Recent estimates suggest that 844 million people worldwide have chronic liver disease, with two million deaths per year and a rising incidence. Iterative liver injury secondary to any cause leads to progressive fibrosis and ultimately results in liver cirrhosis. Notably, the degree of liver fibrosis predicts adverse patient outcomes. Hence, effective antifibrotic therapies for patients with chronic liver disease are urgently required.

Liver fibrosis involves a complex interplay between multiple non-parenchymal cell (NPC) lineages including immune, endothelial and mesenchymal cells spatially located within areas of scarring, termed the fibrotic niche. Despite progress in our understanding of liver fibrogenesis accrued using rodent models, there remains a considerable 'translational gap' between putative targets and effective patient therapies. This is in part due to limited definition of the functional heterogeneity and interactome of cell lineages that contribute to the fibrotic niche of human liver cirrhosis, which is imperfectly recapitulated by rodent models.

Liver cirrhosis is a major cause of death worldwide and is characterized by extensive fibrosis. There are currently no effective antifibrotic therapies available. To obtain a better understanding of the cellular and molecular mechanisms involved in disease pathogenesis and enable the discovery of therapeutic targets, here we profile the transcriptomes of more than 100,000 single human cells, yielding molecular definitions for non-parenchymal cell types that are found in healthy and cirrhotic human liver. We identify a scar-associated TREM2+CD9+ subpopulation of macrophages, which expands in liver fibrosis, differentiates from circulating monocytes and is pro-fibrogenic. We also define ACKR1+ and PLTVAP+ endothelial cells that expand in cirrhosis, are topographically restricted to the fibrotic niche and enhance the transmigration of leucocytes. Multi-lineage modelling of ligand and receptor interactions between the scar-associated macrophages, endothelial cells and PDGFRα+ collagen-producing mesenchymal cells reveals intra-scar activity of several pro-fibrogenic pathways including TNFRSF12A, PDGFR and NOTCH signalling. Our work dissects unanticipated aspects of the cellular and molecular basis of human organ fibrosis at a single-cell level, and provides a conceptual framework for the discovery of rational therapeutic targets in liver cirrhosis.

Single-cell RNA sequencing (scRNA-seq) is delivering a step change in our understanding of disease pathogenesis, allowing the interrogation of individual cell populations at unprecedented resolution. Here, we studied the mechanisms that regulate human liver fibrosis using scRNA-seq.

Single-cell atlas of human liver NPCs

Hepatic NPCs were isolated from healthy and cirrhotic human livers spanning a range of aetiologies of cirrhosis (Fig. 1a, Extended Data Fig. 1a). Leucocytes (CD45+) or other NPC (CD45−) fractions (Extended Data Fig. 1b) were sorted by flow cytometry before scRNA-seq analysis. To discriminate between liver-resident and circulating leucocytes, we also performed scRNA-seq on CD45+CD66b− peripheral blood mononuclear cells (PBMCs) (Extended Data Fig. 1c, g–i). The combined tissue and PBMC dataset was partitioned into clusters (Extended Data Fig. 1d) and annotated using signatures of known lineage markers (Extended Data Fig. 1e, f).
that allows assessment of NPC gene expression between healthy and cirrhotic livers.

**Distinct macrophages inhabit the fibrotic niche**

Previous studies in rodents have highlighted macrophage subpopulations that orchestrate both the progression and regression of liver fibrosis.\(^1\)\(^–\)\(^4\) Clustering of mononuclear phagocytes (MPs) identified ten clusters; annotated as scar-associated macrophages (SAMacs), Kupffer cells (KCs), tissue monocytes (TMs), conventional dendritic cells (cDCs) and cycling (proliferating) cells (Fig. 2a, Extended Data Fig. 4a, Supplementary Note 2). Clusters MP(4) and MP(5)—named SAMac(1) and SAMac(2), respectively—were expanded in cirrhotic livers (Fig. 2b), as confirmed by quantification of the MP cell composition of each liver individually (Fig. 2c).

Clusters MP(6) and MP(7) were enriched in the expression of CD163, MARCO and TIMD4 (Extended Data Fig. 4b); tissue staining confirmed these as KCs (resident liver macrophages), facilitating the annotation of these clusters as KC(1) and KC(2), respectively (Extended Data Fig. 4c). A lack of TIMD4 expression distinguished cluster KC(2) from KC(1) (Extended Data Fig. 4b); cell counting demonstrated TIMD4+ cell numbers to be equivalent between healthy and cirrhotic livers, but showed a loss of MARCO+ cells, consistent with a selective reduction in MARCO-TIMD4+ KC(2) in liver fibrosis (Fig. 2c, Extended Data Fig. 4d, e).

Scar-associated clusters SAMac(1) and SAMac(2) expressed the unique markers TREM2 and CD9 (Fig. 2d, e). These macrophages displayed a hybrid phenotype, with features of both TMs and KCs (Fig. 2d, e), analogous to monocyte-derived macrophages in mouse liver injury models.\(^7\) Flow cytometry confirmed expansion of TREM2+CD9+ macrophages in human fibrotic livers (Fig. 2f, Extended Data Fig. 4f). Conditioned medium from SAMacs after fluorescence-activated cell sorting (FACS) promoted fibrillar collagen expression by primary human hepatic stellate cells (HSCs) (Fig. 2g), indicating that SAMacs have a pro-fibrogenic phenotype. Tissue staining demonstrated the presence of TREM2+CD9+ MARCO+ scarring macrophages topographically localized in collagen-positive scar regions (Fig. 2h, Extended Data Fig. 4g–i), and significantly expanded in cirrhotic livers (Extended Data Fig. 4j, k). Cell counting of stained cirrhotic livers morphologically segmented into regions of fibrotic septae and parenchymal nodules, confirmed SAMac accumulation within the fibrotic niche (Extended Data Fig. 4i).

Local proliferation has a significant role in macrophage expansion at sites of fibrosis in rodent models.\(^8\)\(^–\)\(^10\) Cycling MP cells (Fig. 2a) subclustered into subpopulations of conventional dendritic cells (cDC1 and cDC2), KCs and SAMacs (Extended Data Fig. 4m, Supplementary Table 8). Cycling SAMacs expanded in cirrhosis (Extended Data Fig. 4m), which highlights the potential role of macrophage proliferation in promoting SAMac accumulation in the fibrotic niche.

**Pro-fibrogenic phenotype of SAMacs**

To delineate the functional profile of SAMacs, we visualized co-ordinately expressed gene groups across the MP subpopulations using self-organizing maps (Extended Data Fig. 5a). We identified six optimally differentiating metagene signatures, denoted as A–F (Extended Data Fig. 5a, Supplementary Table 9). Signatures A and B defined SAMacs and were enriched for ontology terms relevant to tissue fibrosis (Extended Data Fig. 5b). These SAMac-defining signatures included genes such as TREM2, IL1B, SPPI, LGALS3, CCR2 and TNFSF12, some of which are known to regulate the function of scar-producing myofibroblasts in fibrotic liver diseases.\(^10\)\(^–\)\(^13\) The remaining MP subpopulations were defined by signature C (KCs), signatures D, E (TMs) and signature F (cDC1); ontology terms matched known functions for the associated cell type (Extended Data Fig. 5b, Supplementary Table 9).

In mice, under homeostatic conditions, embryologically derived self-renewing tissue-resident KCs predominate.\(^14\)\(^–\)\(^16\) However, after...
injury, macrophages derived from circulating monocytes accumulate in the liver and regulate fibrosis\(^8\). The ontogeny of human hepatic macrophage subpopulations is unknown. TREM2\(^+\)CD9\(^+\) SAMacs demonstrate a monocyte-like morphology (Fig. 2h, Extended Data Fig. 4g–i) and a distinct topographical distribution from KCs (Extended Data Fig. 4l). To assess the origin of SAMacs, we performed in silico trajectory analysis on a combined dataset of peripheral blood monocytes and liver-resident MPs. We visualized the transcriptional profile of these cells (Fig. 3a, Extended Data Fig. 5c), mapped them along a pseudotemporal trajectory and interrogated their directionality via spliced and unspliced mRNA ratios (RNA velocity\(^9\)). These analyses suggested a differentiation trajectory from peripheral blood monocytes into either SAMacs or cDCs, with no differentiation from KCs to SAMacs, and no progression from SAMacs to KCs (Fig. 3a, Extended Data Fig. 5c). Additional RNA velocity analyses\(^9\) showed downregulation (negative velocity) of the monocyte gene MNDA in SAMacs, upregulation (positive velocity) of the SAMac marker gene CD9 in TMs, and a lack of KC gene TIMD4 velocity in SAMacs (Extended Data Fig. 5d). Furthermore, assessment of the probabilities of cells in this dataset transitioning into SAMacs indicated a higher likelihood of TMs than KCs differentiating into SAMacs (Fig. 3b). Overall, these data suggest that SAMacs are monocyte-derived, and represent a terminally differentiated cell state within the fibrotic niche.

To characterize the SAMac phenotype further, we identified differentially expressed genes along monocyte differentiation trajectories. We defined three gene co-expression modules, with module 1 representing upregulated genes during blood monocyte-to-SAMac differentiation (Fig. 3c). Module 1 contained multiple pro-fibrogenic genes including SPP1, LGALS3, CCL2, CXCL8, PDGFB and VEGFA\(^10,11\), and displayed ontology terms that are consistent with the promotion of tissue fibrosis and angiogenesis (Fig. 3c, d, Supplementary Table 10). Module 2 contained genes that were downregulated during the differentiation of monocytes to SAMacs (Fig. 3c, Extended Data Fig. 5e), whereas module 3 encompassed a group of upregulated genes during the differentiation from monocytes to cDCs (Fig. 3c, Extended Data Fig. 5f). SAMacs isolated from cirrhotic human livers (Fig. 2f, Extended Data Fig. 4f) demonstrated enhanced protein secretion of several of the mediators identified by transcriptional analysis (Extended Data Fig. 5g) and promoted fibriar collagen expression by primary human HSCs (Fig. 2g), which confirms that SAMacs have a pro-fibrogenic phenotype.

To enable cross-species comparison, we performed scRNA-seq on liver MP cells isolated from control mice or mice treated with chronic carbon tetrachloride (CCl\(_4\))—a mouse model of liver fibrosis\(^2\). MP cells from fibrotic livers were isolated 24 h after the final CCl\(_4\) injection, a time of active fibrogenesis\(^2\). Five MP cell clusters were defined (Extended Data Table 11), and injury-specific cluster mMP(2) was differentiated by high expression of CD9, Trem2, Spp1 and Lgals3 (Extended Data Fig. 6a–d). We confirmed expansion of this CD9\(^+\) mSAMac population in liver fibrosis (Extended Data Fig. 6e, f) and co-culture of mSAMAs with quiescent primary mouse HSCs promoted fibriar collagen expression in HSCs (Extended Data Fig. 6g). Canonical
correlation analysis between human and mouse MP datasets demonstrated that human and mouse SAMacs clustered together (Extended Data Fig. 6i, j) and that this cluster was enriched for SAMac markers CD9, TREM2 and SPP1 (Extended Data Fig. 6j), confirming that mouse SAMacs represent a corollary population to human SAMacs.

