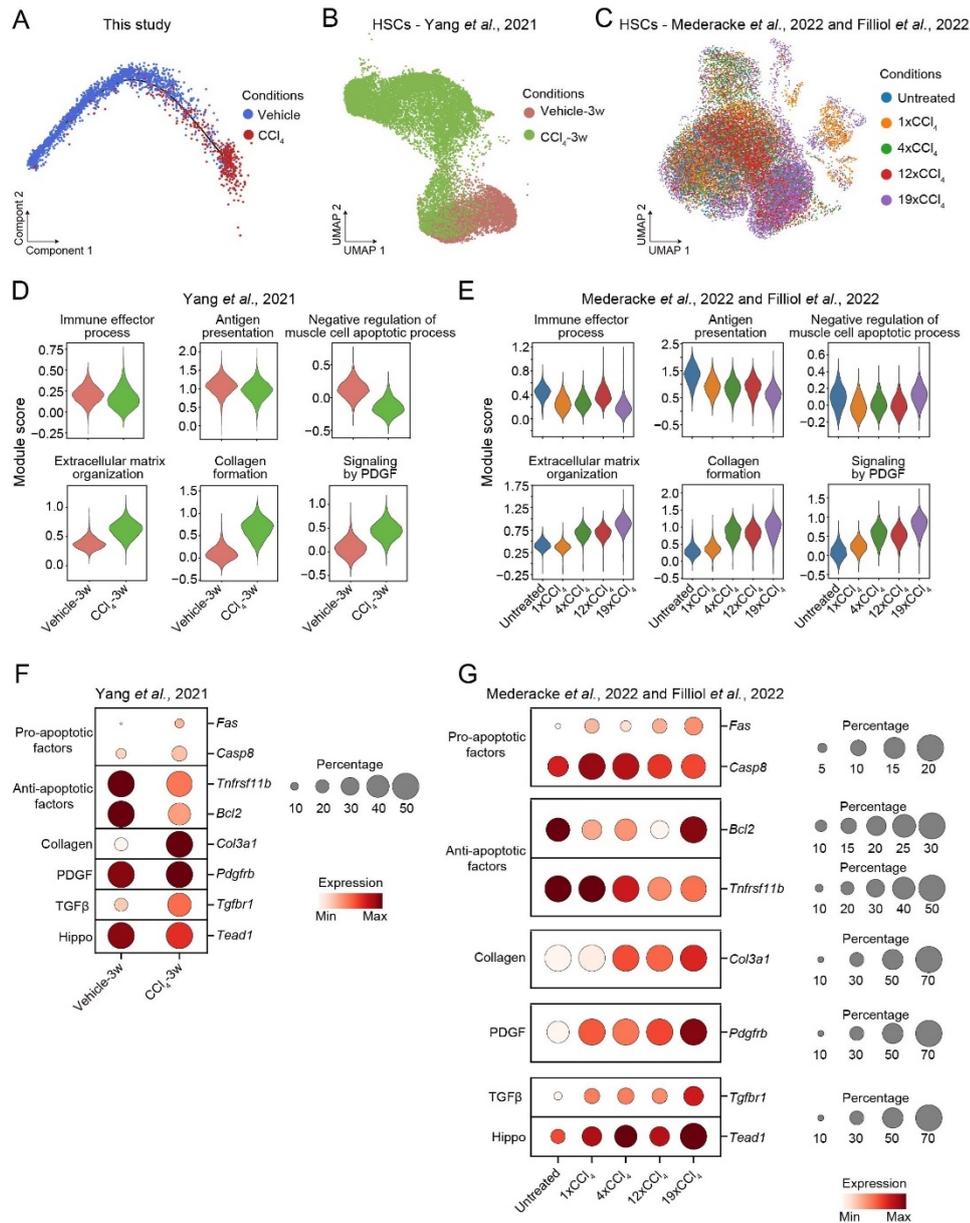
F. Proportion of major cell types among hepatocytes (left) and non-parenchymal cells (right).



Supplementary Figure S2 Hepatocyte responses at different fibrosis stages, related to Figure 2

A. Top: violin plots showing module scores of ErbB signaling (left) and embryonic morphogenesis (right) shown in Figure 2C (top). Bottom: bubble plots showing expression levels of genes in corresponding module for hepatocytes in each condition. *P*-values were calculated by Wilcoxon rank sum test.

- B.** Violin plots showing module scores of enriched up-regulated terms shown in **Figure 2C** (top) in pericentral, midzonal, and periportal hepatocytes.
- C.** UMAP visualization of expression levels of *ErbB4* for the indicated conditions. Red circles indicate cycling hepatocyte cluster.
- D.** Top: violin plots showing module scores of arachidonic acid metabolic process (left) and long chain fatty acid metabolic process (right) shown in **Figure 2C** (bottom). Bottom: bubble plots showing expression levels of genes in the corresponding modules in hepatocytes for the indicated conditions. *P*-values were calculated by Wilcoxon rank sum test.
- E.** Violin plots showing module scores of enriched down-regulated terms shown in **Figure 2C** (bottom) in pericentral, midzonal, and periportal hepatocytes.
- F.** Representative images of immunofluorescence staining for glutamine synthetase (GS, yellow) and DAPI (blue) performed on Vehicle-3w and CCl₄-3w sections. Scale bar, 100 μm.