To identify potential transcriptional regulators of human SAMacs, we defined sets of genes co-expressed with known transcription factors (regulons) along the tissue monocyte-to-macrophage pseudotemporal trajectory and in KCs (Extended Data Fig. 5g, h, Supplementary Table 12). We defined sets of genes co-expressed with known transcription factors (regulons) along the tissue monocyte-to-macrophage pseudotemporal trajectory and in KCs (Extended Data Fig. 5g, h, Supplementary Table 12).

To determine whether SAMacs expand in earlier-stage human liver disease, we analysed cohorts of patients with non-alcoholic fatty liver disease (NAFLD). Application of differential gene expression signatures of human SAMacs, KCs and TMs to a deconvolution algorithm enabled the assessment of hepatic monocyte–macrophage pseudotemporal trajectory and in KCs (Extended Data Fig. 5a, b). This identified regulons and corresponding transcription factors associated with distinct macrophase phenotypes, highlighting HES3 and EGR2 activity in SAMacs.

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In summary, these data demonstrate that TREM2+CD9+ SAMacs derive from the recruitment and differentiation of circulating monocytes, are conserved across species, display a pro-fibrogenic phenotype and expand early in the course of liver disease progression.

**Endothelial subpopulations inhabit the fibrotic niche**

In rodent models, hepatic endothelial cells are known to regulate fibrogenesis. Clustering of human liver endothelial cells identified seven subpopulations (Fig. 4a). Classical endothelial cell markers did not discriminate between the seven clusters, although Endo(1) was distinct in lacking CD34 expression (Extended Data Fig. 8a). To annotate endothelial subpopulations fully (Supplementary Note 3, Extended Data Fig. 8k), we identified differentially expressed markers (Fig. 4c, Supplementary Table 13), determined functional expression profiles (Extended Data Fig. 8g, Supplementary Table 14), performed analysis

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**Fig. 3 | Pro-fibrogenic phenotype of SAMacs.** a, Uniform manifold approximation and projection (UMAP) visualization of 23,075 cells from liver-resident MPs (healthy, n = 5; cirrhotic, n = 5) and blood monocytes (PBMCs, n = 5), annotating monocyte pseudotemporal dynamics (purple to yellow). RNA velocity field (red arrows) visualized using Gaussian smoothing on regular grid. Right, annotation of MP subpopulations and injury condition. b, Transition probabilities per SAMac subpopulation, indicating for each cell the likelihood of transition into either SAMac(1) or SAMac(2), calculated using RNA velocity (yellow, high; purple, low; grey, below threshold of 2 × 10−4). c, Heat map with spline curves fitted to genes differentially expressed across blood monocyte-to-SAMac (right arrow) and blood monocyte-to-cDC (left arrow) pseudotemporal trajectories, grouped by hierarchical clustering (k = 3). Gene co-expression modules (colour) and exemplar genes from each module are labelled (right). d, Spline curve fitted to averaged expression of all genes in module 1 along the monocyte-to-SAMac pseudotemporal trajectory (left), with selected enrichment of Gene Ontology terms (right). P values determined by Fisher’s exact test.

**Fig. 4 | Identifying scar-associated endothelial subpopulations.** a, Clustering 8,820 endothelial cells from healthy (n = 4) and cirrhotic (n = 3) human livers, annotating injury condition (right). b, Fractions of endothelial subpopulations in healthy (n = 4) and cirrhotic (n = 3) livers. c, Heat map of endothelial cluster marker genes (colour coded by cluster and condition), with exemplar genes labelled (right). Columns denote cells; rows denote genes. d, Representative immunofluorescence images (n ≥ 3) of CD34 (red), CLEC4M (white), PLVAP (green) and DAPI (blue) in healthy and cirrhotic human liver. e, Digital pixel quantification of CLEC4M staining in healthy (n = 5) and cirrhotic (n = 8) liver, PLVAP staining in healthy (n = 11) and cirrhotic (n = 11) liver, and ACKR1 staining in healthy (n = 10) and cirrhotic (n = 10) liver. All scale bars, 50 μm. Data are mean ± s.e.m. P values determined by Wald test (b) or two-tailed Mann–Whitney test (e).
of transcription factor regulons (Extended Data Fig. 8h, Supplementary Table 15) and assessed spatial distribution via tissue staining (Fig. 4d, Extended Data Fig. 8j).

Disease-specific endothelial cells Endo(6) and Endo(7) (CD34+PLVAP+VWA1 and CD34+PLVAP+ACKRI, respectively; Fig. 4a–c, Extended Data Fig. 8b) expanded in cirrhotic liver tissue (Fig. 4e) and were restricted to the fibrotic niche (Fig. 4d, e, Extended Data Fig. 8c), allowing annotation as scar-associated endothelia SAEndo(1) and SAEndo(2), respectively. By contrast, CD34+CLEC4M+Endo(3) (annotated as liver sinusoidal endothelial cells), were reduced in cirrhotic livers (Fig. 4b, e). Metagene signature analysis demonstrated that Endo(6) (SAEndo(1)) cells expressed pro-fibrogenic genes including PDGFD, PDGFB, LOX and LOXL2; associated ontology terms included extracellular matrix organization (signature A; Extended Data Fig. 8g). Endo(7) (SAEndo(2)) cells displayed an immunomodulatory phenotype (signature B; Extended Data Fig. 8g). The most discriminatory marker for this cluster, ACKRI, has a role in regulating leucocyte recruitment. We confirmed increased expression of PLVAP, CD34 and ACKRI on endothelial cells isolated from cirrhotic livers (Extended Data Fig. 8d). Flow-based adhesion assays demonstrated that cirrhotic endothelial cells display enhanced leucocyte transmigration (Extended Data Fig. S8e), which was attenuated by ACKRI knockdown (Extended Data Fig. 8f).

**PDGFRα expression defines SAMes cells**

Clustering of human liver mesenchymal cells identified four populations (Fig. 5a, b, Extended Data Fig. 9a, Supplementary Table 16). Cluster Mes(1), distinguished by MYH11 expression (Fig. 5b, Extended Data Fig. 9a), was identified as vascular smooth muscle cells (VSMCs) (Fig. 5c). Mes(4) demonstrated expression of mesothelial markers (Fig. 5b, Extended Data Fig. 9a). Cluster Mes(2) expressed high levels of RGSS (Fig. 5b, Extended Data Fig. 9a), and RGSS staining identified this population as HSCs (Fig. 5c). RGSS+ cells were absent from the fibrotic niche (Fig. 5c). Cluster Mes(3) (distinguished by PDGFRα expression) expressed high levels of fibrillar collagens and pro-fibrogenic genes (Fig. 5b, d, Extended Data Fig. 9a). PDGFRα+ cells expanded in cirrhotic livers (Fig. 5a, e, f) and were mapped to the fibrotic niche (Fig. Sf), enabling annotation as scar-associated mesenchymal (SAMes) cells.

To study SAMes cell heterogeneity, further clustering (Extended Data Fig. 9b) identified two populations of SAMes cells (Extended Data Fig. 9c,d, Supplementary Table 17). OSIR expression distinguished cluster SAMesB (Extended Data Fig. 9c), and labelled a subpopulation of periportal cells in healthy liver and scar-associated cells in the fibrotic niche (Extended Data Fig. 9e, f). Cluster SAMesB also expressed other known portal fibroblast markers (Extended Data Fig. 9g).

In rodent liver fibrosis models, HSCs differentiate into scar-producing myofibroblasts. Pseudotemporal ordering and RNA velocity analyses demonstrated a trajectory from human HSCs to SAMes cells (Extended Data Fig. 9h). Assessment of gene co-expression modules along the HSC-to-SAMES differentiation continuum indicated upregulation of fibrogenic genes including COLIA1, COLIA2, COL3A1 and TIMP1 and downregulation of genes including RGSS, IGFBPS, ADAMTS1 and GEM, which are known to be downregulated in mouse HSC in response to liver injury (Extended Data Fig. 9i).

The multi-lineage interactome in the fibrotic niche

Having defined the populations of scar-associated macrophages, endothelial and mesenchymal cells, we confirmed the close topographical association of these cells within the fibrotic niche (Extended Data Fig. 10a, b), and used CellPhoneDB to perform an unbiased ligand–receptor interaction analysis between these populations.

Numerous statistically significant paracrine and autocrine interactions were detected between ligands and cognate receptors expressed by SAMac, SAEndo and SAMes cells (Supplementary Table 18, Extended Data Fig. 10f–n). To interrogate how scar-associated NPCs regulate fibrosis and to identify tractable therapeutic targets, we focused functional analyses on interactions with SAMes (Fig. 6a, e, Extended Data Fig. 10d). In keeping with our data demonstrating that SAMacs promote fibrillar collagen expression in HSCs (Fig. 2g), SAMacs expressed epidermal growth factor receptor (EGFR) ligands that are known to regulate mesenchymal cell activation (Fig. 6a). In addition, SAMacs expressed the mesenchymal cell mitogens TNFSF12 and PDGFB, signaling to cognate receptors TNFRSF12A and PDGFRα on SAMes (Fig. 6a). We confirmed localization of these ligand–receptor pairs within the fibrotic niche (Fig. 6b). Both TNFSF12 and PDGFB-induced proliferation of primary human HSCs, which was inhibited by blockade of TNFSF12A and PDGFRα, respectively (Fig. 6c, d). Conditioned medium from primary human SAMacs promoted primary human HSC proliferation ex vivo (Extended Data Fig. 10c), demonstrating a functional role for SAMacs in regulating SAMes cell expansion.

SAEndo cells expressed high levels of Notch ligands JAG1, JAG2 and DLL4 interacting with Notch receptor NOTCH3 on SAMes cells (Fig. 6e). NOTCH3 was identified on PDGFRα+ SAMes cells within the fibrotic niche (Fig. 6f), and primary endothelial cells from cirrhotic human liver demonstrated increased expression of JAG1 (Fig. 6g). Co-culture of primary human HSCs and endothelial cells from cirrhotic livers promoted fibrillar collagen production by HSCs, which was inhibited by addition of...
of the Notch-signalling inhibitor dibenzazepine (Fig. 6h). Furthermore, knockdown of NOTCH3 expression in primary human HSCs resulted in reduced fibrillar collagen expression (Fig. 6i).