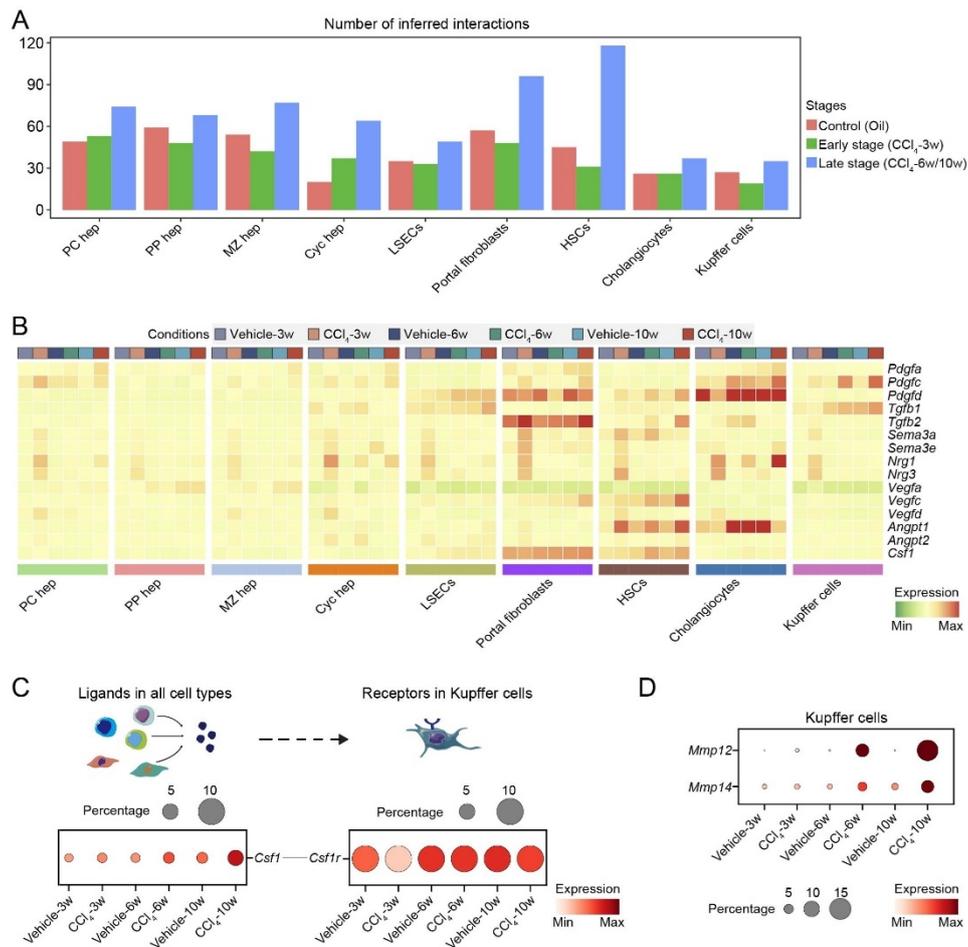


Supplementary Figure S3 Reanalysis of published scRNA-seq CCl₄-induced injury liver datasets, related to Figure 3

- A.** Monocle2-based trajectory reconstruction of HSC activation during liver fibrosis (our own dataset) colored by group.
- B.** UMAP visualization of HSCs taken from the scRNA-seq dataset of Yang et al. (2021) colored by group.
- C.** UMAP visualization of HSCs taken from the scRNA-seq datasets of Filliol et al. (2022) and Mederacke et al. (2022) colored by group.
- D.** Violin plots showing module scores of selected pathways from the functional enrichment shown in **Figure 3D** in HSCs taken from the scRNA-seq dataset of Yang et al. (2021).
- E.** Violin plots showing module scores of selected pathways from the functional