In summary, our unbiased dissection of the key ligand–receptor interactions between scar-associated NPCs highlights TNFRSF12A, PDGFRα and Notch signalling as important regulators of mesenchymal cell function within the human liver fibrotic niche.

Discussion

Here, using scRNA-seq and spatial mapping, we resolve the fibrotic niche of human liver cirrhosis, identifying pathogenic subpopulations of TREM2+CD9+ macrophages, ACKR1+ and PLVAP+ endothelial cells and PDGFRα+ collagen-producing myofibroblasts. We dissect a complex, pro-fibrotic interactome between multiple scar-associated cell lineages and identify highly relevant intra-scar pathways that are potentially druggable. In this era of precision medicine, this unbiased multi-lineage approach should inform the design of highly targeted combination therapies that will very likely be necessary to achieve effective antifibrotic potency.

Application of our novel scar-associated cell markers could also potentially inform molecular pathology-based patient stratification, which is fundamental to the prosecution of successful antifibrotic clinical trials. Our work illustrates the power of single-cell transcriptomics to decode the cellular and molecular basis of human organ fibrosis, providing a conceptual framework for the discovery of relevant therapeutic targets to treat patients with a broad range of fibrotic diseases.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions; and statements of data and code availability are available at [https://doi.org/10.1038/s41586-019-1631-3].

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METHODS

Study subjects
Local approval for procuring human liver tissue and blood samples for scRNA-seq, flow cytometry and histological analysis was obtained from the NRS BioResource and Tissue Governance Unit (study number SR574), following review at the East of Scotland Research Ethics Service (reference 15/ES/0094). All subjects provided written informed consent. Healthy background non-lesional liver tissue was obtained intraoperatively from patients undergoing surgical liver resection for solitary colorectal metastasis at the Hepatobiliary and Pancreatic Unit, Department of Clinical Surgery, Royal Infirmary of Edinburgh. Patients with a known history of chronic liver disease, abnormal liver function tests or those who had received systemic chemotherapy within the last four months were excluded from this cohort. Cirrhotic liver tissue was obtained intraoperatively from patients undergoing orthotopic liver transplantation at the Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh. Blood from patients with a confirmed diagnosis of liver cirrhosis were obtained from patients attending the Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh. Patients with liver cirrhosis due to viral hepatitis were excluded from the study. Patient demographics are summarized in Extended Data Fig. 1a. Isolation of primary hepatic macrophage subpopulations and endothelial cells from healthy and cirrhotic livers for cell culture and analysis of secreted mediators was performed at the University of Birmingham, UK. Local ethical approval was obtained (reference 06/Q2708/11, 06/Q2702/61) and all patients provided written, informed consent. Liver tissue was acquired from explanted diseased livers from patients undergoing orthotopic liver transplantation, resected liver specimens or donor livers rejected for transplant at the Queen Elizabeth Hospital, Birmingham. For histological assessment of NAFLD biopsies, anonymized unstained formalin-fixed paraffin-embedded liver biopsy sections encompassing the complete NAFLD spectrum were provided by the Lothian NRS Human Annotated Bioresource under authority from the East of Scotland Research Ethics Service REC 1, reference 15/ES/0094.

Human tissue processing
Importantly, to minimize artefacts, we developed a rapid tissue processing pipeline, obtaining fresh non-lesional liver tissue taken by wedge biopsy before the interruption of the hepatic vascular inflow during liver surgery or transplantation, and immediately processing this for FACS. This enabled a workflow time of under three hours from patient to single-cell droplet encapsulation.

For human liver scRNA-seq and flow cytometry analyses, a wedge biopsy of non-lesional fresh liver tissue (2–3 g) was obtained by the operating surgeon. This was immediately placed in HBSS (Gibco) on ice. The tissue was then transported directly to the laboratory and dissociation routinely commenced within 20 min of the liver biopsy. To enable paired histological assessment, a segment of each liver specimen was also fixed in 4% neutral-buffered formalin for 24 h followed by paraffin-embedding. Additional liver samples, obtained via the same method, were fixed in an identical manner and used for further histological analysis. For human macrophage cell sorting and endothelial cell isolation, liver tissue (40 g) was used from cirrhotic patients undergoing orthotopic liver transplantation or control samples from donor liver or liver resection specimens.

Mice
Adult male C57BL/6JCrI mice aged 8–10 weeks were purchased from Charles River. Mice were housed under specific pathogen-free conditions at the University of Edinburgh. All experimental protocols were approved by the University of Edinburgh Animal Welfare and Ethics Board in accordance with UK Home Office regulations. Liver fibrosis was induced with 4 weeks (nine injections) of twice-weekly intraperitoneal CC14, at a dose of 0.4 μl g−1 body weight, diluted 1:3 in olive oil as previously described. Mice were randomly assigned to receive CCl4, or to serve as healthy controls. No sample size calculation or blinding was performed. Liver tissue was obtained 24 h after the final CCl4 injection, a time of active fibrogenesis. Comparison was made to age-matched uninjured mice.

Preparation of single-cell suspensions
For human liver scRNA-seq, liver tissue was minced with scissors and digested in 5 mg ml−1 pronase (Sigma-Aldrich, PS147-5G), 2.93 mg ml−1 collagenase D (Roche, 11088882001), and 0.019 mg ml−1 DNase (Roche, 10104159001) at 37 °C for 30 min with agitation (200–250 r.p.m.), then strained through a 120-μm nylon mesh along with PEB buffer (PBS, 0.1% BSA and 2mM EDTA) including DNase (0.019 mg ml−1). Thereafter, all processing was done at 4 °C. The cell suspension was centrifuged at 400 g for 7 min, supernatant removed, cell pellet resuspended in PEB buffer and DNase added (0.019 mg ml−1), followed by additional centrifugation (400 g, 7 min). Red blood cell lysis was performed (BioLegend, 420301), followed by centrifugation (400g, 7 min), resuspension in PEB buffer and staining through a 35-μm filter. Following another centrifugation at 400g for 7 min, cells were blocked in 10% human serum (Sigma-Aldrich, H4522) for 10 min at 4 °C before antibody staining.

For human liver macrophage flow cytometry analysis and cell sorting, and for mouse liver macrophage flow cytometry, cell sorting and scRNA-seq, single-cell suspensions were prepared as previously described, with minor modifications. In brief, liver tissue was minced and digested in an enzyme cocktail 0.625 mg ml−1 collagenase D (Roche, 11088882001), 0.85 mg ml−1 collagenase V (Sigma-Aldrich, C9263-1G), 1 mg ml−1 dispase (Gibco, Invitrogen, 17105-041) and 30 U ml−1 DNase (Roche, 10104159001) in RPMI-1640 at 37 °C for 20 min (mouse) or 45 min (human) with agitation (200–250 r.p.m.), before being passed through a 100-μm filter. After lysis of red blood cells (BioLegend, 420301), cells were washed in PEB buffer and passed through a 35-μm filter. Before the addition of antibodies, cells from human samples were blocked in 10% human serum (Sigma-Aldrich, H4522) and mouse samples were blocked in anti-mouse CD16/32 antibody (1:100; BioLegend, 101302) and 10% normal mouse serum (Sigma, M5905) for 10 min at 4 °C.

For human PBMC scRNA-seq, 4.9-ml peripheral venous blood samples were collected in EDTA-coated tubes (Sarstedt, S-Monovette 4.9ml K3E) and placed on ice. Blood samples were transferred into a 50-ml Falcon tube. After lysis of red blood cells (BioLegend, 420301), blood samples were then centrifuged at 500g for 5 min and supernatant was removed. Pelleted samples were then resuspended in staining buffer (PBS plus 2% BSA; Sigma-Aldrich) and centrifugation was repeated. Samples were then resuspended in 10% human serum (Sigma-Aldrich, H4522) in staining buffer on ice for 30 min. Cells were then resuspended in staining buffer and passed through a 35-μm filter before antibody staining.

Flow cytometry and cell sorting
Incubation with primary antibodies was performed for 20 min at 4 °C. All antibodies, conjugates, lot numbers and dilutions used in this study are presented in Supplementary Table 19. After antibody staining, cells were washed with PBS buffer. For human macrophage flow cytometry analysis and cell sorting, cells were then incubated with streptavidin-BV711 for 20 min at 4 °C (BioLegend 405241; 1:200). For human and mouse cell sorting (FACS) and mouse flow cytometry analysis, cell viability staining (DAPI; 1:1,000) was then performed, immediately before acquiring the samples.

Human cell sorting for scRNA-seq was performed on a BD Influx (Becton Dickinson). Viable single CD45 (leucocytes) or CD45+ (other non-parenchymal cells) cells were sorted from human liver tissue (Extended Data Fig. 1b) and viable CD45– CD66b+ (PBMC) cells were sorted from peripheral blood (Extended Data Fig. 1c) and processed for droplet-based scRNA-seq.

To generate conditioned medium from cirrhotic liver macrophage subpopulations, cells were sorted on a BD FACSAria Fusion (Becton Dickinson). Sorted SAMacs (viable CD45 Lin HLA-DR CD14+ CD16– CD163 TREM2+ CD95+), TMs (viable CD45 Lin HLA-DR CD14+ CD16+ CD163 TREM2 CD95–) were then cultured in serum-free media for three days before being conditioned for 72 h.
CD163+TREM2+CD9+ and KCs (viable CD45Lin HLA-DR CD14+CD16+CD163+CD9+) were plated in 12-well plates (Corning, 3513) in DMEM (Gibco, 41965039) containing 2% fetal bovine serum (FBS; Gibco, 10500056) at 1 x 10^6 cells per ml for 24 h at 37°C 5% CO2. Control wells contained medium alone. Conditioned medium was collected, centrifuged at 400g for 10 min, and supernatant was stored at -80°C.

For human macrophage flow cytometry analysis, after surface antibody staining, cells were stained with Zombie NIR fixable viability dye (BioLegend, 423105) according to the manufacturer’s instructions. Cells were washed in PBS then fixed in Intracellular (IC) Fixation Buffer (Thermo Fisher, 00-8222-49) for 20 min at 4°C. Fixed samples were stored in PBS at 4°C until acquisition. Flow cytometry acquisition was performed on a six-laser Fortessa flow cytometer (Becton Dickinson). The gating strategy is shown in Extended Data Fig. 4f and Fig. 2f.

Mouse macrophage cell sorting for scRNA-seq and co-culture experiments was performed on a BD FACSAria (Becton Dickinson). For scRNA-seq, viable CD45+Lin(CD3, NK1.1, Ly6G, CD19)- cells were sorted from healthy (n = 3) and CCl4-treated (n = 3) mice and processed for droplet-based scRNA-seq. For transwell co-culture, viable CD45+Lin CD11b F4/80 TIMD4+CD9- (SAMacs) or CD9+ (TMcs) cells were sorted from CCl4-treated mice (Extended Data Fig. 6e). Flow cytometry analysis on macrophages from healthy and CCl4-treated mice was also performed on a BD FACSAria (Becton Dickinson), using the same gating strategy (Extended Data Fig. 6e). All flow cytometry data were analysed using FlowJo software (Treestar).

**Luminex assay**
Detection of CCL2, galectin-3, IL-1β, CXCL8 and osteopontin (SPP1) and CD163 proteins in conditioned medium from human liver macrophage subpopulations was performed using a custom human luminex assay (R&D Systems), according to the manufacturer’s protocol. Data were acquired using a Bio-Plex 200 (Bio-Rad) and are presented as MFI for each analyte.

**Cell culture**
Primary human HSCs (ScienCell, 5300) were cultured in stellate cell medium (SteCM, ScienCell, 3301) on poly-L-lysine (Sigma, P4832)-coated 3T3 tissue culture flasks, according to the supplier’s protocol. All experiments were performed using cells between passages 3 and 5. For assessment of fibrillar collagen gene expression, HSCs were plated at 75,000 cells per well in 24-well plates (Corning, 3513) in DMEM (Gibco, 21969-035) with 20 μM HEPES (Sigma, H3375), 2 mM glutamine (Sigma, G6784), VEGF (10 ng ml⁻¹; Peprotech, 100-39). Expression was determined empirically using the FlexiTube GeneSolution kit (Qiagen, GS4854). HSCs were treated with control siRNA (Qiagen, 1027280) and siRNA for NOTCH3 (Qiagen, HS NOTCH3 3, SI00009513; knockdown 83%) were then assessed for fibrillar collagen gene expression.

**Mouse HSC activation**
Primary mouse HSCs were isolated from healthy mice as previously described28. In brief, after cannulation of the inferior vena cava, the portal vein was cut to allow retrograde step-wise perfusion with pronase (Sigma, P5147) and collagenase D (Roche, 11088882001) containing solutions, before ex vivo digestion in a solution containing pronase, collagenase D and DNAse (Roche, 10104159001). HSCs were isolated from the digest solution by Histodenz (Sigma, D2158-100G) gradient centrifugation. HSCs were plated at a density of 400,000 cells per well in a 24-well plate (Corning, 3524) in HSC medium containing 10% FBS. After overnight culture, cells were washed with PBS and cultured in HSC medium containing 2% FBS. For macrophage coculture, transwell inserts (0.4-μm polyester membrane; Costar, 3470) were then placed above adherent HSCs. FACs-sorted CD9+ mouse SAMacs or CD9- mouse TMcs from CCl4-treated mice were resuspended in HSC medium containing 2% FBS at 400,000 cells per ml and 200,000 cells were added to the top of the transwell insert. Co-culture proceeded for 48 h and HSCs were collected for RNA. Quiescent HSCs (collected at the start of co-culture) were used as a control population.

**Isolation of human liver endothelial cells**
Human liver endothelial cells were isolated from cirrhotic explant livers and non-fibrotic control donor liver as previously described26. In brief, endothelial cells were cultured on plasticware coated with rat-tail collagen (Sigma, C3867) in complete endothelial medium consisting of endothelial basal medium (Thermo Fisher, 1110044) containing 10% heparin-inactivated human serum (tcsBiosciences, CS100-500), 100 U penicillin, 100 μg ml⁻¹ streptomycin, 2 mM glutamine (Sigma, G6784), VEGF (10 ng ml⁻¹; Peprotech, 100-20) and 10 ng ml⁻¹ HGF (10 ng ml⁻¹; Peprotech, 100-39). Expression of PLVAP, CD34, ACKR1 and JAG1 was assessed using flow cytometry.

**Flow-based adhesion assays**
Flow-based adhesion assays were performed as previously described11,12. In brief, endothelial cells from healthy and cirrhotic liver were seeded onto a rat-tail collagen-coated Ibidi slide V14 (Ibidi, 80606) at a density to give a monolayer and incubated overnight. Peripheral blood was collected from healthy donors in EDTA-coated tubes. PBMCs were isolated using a lymphocyte density gradient (Cedarlane Laboratories) then washed in PBS containing 1 mM Ca²⁺, 0.5 mM Mg²⁺ and 0.1% bovine serum albumin. Monocytes were enriched from PBMCs using a pan-monocyte isolation kit (Miltenyi Biotech, 130-096-337) according
to the manufacturer’s protocol. For flow-based adhesion assays, cells were resuspended at 10^4 cells per millilitre in endothelial basal media (Thermo Fisher, 1111044) containing 0.15% BSA, then perfused over the endothelial cell monolayer for 5 min at 0.28 ml min⁻¹. Non-adherent cells were washed off during 5 min perfusion of 0.15% BSA human basal endothelial medium and 10 random non-overlapping images were randomly recorded from each channel. Total adherent (bright-phase; expressed as cell number per mm² per million cells perfused) and transmigrating cells (dark-phase; expressed as percentage total adherent cells) on an endothelial cell monolayer from each patient were counted and quantified as previously described²².

Gene knockdown in endothelial cells

Knockdown of ACKR1 and PLVAP gene expression in human cirrhotic endothelial cells was performed using siRNA as previously described²². In brief, siRNA duplexes for PLVAP, ACKR1 or negative control (Qiagen, 1027280) with Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher, 13778075) were prepared in OptiMEM (Thermo Fisher, 39880070) according to the manufacturer’s recommendations, and used at a concentration of 25 nM. Cells were exposed to the duplex for 4 h at 37°C, after which time the medium was replaced with endothelial basal medium containing 10% heat-inactivated human serum for 24 h. The medium was then replaced with complete endothelial medium and incubated at 37°C with 5% CO₂ for a further 24 h. Knockdown efficacy was assessed by flow cytometry and the MF1 (Extended Data Fig. 8f). The best siRNA for knockdown was determined empirically using the FlexiTube GeneSolution Kit (Qiagen, GS3483 (PLVAP) and GS2332 (ACKR1)). For flow-based adhesion assays, siRNAs against PLVAP (Qiagen, HS_PLVAP_1, SI00667547; knockdown 50.6%), ACKR1 (Qiagen, HS_Fy_5, SI02627667; knockdown 37.7%) or control siRNA were selected. Then, 90,000 endothelial cells from cirrhotic patients (n = 6) were seeded into channels of a rat-tail collagen-coated Ibidi slide VI0.4 and gene knockdown was performed, followed by flow-based adhesion assay as described above.

Co-culture of endothelial cells and HSCs

HSCs (15,000 cells) were seeded onto an Ibidi slide VI0.4 with and without primary human endothelial cells (15,000 cells) from individual patients with cirrhosis (n = 3) in complete endothelial medium. After 2 h, all growth factor supplements were removed and cells were cultured for a further 72 h in endothelial basal medium containing 10% heat-inactivated human serum with or without the Notch signalling inhibitor dibenzazepine (10 μM; Bio-Techne, 4489/10) or vehicle (DMSO) control. Cells were fixed in 4% paraformaldehyde (PFA) for 30 min, permeabilized with 0.3% Triton X-100 in PBS for 5 min and blocked with 10% goat serum in PBS for 30 min followed by primary antibody incubation (mouse anti-PECAM and rabbit anti-collagen 1; see Supplementary Table 19) for 1 h. Cells were washed in 0.1% Triton X-100 in PBS followed by addition of fluorescently conjugated secondary antibodies (1:500 dilution) for 1 h. Cells were mounted with Pro-long Gold anti-fade DAPI, images were taken on the Confocal Microscope Zeiss LSM780, and the collagen I staining area was quantified using IMARIS.

RNA extraction and RT–qPCR

RNA was isolated from HSCs using the RNeasy Plus Micro Kit (Qiagen, 74034) and cDNA synthesis performed using the Quantitect Reverse Transcription Kit (Qiagen, 205313) according to the manufacturer’s protocol. Reactions were performed in triplicate in 384-well plate format and were assembled using the QIAgility automated pipetting system (Qiagen). RT–qPCR for human HSCs was performed using PowerUp SYBR Green Master Mix (Thermo Fisher, A25777) with the following primers (all Qiagen): GAPDH (QT00079247), COL1A1 (QT00037793), COL3A1 (QT0008233) and NOTCH3 (QT00003374). RT–qPCR for mouse HSCs was performed using TaqMan Fast Advanced Master Mix (Thermo Fisher, 4444537) with the following primers: Gapdh (Thermo Fisher, Mm99999915_g1) and Col3a1 (Thermo Fisher, Mm00802300_m1). Samples were amplified on an ABI 7900HT FAST PCR system (Applied Biosystems, Thermo Fisher Scientific). Data were analysed using Thermo Fisher Connect cloud qPCR analysis software (Thermo Fisher Scientific). The 2⁻ΔΔCT quantification method, using GAPDH for normalization, was used to estimate the amount of target mRNA in samples, and expression calculated relative to average mRNA expression levels from control samples.

Immunohistochemistry, immunofluorescence and single-molecule FISH

Formalin-fixed paraffin-embedded human liver tissue was cut into 4-μm sections, dewaxed, rehydrated, then incubated in 4% neutral-buffered formalin for 20 min. After heat-mediated antigen retrieval in pH 6 sodium citrate (microwave; 15 min), slides were washed in PBS and incubated in 4% hydrogen peroxide for 10 min. Slides were then washed in PBS, blocked using protein block (GeneTex, GTX30963) for 1 h at room temperature before incubation with primary antibodies for 1 h at room temperature. A full list of primary antibodies and conditions is shown in Supplementary Table 19. Slides were washed in PBS plus 0.1% Tween 20 (PBST; Sigma-Aldrich, P1379) then incubated with ImmPact HRP Polymer Detection Reagents (depending on species of primary; rabbit, MP-7401; mouse, MP-6402-15; goat, MP-7403; all Vector Laboratories) for 30 min at room temperature. Slides were washed in PBS followed by detection. For DAB staining, sections were incubated with DAB (DAKO, K3468) for 5 min and washed in PBS before a haematoxylin (Vector Laboratories, H3404) counterstain. For multiplex immunofluorescence staining, following the incubation with ImmPact and PBS wash, initial staining was detected using Cy3, Cy5, or fluorescein tyramide (Perkin-Elmer, NE-740021KIT) at a 1:1,000 dilution. Slides were then washed in PBST followed by further heat treatment with pH 6 sodium citrate (15 min), washes in PBS, protein block, incubation with the second primary antibody (incubated overnight at 4°C), ImmPact Polymer and tyramide as before. This sequence was repeated for the third primary antibody (incubated at room temperature for 1 h) and a DAPI-containing mountant was then applied (Thermo Fisher Scientific, P36931).