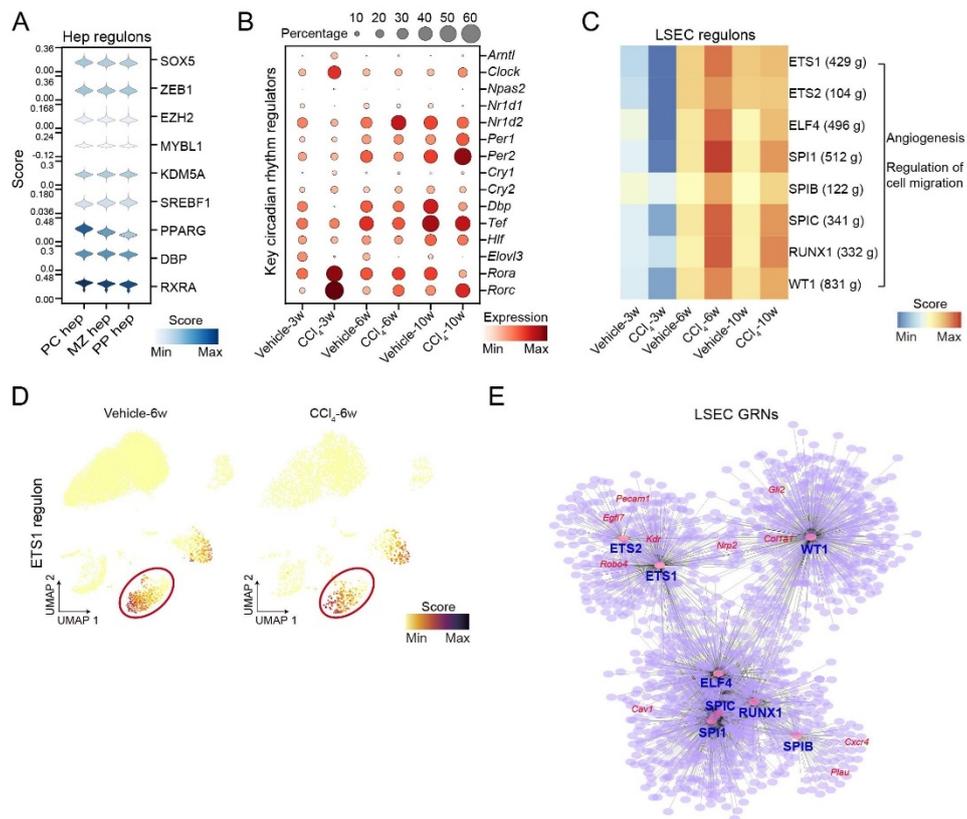
enrichment shown in **Figure 3D** in HSCs taken from the scRNA-seq datasets of Filliol et al. (2022) and Mederacke et al. (2022).

- F. Bubble plots showing expression levels of selected genes belonging to the indicated pathways in HSCs taken from the scRNA-seq dataset of Yang et al. (2021).
- G. Bubble plots showing expression levels of selected genes belonging to the indicated pathways in HSCs taken from the scRNA-seq datasets of Filliol et al. (2022) and Mederacke et al. (2022).



Supplementary Figure S4 Dynamics of cell-cell communication at different fibrosis stages, related to Figure 5

- A. Bar plots showing the number of inferred interactions detected in each cell type at each stage by CellChat.
- B. Heatmap showing average expression levels of ligands indicated in **Figure 5C, 5D and S4C** in each cell type for indicated conditions.
- C. Top: schematic of ligands interacting with the corresponding receptors in Kupffer cells. Bottom: bubble plot showing expression levels of *Csf1* in all cell types (left) and the corresponding receptor (*Csf1r*) in Kupffer cells (right) for indicated conditions.
- D. Bubble plot showing expression levels of *Mmp12* and *Mmp14* in Kupffer cells for the indicated conditions.



Supplementary Figure S5 SnRNA-seq analysis of gene regulatory dynamics in LSECs at different fibrosis stages, related to Figure 6

- A.** Violin plots showing regulon activity in pericentral, midzonal, and periportal hepatocytes.
- B.** Bubble plot showing expression levels of key circadian rhythm genes in hepatocytes for the indicated conditions.
- C.** Heatmap showing average regulon activity of LSECs for the indicated conditions. Representative enriched GO terms related to the corresponding regulons are indicated on the right side.
- D.** UMAP visualization showing regulon activity of ETS1 for the indicated conditions. Red circles indicate LSEC cluster.
- E.** GRNs in LSECs visualized by Cytoscape. Selected target genes of each regulon are labeled in the network.

SUPPLEMENTARY TABLES

Supplementary Table S1 Benchmarking of data generated in this study and previous scRNA-seq studies for CCl₄-induced liver fibrosis

Supplementary Table S2 DEGs in hepatocytes of CCl₄-treated group compared to control group at each time point

Supplementary Table S3 Functional enrichment of stage-specific genes in hepatocytes, HSCs, and LSECs

Supplementary Table S4 Gene modules learned by functional enrichment

Supplementary Table S5 Psupertime gene clustering of DEGs in HSCs

Supplementary Table S6 Psupertime gene clustering of DEGs in LSECs

Supplementary Table S7 Inferred cell-cell interactions during CCl₄-induced progressive liver fibrosis

Supplementary Table S8 Regulons and their corresponding target genes

Supplementary Tables S1–S8 are listed as separate Excel files due to their large size.