For AMEC staining (only CLEC4A immunohistochemistry), all washes were carried out with TBST (dH₂O, 200 mM Tris, 1.5 mM CaCl₂, 1% Tween-20 (all Sigma-Aldrich) pH 7.5) and peroxidase blocking was carried out for 30 min in 0.6% hydrogen peroxide in methanol. Sections were incubated with AMEC (Vector Laboratories, SK-4285) for 20 min and washed in TBST before a haematoxylin (Vector Laboratories, SK-4285) counterstain.

For combined single-molecule fluorescent in situ hybridization (smFISH) and immunofluorescence, detection of TREM2 was performed using the RNAscope 2.5 LS Reagent Kit BrownAssay (Advanced Cell Diagnostics) in accordance with the manufacturer’s instructions. In brief, 5-μm tissue sections were dewaxed, incubated with endogenous enzyme block, boiled in pre-treatment buffer and treated with protease, followed by target probe hybridization using the RNAscope LS 2.5 HS-TREM2 (420498, Advanced Cell Diagnostics) probe. Target RNA was then detected with Cy3 tyramide (Perkin-Elmer, NE-74001KIT) at a 1:1,000 dilution. The sections were then processed through pH 6 and pH 7.5 sodium citrate heat-mediated antigen retrieval, hydrogen peroxide treatment and protein block (all as for multiplex immunofluorescence staining as above). MND antibody was applied overnight at 4°C, completed using a secondary ImmPact HRP Anti-Rabbit Peroxidase IgG (Vector Laboratories, MP-7401), visualized using a fluorescein tyramide (Perkin-Elmer, NE-741001KIT) at a 1:1,000 dilution and stained with DAPI.

Bright-field and fluorescently stained sections were imaged using the slide scanner AxioScan.Z1 (Zeiss) at 20× magnification (40× magnification for smFISH). Images were processed and scale bars added using Zen Blue (Zeiss) and Fiji software²⁴.

Cell counting and image analysis

Automated cell counting was performed using QuPath software²⁴. In brief, DAB-stained whole tissue section slide-scanned images (CZF files)
were imported into QuPath. Cell counts were carried out using the positive cell detection tool, detecting haematoxylin-stained nuclei and then thresholding for positively stained DAB cells, generating DAB-positive cell counts per mm² tissue. Identical settings and thresholds were applied to all slides for a given stain and experiment. For cell counts of fibrotic septae versus parenchymal nodules, the QuPath segmentation tool was used to segment the DAB-stained whole tissue section into fibrotic septae or non-fibrotic parenchymal nodule regions using tissue morphological characteristics. Positive cell detection was then applied to the fibrotic and non-fibrotic regions in turn, providing DAB-positive cell counts per mm² in fibrotic septae and non-fibrotic parenchymal nodules for each tissue section.

Digital morphometric pixel analysis was performed using the Trainable Weka Segmentation (TWS) plugin in Fiji software. In brief, each stained whole tissue section slide-scanned image was converted into multiple TIFF files in Zen Blue software (Zeiss). TIFF files were imported into Fiji and TWS plugin trained to produce a classifier which segments images into areas of positive staining, tissue background and white space. The same trained classifier was then applied to all TIFF images from every tissue section for a particular stain, providing a percentage area of positive staining for each tissue section. For digital morphometric quantification of positive staining of fibrotic septae versus parenchymal nodules, TIFF images were segmented into fibrotic septae or non-fibrotic parenchymal nodule regions using tissue morphological characteristics, followed by analysis using the TWS plugin in Fiji software.

Histological assessment of NASH sections
Sections stained with haematoxylin and eosin or picrosirius red were whole-slide imaged using a NanoZoomer imager (Hamamatsu Photonics). Images of stained sections were independently scored by a consultant liver transplant histopathologist (T.J.K.) at the national liver transplant centre with experience in trial scoring by applying the ordinal NAFLD activity score. For observer-independent quantification of the area of positive picrosirius red staining, images were split using ndsplit into tiles of ×5 magnification before the application of a classifier that had been trained by the liver histopathologist using the machine learning WEKA plugin in Fiji, as previously described. All analysis was undertaken blinded to all other data.

Droplet-based scRNA-seq
Single cells were processed through the Chromium Single Cell Platform using the Chromium Single Cell 3’ Library and Gel Bead Kit v2 (10X Genomics, PN-120237) and the Chromium Single Cell A Chip Kit (10X Genomics, PN-120236) as per the manufacturer’s protocol. In brief, single cells were sorted into PBS plus 0.1% BSA, washed twice and counted using a Bio-Rad TC20. Then, 10,800 cells were added to each lane of the 10X chip. The cells were partitioned into Gel Beads in Emulsion in the Chromium instrument, in which cell lysis and bar-coded reverse transcription of RNA occurred, followed by amplification, fragmentation and 5’ adaptor and sample index attachment. Libraries were sequenced on an Illumina HiSeq 4000.

Computational analysis
In total, we analysed 67,494 human cells from healthy (n = 5) and cirrhotic (n = 5) livers, 30,741 PBMCs from patients with cirrhosis (n = 4) and compared our data with a publicly available reference dataset of 8,381 PBMCs from a healthy donor. We estimated cell-containing partitions and associated unique molecular identifiers (UMIs), using the Cell Ranger v2.1.0 Single-Cell Software Suite from 10X Genomics. Genes expressed in fewer than three cells in a sample were excluded, as were cells that expressed fewer than 300 genes or mitochondrial gene content >30% of the total UMI count. We normalized by dividing the UMI count per gene by the total UMI count in the corresponding cell and log-transforming. Variation in UMI counts between cells was regressed according to a negative binomial model, before scaling and centring the resulting value by subtracting the mean expression of each gene and dividing by its standard deviation (E_t), then calculating ln(10^c / E_t^+ 1).

Dimensionality reduction, clustering and differential expression analysis
We performed unsupervised clustering and differential gene expression analyses in the Seurat R package v.2.3.0. In particular, we used shared nearest neighbour graph-based clustering, in which the graph was constructed using from 2 to 11 principal components as determined by dataset variability shown in principal component analysis (PCA); the resolution parameter to determine the resulting number of clusters was also tuned accordingly. To assess cluster similarity we used the ‘BuildClusterTree’ function from Seurat.

In total, we present scRNA-seq data from ten human liver samples (named healthy 1–5 and cirrhotic 1–5), five human blood samples (n = 4 cirrhotic named blood 1–4 and n = 1 healthy named PBMC8K; pbmc8k dataset sourced from single-cell gene expression datasets hosted by 10X Genomics), and two mouse liver samples (n = 3 uninjured and n = 3 fibrotic). For seven human liver samples (healthy 1–4 and cirrhotic 1–3), we performed scRNA-seq on both leucocytes (CD45*) and other non-parenchymal cells (CD45*); for the remaining three human livers (healthy 5, cirrhotic 4–5) we performed scRNA-seq on leucocytes only (Extended Data Fig. 2e, f).

Initially, we combined all human scRNA-seq datasets (liver and blood) and performed clustering analysis with the aim of isolating a population of liver-resident cells, by identifying contaminating circulatory cells within datasets generated from liver digests and removing them from downstream analysis. Specifically, we removed from our liver datasets cells that fell into clusters 1 and 13 of the initial dataset in Extended Data Fig. 1d.

Using further clustering followed by signature analysis, we interrogated this post-processed liver-resident cell dataset for robust cell lineages. These lineages were isolated into individual datasets, and the process was iterated to identify robust lineage subpopulations. At each stage of this process we removed clusters expressing more than one unique lineage signature in more than 25% of their cells from the dataset as probable doublets. This resulted in removal of 1,351 cells. Where the cell proliferation signature identified distinct cycling subpopulations, we re-clustered these again to ascertain the identity of their constituent cells.

The mouse scRNA-seq datasets were combined, clustered and interrogated for cell lineages in a similar manner to their human counterparts. All heat maps, distributed stochastic neighbour embedding (t-SNE) and UMAP visualizations, violin plots and dot plots were produced using Seurat functions in conjunction with the ggplot2, pheatmap and grid R packages. t-SNE and UMAP visualizations were constructed using the same number of principal components as the associated clustering, with perplexity ranging from 30 to 300 according to the number of cells in the dataset or lineage. We conducted differential gene expression analysis in Seurat using the standard AUC classifier to assess significance. We retained only those genes with a log-transformed fold change of at least 0.25 and expression in at least 25% of cells in the cluster under comparison.

Defining cell lineage signatures
For each cell, we obtained a signature score across a curated list of known marker genes per cell lineage in the liver (Supplementary Table 2). This signature score was defined as the geometric mean of the expression of the associated signature genes in that cell. Lineage signature scores were scaled from 0 to 1 across the dataset, and the score for each cell
with a signature less than a given threshold (the mean of said signature score across the entire dataset) was set to 0.

**Batch effect and quality control**
To investigate agreement between samples, we extracted the average expression profile for a given cell lineage in each sample, and calculated the Pearson correlation coefficients between all possible pairwise comparisons of samples per lineage.**

**Imputing dropout in T cell and ILC clusters**
To impute dropout of low-abundance transcripts in our T cell and ILC clusters so that we might associate them with known subpopulations, we downsampled to 7,380 cells from 36,900 and applied the scImpute R package v.0.0.8**

**Analysing functional phenotypes of scar-associated cells**
For further analysis of function we adopted the self-organizing maps approach as implemented in the SCRAT R package v.1.0.0**

**Inferring injury dynamics and transcriptional regulation**
To generate cellular trajectories (pseudotemporal dynamics) we used the monocle R package v.2.6.1**

**Canonical correlation analysis**
To compare human and mouse populations of monocyctic phagocytes, we used canonical correlation analysis as implemented in Seurat**

**Deconvolution of whole liver microarray data**
To assess the macrophage composition of early-stage NAFLD, we performed deconvolution analysis on publicly available microarray data from annotated liver biopsy specimens taken across the NAFLD disease spectrum (GEO accession GSE48452)**

**Statistics and reproducibility**
To assess whether our identified subpopulations were significantly overexpressed in injury, we posited the proportion of injured cells in each cluster as a random count variable using a Poisson process, as previously described. We modelled the rate of detection using the total number of cells in the lineage profiled in a given sample as an offset, with the condition of each sample (healthy versus cirrhotic) provided as a
covariate factor. The model was fitted using the R command 'glm' from the stats package. The P-value for the significance of the proportion of injured cells was assessed using a Wald test on the regression coefficient.

Remaining statistical analyses were performed using GraphPad Prism. Comparison of changes between two groups was performed using a Mann–Whitney test (unpaired; two-tailed) or a Wilcoxon matched-pairs signed rank test (paired; two-tailed). Comparison of changes between multiple groups was performed using a Kruskal–Wallis and Dunn, one-way ANOVA and Tukey or repeated measures one-way ANOVA and Tukey tests. Correlations were preformed using Pearson correlation and best-fit line plotted using linear regression. P < 0.05 was considered statistically significant. All immunofluorescence stains were repeated in a minimum of three patients and representative images are displayed.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
Our expression data are freely available for user-friendly interactive browsing online at http://www.livercellatlas.mvm.ed.ac.uk. CellPhoneDB is available at www.CellPhoneDB.org. All raw sequencing data have been deposited in the Gene Expression Omnibus (GEO) under accession GSE136103.

Code availability
R scripts enabling the main steps of the analysis are available from the corresponding authors on reasonable request.

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Author contributions
P.R. performed experimental design, tissue procurement, data generation, data analysis and interpretation, and manuscript preparation. R.D. performed experimental design, data generation and data analysis; E.F.D., K.P.M., B.E.P.H., M.B., J.A.M. and N.T.L. performed data generation and analysis; J.R.P. generated the interactive online browser; M.E. and R.V.-T. assisted with CellPhoneDB analyses and critically appraised the manuscript; T.I.K. performed pathological assessments and provided intellectual contribution; N.O.C., J.A.F. and P.N.N. provided intellectual contribution; C.J.W. performed tissue procurement, data generation, interpretation and intellectual contribution; J.R.W.-K. performed computational analysis with assistance from J.R.P. and R.S.T. and advice from C.P.P., J.C.M. and S.A.T.; J.R.W.-K. also helped with manuscript preparation, and C.P.P., J.C.M. and S.A.T. critically appraised the manuscript; E.M.H., D.I.M. and S.J.W. procured human liver tissue and critically appraised the manuscript; J.P.I., F.T. and J.W.P. provided intellectual contribution and critically appraised the manuscript; N.C.H. conceived the study, designed experiments, interpreted data and prepared the manuscript.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | Strategy for isolation of human liver non-parenchymal cells. a, Patient demographics and clinical information. Data are mean ± s.e.m. b, Flow cytometry gating strategy for isolation of leucocytes (CD45+) and other non-parenchymal cells (CD45-) from human liver; representative plots from ten livers. c, Flow cytometry gating strategy for isolation of PBMCs; representative plots from four patients. d, Clustering 103,568 cells from healthy (n = 5) and cirrhotic (n = 5) livers, healthy PBMCs (n = 1) and cirrhotic PBMCs (n = 4) (left), annotating the source (PBMC versus liver; middle) and cell lineage inferred from known marker gene signatures (right). e, Dot plot annotating PBMC and liver clusters by lineage signatures. Circle size indicates cell fraction expressing signature greater than mean; colour indicates mean signature expression (red, high; blue, low). f, CXCR4 gene expression in single cells derived from blood or liver tissue, divided by cell lineage. Bottom right, representative immunofluorescence image (n ≥ 3) of CXCR4 (green) and DAPI (blue) in human liver; arrows denote CXCR4- cells in the lumen of a blood vessel. Scale bar, 50 μm. g, Violin plots showing the number of unique genes (nGene), number of total unique molecular identifiers (nUMI) and mitochondrial gene fraction expressed in five PBMC samples. Black lines denote the median. h, Pie charts showing the proportion of cell lineages per PBMC sample. i, Box and whisker plots showing the agreement in expression profiles across five PBMC samples. Pearson correlation coefficients between average expression profiles for cells in each lineage, across all pairs of samples. Black bars denote the median; box edges denote the twenty-fifth and seventy-fifth percentiles; whiskers denote the full range.
Extended Data Fig. 2 | Quality control and annotation of human liver-resident cells. a, Lineage signature expression across 66,135 liver-resident cells from healthy (n = 5) and cirrhotic (n = 5) human livers (red, high; blue, low). b, Dot plot annotating liver-resident cell clusters by lineage signature. Circle size indicates cell fraction expressing signature greater than mean; colour indicates mean signature expression (red, high; blue, low). c, Violin plots of the number of unique genes (left), number of total UMIs (middle) and mitochondrial gene fraction (right) across 66,135 liver-resident cells from healthy (n = 5) and cirrhotic (n = 5) livers. Black lines denote the median. d, Pie charts of the proportion of cell lineage per liver sample. e, Box and whisker plots of the agreement in expression profiles across healthy (n = 5) and cirrhotic (n = 5) liver samples, as in Extended Data Fig. 1i. f, t-SNE visualization of liver-resident cells per liver sample, with cirrhotic samples annotated by aetiology of underlying liver disease. ALD, alcohol-related liver disease; PBC, primary biliary cholangitis.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Annotating human liver lymphoid cells. 

a, Clustering of 36,900 T cells and ILCs (left) from healthy (n = 5) and cirrhotic (n = 5) human livers, annotating the injury condition (right). NK, natural killer cell; cNK, cytotoxic NK cell. 
b, Fractions of T cell and ILC subpopulations in healthy (n = 5) and cirrhotic (n = 5) livers. 
c, Selected gene expression in 36,900 T cells and ILCs. 
d, Heat map of T cell and ILC cluster marker genes (colour-coded by cluster and condition), with exemplar genes labelled (right). Columns denote cells; rows denote genes. 
e, t-SNE visualizations of downsampled T cell and ILC dataset (7,380 cells from healthy (n = 5) and cirrhotic (n = 5) human livers) before and after imputation (scImpute); annotating data used for visualization and clustering, inferred lineage and injury condition. No additional heterogeneity was observed after imputation. 
f, Clustering 2,746 B cells and plasma cells (left) from healthy (n = 5) and cirrhotic (n = 5) human livers, annotating the injury condition (right). 
g, Heat map of B cell and plasma cell cluster marker genes (colour-coded by cluster and condition), with exemplar genes labelled (right). Columns denote cells; rows denote genes. 
h, Fractions of B cell and plasma cell subpopulations in healthy (n = 5) and cirrhotic (n = 5) livers. Data are mean ± s.e.m. P values determined by Wald test (b).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Annotating human liver MPs. a, Clustering and selected genes expressed in 10,737 MPs from healthy (n=5) and cirrhotic (n=5) human livers. b, Scaled gene expression of KC cluster markers across MP cells from healthy (n=5) and cirrhotic (n=5) livers. c, Representative immunofluorescence images (n≥3) of TIMD4 (red), CD163 (white), MARCO (green) and DAPI (blue) in healthy and cirrhotic liver; arrows denote CD163/MARCO/TIMD4+ cells. d, Immunohistochemistry (left) and cell counts (right) of TIMD4 expression in healthy (n=12) and cirrhotic (n=9) human liver. e, Immunohistochemistry (left) and cell counts (right) of MARCO expression in healthy (n=8) and cirrhotic (n=8) liver. f, Flow cytometry gating strategy for identifying KCs, TMs and SAMacs in healthy (n=2) and cirrhotic (n=3) liver. SAMacs are detected as TREM2+CD9+ cells within the TM and SAMac gate (see Fig. 2f). g, Representative immunofluorescence images (n≥3) of TREM2 (red), MNDA (white), collagen 1 (green) and DAPI (blue) in cirrhotic liver. h, Representative images (n=2) of TREM2 (smFISH; red), MNDA (immunofluorescence; green) and DAPI (blue) in cirrhotic liver. i, Representative immunofluorescence images (n≥3) of CD9 (red), MNDA (white), collagen 1 (green) and DAPI (blue) in cirrhotic liver. j, Immunohistochemistry (top) and cell counts (bottom) of TREM2 expression in healthy (n=10) and cirrhotic (n=9) liver. k, Immunohistochemistry (top) and cell counts (bottom) of CD9 expression in healthy (n=12) and cirrhotic (n=10) liver. l, Top, exemplar tissue segmentation of cirrhotic liver section into fibrotic septae (orange) and parenchymal nodules (purple). Bottom, cell counts based on immunohistochemistry analysis of TREM2 (n=9), CD9 (n=11), TIMD4 (n=9) and MARCO (n=7) in parenchymal nodules and fibrotic septae. m, Top, clustering and annotation of 208 cycling MP cells from healthy (n=5) and cirrhotic (n=5) livers, with scaled gene expression of MP subpopulation markers across four clusters of cycling MP cells. Bottom, fractions of cycling MP subpopulations in healthy (n=5) and cirrhotic (n=5) livers. All scale bars, 50 μm. Data are mean±s.e.m. P values determined by two-tailed Mann–Whitney (e, j, k).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Phenotypic characterization of mononuclear phagocytes in healthy and cirrhotic human livers. a, Top, self-organizing map (60 × 60 grid) of smoothed scaled metagene expression of 10,737 MPs from healthy (n = 5) and cirrhotic (n = 5) livers. In total, 20,952 genes, 3,600 metagenes and 44 signatures were identified. A–F denote metagene signatures overexpressed in one or more MP subpopulations. Bottom, smoothed mean metagene expression profile for each MP subpopulation. b, Radar plots (left), exemplar genes (middle) and selected GO enrichment (right) of metagene signatures A–F showing distribution of signature expression across MP subpopulations from 10,737 MP cells. c, Diffusion map (DM) visualization of blood monocytes and liver-resident MP lineages (23,075 cells from healthy (n = 5) and cirrhotic (n = 5) liver samples and PBMCs (n = 5)), annotating monocle pseudotemporal dynamics (purple to yellow). Top, RNA velocity field (red arrows) visualized using Gaussian smoothing on regular grid. Bottom, annotation of MPs by subpopulation and injury condition. d, Unspliced–spliced phase portraits (top): 23,075 cells coloured and visualized as in Fig. 3a; monocyte (MNDA), SAMac (CD9) and KC (TIMD4) marker genes. Cells plotted above or below the steady-state (black dashed line) indicate increasing or decreasing expression of gene, respectively. Spliced expression profile for stated genes (middle row; red, high, blue, low). Unspliced residuals for stated genes (bottom row), positive (red) indicating expected upregulation, negative (blue) indicating expected downregulation. MNDA displays negative velocity in SAMacs; CD9 displays positive velocity in monocytes and SAMacs; TIMD4 velocity is restricted to KCs. e, Cubic smoothing spline curve fitted to averaged expression of all genes in module 2 from the blood monocyte-to-SAMac pseudotemporal trajectory (see Fig. 3c), with selected GO enrichment (right). f, Cubic smoothing spline curve fitted to averaged expression of all genes in module 3 from the blood monocyte-to-cDC pseudotemporal trajectory (see Fig. 3c), with selected GO enrichment (right). g, Luminex assay showing quantification of levels of stated proteins in culture medium from FACS-isolated SAMacs (n = 3), TMs (n = 2) and KCs (n = 2). Control denotes medium alone (n = 2). Data are mean ± s.e.m. h, Heat map of transcription factor regulons across MP pseudotemporal trajectory and in KCs (colour-coded by MP cluster, condition and pseudotime), with selected regulons labelled (right). Columns denote cells; rows denote genes. i, Scaled regulon expression of selected regulons across MP clusters from healthy (n = 5) and cirrhotic (n = 5) livers. All P values determined by Fisher’s exact test.
Extended Data Fig. 6 | Characterization of macrophages in mouse liver fibrosis. 

a, Clustering and annotating 3,250 mouse (m)MPs from healthy (n = 3) and fibrotic (4 weeks CCl4 treatment; n = 3) livers. 

b, Annotating mouse MP cells by injury condition.

c, Heat map of mouse MP cluster marker genes (top; colour-coded by cluster and condition), with exemplary genes labelled (right). Columns denote cells; rows denote genes. 

d, Selected genes expressed in 3,250 mouse MPs.

e, Representative flow cytometry plots of the gating strategy (n = 8 from two independent experiments) for identifying mouse KCs, CD9− TMs and CD9+ SAMacs in fibrotic mice.

f, Quantifying mouse macrophage subpopulations by flow cytometry in healthy (n = 6) and fibrotic (n = 8) mouse livers from two independent experiments. The macrophage subpopulation (x axis) is shown as a percentage of total viable CD45+ cells (y axis). Data are mean ± s.e.m. 

P values determined by two-tailed Mann–Whitney test.

g, Co-culture of primary mouse HSCs from uninjured livers and either FACS-isolated CD9+ mouse TMs or CD9+ mouse SAMacs from fibrotic livers (n = 8 mice; two independent experiments).

Right, qPCR of Col3α1 expression in HSCs; expression relative to mean expression of quiescent HSC. 

P value determined by two-tailed Wilcoxon test.

h, Clustering 3,250 mouse MPs and 10,737 human (h)MPs into five clusters using canonical correlation analysis. 

i, Annotation of human and mouse macrophage subpopulations from 3,250 mouse MPs and 10,737 human MPs. 

j, Selected genes expressed in 3,250 mouse MPs and 10,737 human MPs.
Extended Data Fig. 7 | SAMac expansion in human NASH. 

a–d, Deconvolution of publicly available whole liver microarray data \((n = 73)\) assessed for frequency of SAMacs, KCs and TMs using the Cibersort algorithm. 

a, Macrophage composition. GEO accession numbers are shown on the x axis; the fraction of monocyte–macrophages is shown on the y axis. Liver phenotypes are annotated at the top. 
b, Frequency of SAMacs in control \((n = 14)\), healthy obese \((n = 27)\), steatosis \((n = 14)\) and NASH \((n = 18)\) livers. 
c, Left, frequency of SAMacs in patients with histological NAFLD activity scores (NAS) of 0 \((n = 37)\), 1–3 \((n = 19)\) and 4–7 \((n = 17)\). Right, frequency of SAMacs in patients with histological fibrosis scores of 0 \((n = 46)\), 1 \((n = 20)\) and 2–4 \((n = 5)\). 
d, Left, frequency of SAMacs in female \((n = 58)\) and male \((n = 15)\) patients. Middle, frequency of SAMacs in patients aged 23–39 \((n = 22)\), 40–49 \((n = 29)\) and 50–80 \((n = 22)\). Right, frequency of SAMacs in patients with a body mass index (BMI) of 17–30 \((n = 18)\), 31–45 \((n = 28)\) and 46–70 \((n = 27)\). 

e, Left, immunohistochemistry of CD9 and TREM2 expression in NAFLD liver biopsy sections. Scale bars, 50 μm. Right, cell counts of CD9 and TREM2 expression. CD9: NAS 1–3 \((n = 13)\), NAS 4–8 \((n = 21)\). TREM2: NAS 1–3 \((n = 12)\), NAS 4–8 \((n = 16)\). 
f, Correlation of cell counts with picrosirius red (PSR) digital morphometric pixel quantification in NAFLD liver biopsy tissue with CD9 staining (top; \(n = 39\)) or TREM2 staining (bottom; \(n = 32\)). Data are mean ± s.e.m. 

\(P\) values determined by Kruskal–Wallis and Dunn test (b, c), two-tailed Mann–Whitney test (e), or Pearson’s correlation and linear regression (f).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Phenotypic characterization of endothelial cells in healthy and cirrhotic human livers. 

**a**, Clustering and selected genes expressed in 8,020 endothelial cells from healthy (n = 4) and cirrhotic (n = 3) human livers. **b**, Scaled gene expression of endothelial cluster markers across endothelial cells from healthy (n = 4) and cirrhotic (n = 3) livers. **c**, Top, digital pixel quantification of PLVAP immunostaining of cirrhotic liver sections (n = 10) in parenchymal nodules and fibrotic septae. Bottom, ACKR1 immunostaining of cirrhotic liver sections (n = 10) in parenchymal nodules and fibrotic septae. 

**d**, Flow cytometry analysis of PLVAP, CD34 and ACKR1 in endothelial cells from healthy (n = 4, grey) or cirrhotic (n = 7, red) livers. Top, representative histograms; bottom, MFI values. 

**e**, Flow-based adhesion assay. Peripheral blood monocytes assessed for adhesion to primary human liver endothelial cells (top) and the percentage of adherent monocytes that transmigrate (bottom); endothelial cells isolated from healthy (n = 5) or cirrhotic (n = 4) livers. 

**f**, Endothelial cell gene knockdown. Cirrhotic endothelial cells were treated with siRNA against PLVAP (n = 6) or ACKR1 (n = 5) or with control siRNA (n = 6). Top, representative flow cytometry histograms for stated markers, with comparison to isotype control antibody. Bottom, flow-based adhesion assay, with PBMCs assessed for adhesion (bottom left) and the percentage of adherent cells that transmigrate (bottom right) after siRNA treatment of endothelial cells. 

**g**, Top left, self-organizing map (60 × 60 grid) of smoothed scaled metagene expression of endothelial lineage. In total, 21,237 genes, 3,600 metagenes and 45 signatures were identified. A–E denote metagene signatures overexpressed in one or more endothelial subpopulations. Bottom left, smoothed mean metagene expression profile for each endothelial subpopulation. Middle, radar plots of metagene signatures A–E showing distribution of signature expression across endothelial subpopulations, exemplar genes (middle) and Gene Ontology enrichment (right). **h**, Heat map of endothelial subpopulation transcription factor regulon expression (colour-coded by cluster and condition) across 8,020 endothelial cells from healthy (n = 4) and cirrhotic (n = 3) human livers. Exemplar regulons are labelled (right). Columns denote cells; rows denote regulons. 

**i, t-SNE visualization of endothelial lineage (8,020 cells from healthy (n = 4) and cirrhotic (n = 3) livers), annotating monocle pseudotemporal dynamics (purple to yellow; grey indicates lack of inferred trajectory). RNA velocities (red arrows) visualized using Gaussian smoothing on regular grid. **j**, Representative immunofluorescence images (n ≥ 3) of RSPO3, PDPN, AIF1L, VWA1 or ACKR1 (red), CD34 (white), PLVAP (green) and DAPI (blue) in healthy and cirrhotic liver. Scale bars, 50 μm. 

**k**, Annotation of 8,020 endothelial cells by subpopulation and injury condition. LSEC, liver sinusoidal endothelial cells. Data are mean ± s.e.m. P values determined by two-tailed Wilcoxon test (c), two-tailed Mann–Whitney test (d, e), Kruskal–Wallis and Dunn test (f), or Fisher’s exact test (g).
**Extended Data Fig. 9 | Characterization of mesenchymal cells in healthy and cirrhotic human livers.** a. Selected genes expressed in 2,318 mesenchymal cells from healthy (n = 4) and cirrhotic (n = 3) human livers. b. Clustering 319 SAMes into two further subclusters. c. Heat map of SAMes subcluster marker genes (colour-coded by cluster and condition), with exemplar genes labelled (right). Columns denote cells; rows denote genes. d. Fractions of SAMes subpopulations in healthy (n = 4) and cirrhotic (n = 3) livers. e, f. Representative immunofluorescence images (n ≥ 3) of OSR1 (red), collagen 1 (green) and DAPI (blue) in portal region of healthy liver (e) or fibrotic niche of cirrhotic liver (f). Scale bars, 50 μm. g. Scaled gene expression of selected genes across 2,318 mesenchymal cells from healthy (n = 4) and cirrhotic (n = 3) livers. h, i. t-SNE visualization of 1,178 HSCs and SAMes from healthy (n = 4) and cirrhotic (n = 3) livers annotated by monocle pseudotemporal dynamics (purple to yellow). RNA velocity field (red arrows) visualized using Gaussian smoothing on regular grid. j. Heat map of cubic smoothing spline curves fitted to genes differentially expressed across HSC-to-SAMes pseudotemporal trajectories, grouped by hierarchical clustering (k = 2); colour-coded by pseudotime and condition (top). Gene co-expression modules (colour) and exemplar genes are labelled (right). Data are mean ± s.e.m. P-values determined by Wald test (d).
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Characterization of the cellular interactome in the fibrotic niche. a, b, Representative immunofluorescence images \((n \geq 3)\) of fibrotic niche in cirrhotic liver. a, TREM2 (red), PLVAP (white), PDGFRα (green) and DAPI (blue). b, TREM2 (red), ACKR1 (white), PDGFRα (green) and DAPI (blue).

c, Proliferation assay. Human HSCs were treated with conditioned medium from primary hepatic macrophage subpopulations SAMac \((n = 2)\), TMs \((n = 2)\), KCs \((n = 2)\) or control medium \((n = 2)\). The AUC of the percentage change in HSC number over time (hours) is shown on the y axis. Data are mean \(\pm\) s.e.m. d, Circle plot showing potential interaction magnitude from ligands expressed by SAMacs and SAEndos to receptors expressed on SAMes. e, Circle plot showing potential interaction magnitude from ligands expressed by SAMes to receptors expressed on SAMacs and SAEndos. f, Dot plot of ligand–receptor interactions between SAMacs \((n = 7\) human livers), SAMacs \((n = 10\) human livers) and SAEndos \((n = 7\) human livers). Ligand (red) and cognate receptor (blue) shown on the x axis; populations that express ligand (red) and receptor (blue) are shown on the y axis; circle size denotes \(P\) value (permutation test); colour (red, high; yellow, low) denotes average ligand and receptor expression levels in interacting subpopulations. g, Top, representative immunofluorescence image \((n \geq 3)\) of CCL2 (red), CCR2 (white), PDGFRα (green) and DAPI (blue) in fibrotic niche in cirrhotic liver; arrows denote CCL2+PDGFRα+ cells. Bottom, representative immunofluorescence image \((n \geq 3)\) of ANGPT1 (red), TEK (white), PDGFRα (green) and DAPI (blue) in fibrotic niche in cirrhotic liver; arrows denote ANGPT1+PDGFRα+ cells. h, Circle plot denotes potential interaction magnitude from ligands expressed by SAMacs to receptors expressed on SAEndos. i, Dot plot of ligand–receptor interactions between SAMacs \((n = 10\) human livers) and SAEndos \((n = 7\) human livers) as in f. j, Representative immunofluorescence image \((n \geq 3)\) of TREM2 (red), FLT1 (white), VEGFA (green) and DAPI (blue) in fibrotic niche in cirrhotic liver; arrows denote TREM2’VEGFA’ cells. k, Circle plot of the potential interaction magnitude from ligands expressed by SAEndos to receptors expressed on SAMacs. l, Dot plot of ligand–receptor interactions between SAEndo \((n = 7\) human livers) and SAMacs \((n = 10\) human livers) as in f. m, Top, representative immunofluorescence image \((n \geq 3)\) of TREM2 (red), CD200 (white), CD200R (green) and DAPI (blue) in fibrotic niche in cirrhotic liver; arrows denote TREM2’CD200R’ cells. Bottom, representative immunofluorescence image \((n \geq 3)\) of TREM2 (red), DLL4 (white), NOTCH2 (green) and DAPI (blue) in fibrotic niche in cirrhotic liver; arrows denote TREM2’NOTCH2’ cells. All scale bars, 50 \(\mu m\).
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Initial processing of single-cell RNA-sequencing data was performed using the commercial Cell Ranger pipeline (10X Genomics, version 2.1.0, see Methods). Subsequent analysis was performed using the open-source R programming language (version 3.4.1). BD FACSDiva software was used for cell sorting on BD Influx equipment. BD FACSDiva software was used for flow cytometry on BD LSR Fortessa equipment and for cell sorting on BD FACSAria Fusion and FACSAria II L. Fluorescent and brightfield microscopy images were acquired using Zen Blue software (Zeiss) on an Axiocam2 Z1 instrument (Zeiss) or Confocal Microscope Zeiss LSM780. Limuxin data was acquired on a Bio-Plex 200 (Bio-Rad). Cell proliferation data was acquired on an Incucyte ZOOM live cell analysis system (Essen biosciences). RT-qPCR data was acquired on ABI 7900HT FAST PCR system (Applied Biosystems).

Data analysis

 Immunofluorescent images were processed and analysed using Zen Blue software (Zeiss) and Fiji software (ImageJ version 2.00). Cell proliferation data were analysed on the Incucyte proprietary analysis software (version 2018A). Immunohistochemistry images were analysed using QuPath software (version 0.3.2) for automated cell counting and using Fiji software (ImageJ version 2.00) with Trainable Weka Segmentation plugin (see Methods). Co-culture immunocytochemistry data was analysed using Imaris x64 (version 8.1.2). Flow cytometry analysis was performed using Flowjo software (version 10.2). RT-qPCR data was analysed using ThermoFisher Connect cloud qPCR software (version 2019.1.6-Q1-19-build4). Statistical analysis was performed either in R (version 3.4.1) or using Graphpad Prism software (version 7.0a). Single-cell RNA-sequencing analysis was performed in R, based around the following packages: Seurat 3.2.0, scimpute 0.0.8, SCATR 1.0.0, monocle 2.6.1, scater 1.4.0, velocyt 0.6.0, SCENIC 0.1.7 (see Methods). We also made use of the CellPhoneDB repository of ligands, receptors, and interactions. Deconvolution was performed using Cicerosr. Gene Ontology enrichment analysis was performed using PANTHER 13.1.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Our expression data will be freely available for user-friendly interactive browsing online at www.livercellatlas.mvm.ed.ac.uk. CellPhoneDB is available at www.CellPhoneDB.org, along with lists of membrane proteins, ligands and receptors, and heteromeric complexes. All raw sequencing data have been deposited in the Gene Expression Omnibus (GEO Accession GSE136103). We make available as Supplementary Tables: lists of lineage-specific genes for signature analysis (Extended Data Figure 1e, 2b); lists of marker genes and regulons from clustering results (Figure 1e, 2d, 4c, 5b, Extended Data Figure 3d, e, g, 5h, 6c, 8h, 9c); lists of module / signature genes from trajectory and self-organising map analyses and corresponding lists of gene ontology terms from enrichment analysis (Figure 3c, d, Extended Data Figure 5a, b, e, f, g); lists of significant interactions in the fibrotic niche as identified using CellPhoneDB (Figure 6a, e, Extended Data Figure 10f, i, l).

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- Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: In total, we present scRNA-seq data from ten human liver samples (5 healthy and 5 cirrhotic), five human blood samples (4 cirrhotic and 1 healthy named PIMCRK); Pimc8k dataset sourced from single-cell gene expression datasets hosted by 10X Genomics) and two mouse samples (3 healthy and 3 fibrotic). No statistical methods were used to predetermine sample size. Patients were selected to give a balanced representation of healthy and cirrhotic liver cells and to provide sufficient cells of each lineage to facilitate more detailed analysis. Histology, flow cytometry, immunex, RT-qPCR and cell proliferation analysis were performed on multiple independent biological replicates (as shown in figure legends).

- **Data exclusions**: Described in detail in Methods. Exclusion criteria were determined following initial assessment and QC of the data. Low gene expression (fewer than 300 genes) or a high mitochondrial gene content (>50% of the total UMI count) are indicators of outlier low quality cells and were excluded. At each stage of the analysis we used signature analysis to identify and exclude potential doublet clusters.

- **Replication**: All experimental findings reported here were successfully replicated across multiple biological samples (as reported in each figure legend). All immunofluorescence was performed on a minimum of 3 liver samples to identify representative images.

- **Randomization**: One group of randomly selected healthy livers and another group of randomly selected cirrhotic livers were analysed in this study. All subsequent analyses were performed in randomly selected healthy or cirrhotic liver samples. For mouse experiments, age-matched littermate mice were randomly assigned to be healthy controls or to receive carbon tetrachloride.

- **Blinding**: Blinding to the origin of the tissue samples was not possible. All analyses were performed in an automated manner across conditions.

Reporting for specific materials, systems and methods

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### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology         |
| ☒   | Animals and other organisms |
| ☒   | Human research participants |
| ☒   | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging |
Antibodies

Antibodies used
All antibodies used in this work, clone, application, supplier and lot number are listed in Supplementary Table 19.

Validation
All antibodies used are commercially available and validated by the vendor for the assay and species used in this study. Specific validation information for each antibody is available on the vendors website. The specificity of each primary flow cytometry antibody was validated by staining directly against species-matched isotype and unstimulated controls. Validation of each primary antibody used for immunostaining was performed by comparison to species-matched isotype antibodies and unstimulated controls.

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research.

Laboratory animals
Male C57BL/6J/crl mice aged 8 to 10 weeks

Wild animals
Study did not involve wild animals

Field-collected samples
Study did not involve samples collected in the field

Ethics oversight
All experiments were performed in accordance with UK Home Office regulations.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics
Please see Extended Data Figure 1a for the clinical characteristics of patients used for single-cell RNA sequencing.

Recruitment
Patients were recruited as described in Methods. Healthy background non-lesional liver tissue was obtained intraoperatively from patients undergoing surgical liver resection for solitary colorectal metastasis at the Hepatobiliary and Pancreatic Unit, Department of Clinical Surgery, Royal Infirmary of Edinburgh. Patients with a known history of chronic liver disease, abnormal liver function tests or those who had received systemic chemotherapy within the last four months were excluded from this cohort. Cirrhotic liver tissue was obtained intraoperatively from patients undergoing orthotopic liver transplantation at the Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh. Blood from patients with a confirmed diagnosis of liver cirrhosis were obtained from patients attending the Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh. Patients with liver cirrhosis due to viral hepatitis were excluded from the study. For cell sorting of macrophages or isolation of human endothelial cells, liver tissue was acquired from explanted diseased livers from patients undergoing orthotopic liver transplantation, resected liver specimens or donor livers rejected for transplant at the Queen Elizabeth Hospital, Birmingham.

Ethics oversight
NRS BioResource and Tissue Governance Unit (Study Number SR574), following review at the East of Scotland Research Ethics Service (Reference 15/ES/0094)
For University of Birmingham samples, separate local ethical approval was obtained (Reference 06/Q2708/11, 06/Q2702/61).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axes scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Please see Methods for detailed sample preparation protocol for FACS and flow cytometry

Instrument
BD Influx and BD FACSAriiI were used for cell sorting at University of Edinburgh. BD LSR Fortessa was used for flow cytometry analysis. BD FACSArii Fusion for cell sorting at the University of Birmingham

Software
BD FACS Software software was used for cell sorting on BD Influx equipment. BD FACS Diva software for cell sorting on BD FACSArii and BD FACSArii Fusion. BD FACS Diva software was used for flow cytometry on BD LSR Fortessa equipment. Flow
Cytometry analysis was performed using FlowJo software (version 10.2).

| Cell population abundance | Sort purity was routinely over 95% on post-sort checks |
|----------------------------|-------------------------------------------------------|

**Gating strategy**

Please see Methods and Extended Data Figures 1b,c, 4f and 6e for gating strategies. Initial gating for all experiments: Cells (FSC-A vs SSC-A), Singlets (FSC-A vs FSC-H [or FSC-A vs TPW for BD Influx]), Viable (SSC-A vs viability dye [see methods]). For human PIMC sort, CD45+ CD66b+ cells were sorted. For human liver single-cell RNA-seq sorting, CD45+ cells (leukocytes) or CD45− cells (other NPCs) were sorted. For human liver macrophage flow cytometry quantification and cell sorting, tissue monocyte-macrophages were identified as CD45+, Lin− (CD3, CD335, CD19, CD66b, ULRA4, CD326), HLA-DR+, CD1C+, CD14+ and/or CD16+ cells. SAM were then identified as CD163− TREM2− CD9+, KCs were identified as CD163+ CD9− and TMO were identified as CD163−. For mouse liver single-cell RNA-seq sorting, tissue mononuclear phagocytes identified as CD45+ Lin− (CD3, NK1.1, Ly6G, CD19) cells were sorted. For mouse liver macrophage cell sorting, CD45+ Lin− CD11b+ F4/80+ TIMD4− CD9− or CD9+ cells were sorted from CCA-treated mice. For human liver endothelial cell flow cytometry, cultured endothelial cells were stained with antibodies to PLVAP, ACKR1, JAG1 and CD34. Gates and boundaries were defined by comparison to FMO and unstained samples.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.