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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.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	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
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

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: Quantitative data were collected primarily into Microsoft excel.

Data analysis	For scRNA-seq analysis For MARS-seq, demultiplexing and filtering was performed as previously described (DOI: 10.1038/s41596-019-0164-4), with the following adaptations. Mapping of reads was performed using HISAT (version 0.1.6); reads with multiple mapping positions were excluded. Reads were associated with genes if they were mapped to an exon, using the Ensembl gene annotation database (EMBL release 90). The level of spurious unique molecular identifiers (UMIs) in the data were estimated by using statistics on empty MARS-seq wells. For both MARS-seq and 10x, cells with fewer than 500 UMIs or more than 20% mitochondrial genes or mitochondrial genes being the most differential were discarded from the analysis. For PIC-seq, cell with fewer than 1000 UMIs were discarded. All downstream analysis was performed in R (version R-4.0.4). The metacell pipeline (https://github.com/tanaylab/metacell) was used to derive informative genes and compute cell-to-cell similarity, to compute K-nn graph covers and derive distribution of RNA in cohesive groups of cells (or metacells), and to derive strongly separated clusters using bootstrap analysis and computation of graph covers on resampled data. We removed specific mitochondrial genes, immunoglobulin genes, and genes linked with poorly supported transcriptional models (annotated with the prefix ''Rp-''). Gene features were selected using the parameter Tvm = 0.3 and a minimum total UMI count > 50. We subsequently performed hierarchical clustering of the correlation matrix between metacells and grouped them into clusters representing cell types and states. We used K = 100, 500 bootstrap iterations and otherwise standard parameters. All downstream analyses were performed in R (version R-4.0.4). PIC-seq (https://github.com/aygoldberg/PIC-seq) was used for PIC-seq analysis (Fig.5). Graphs for non-scRNA-seq analyses were created using GraphPad Prism 8. Graohs correlating cDC1 cell abundance with liver pathology correlation graphs were created in R (version R-4.0.4). Flow cytometry was analyzed using BD FACS-Diva software BD FACSDiva software v.9.0 (BD Bioscience) and FlowJo software v.10.7.1 (FlowJo, LLC). Immunofluorescent Images were acquired and processed with the same threshold settings across samples using Imaris 9.7 (Bitplane). Non-fluorescent images were analysed using Aperio Image Scope software 12.4.0.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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

The scRNA-seq datasets generated and analyzed in this manuscript are available at GEO submission GSE169447.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	pilot experiments and previously published results were used to estimate the samples size such that appropriate statistical tests would yiled significant results (DOI: 10.1016/j.cell.2019.05.054, DOI: 10.1038/s41586-021-03362-0). the exact n numbers are indicated in figure legends.
Data exclusions	in scRNA-seq analysis, cells which failed to pass quality control (min 500 UMIs, 1000UMIs for the PIC-seq experiments) were excluded. Cells which passed the QC in scRNA-seq experiment in Fig. 1 but were annotated as non-immune (hepatocytes, epithelial), were also excluded from final analysis.
Replication	Main findings from the scRNA-seq experiments, except PIC-seq, were confirmed using other methods. PIC-seq experiment was performed in three independent repetitions. All non-scRNA-seq experiments were replicated at least twice, except if the experiment's goal was to confirm previously observed phenotypes, such as H&E staining in fig. 3d, 3g, 4a, confirming previous findings as reported e.g. DOI: 10.1038/s41586-021-03362-0, 10.1002/hep.21655, 10.1038/emm.2016.123, 10.4196/kjpp.2014.18.4.333.). All attempts of replication were successful.
Randomization	Mice (littermates, sorted according to their genotypes) were allocated to diet regiments at random. In fig 6, CDHFD were assigned to anti-XCL1 and IgG groups following the evaluation of ALT/ASP activity in their sera (Extended data fig. 8a). For human liver samples groups were allocated based on pathology (NAS-score) in H&E staining, which was evaluated by an experienced liver pathologist (Prof. Dr. Achim Weber, University of Zürich). For human blood samples the diagnosis of NAFLD was based on ultrasound evaluation and NASH diagnosis was based on the presence of ballooning, steatohepatitis and fibrosis on liver biopsy, when it was available, or on the presence of fibrosis by noninvasive liver assessment. These evaluations were performed by experienced doctors in the Chaim Sheba Tel-Hashomer Liver Diseases Center.
Blinding	in mouse experiments and human analysis the experimenter was using a running sample number and was blinded to the genotype, treatment, or classification based on phenotype (human NAFLD/NASH) during the experiment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

For marker-based validations and sorting of specific DC and DC progenitor populations, mouse samples were stained using the following antibodies. PE-Dazzle-conjugated CD45 (1:1000; Biolegend, clone 30-F11) or FITC-conjugated CD45 (1:100; eBioscience, clone 30-F11) marking leukocytes. eFluor450-conjugated CD3 (eBioscience; 1:50; clone 17A2), CD19 (eBioscience; 1:150; clone 1D3), B220 (eBioscience; 1:150; clone RA3-6B2), NK1.1 (eBioscience; 1:150; clone: PK136), Ly6G (eBioscience; 1:150; clone 1A8) and Ter119 (eBioscience; 1:150; clone TER-119) to exclude T, B, NK, neutrophils and erythrocytes. PE or PE-Dazzle-conjugated CD11c (clone N418), FITC- or APC-Cy7 conjugated I-A/I-E (Biolegend; 1:150; clone M5/114.15.2), Brilliant Violet 650-conjugated CD11b (Biolegend; 1:200; clone M1/70), PE-conjugated CD103 (Biolegend; 1:200; clone 2E7), APC-conjugated XCR1 (Biolegend; 1:20; clone ZET), APC-conjugated CCR7 (Biolegend; 1:20; clone 4B12), PE/Cy7-conjugated CD83 (Biolegend; 1:20; clone Michel-19), PE-conjugated CD135 (FLT3; eBioscience; 1:150; clone A2F10), PE-Cy7- or PE-conjugated CD115 (eBioscience; 1:150; clone AFS98), APC/Cy7-conjugated Ly6C (Biolegend; 1:200; clone HK1.4), APC/Cy7- conjugated c-kit (Biolegend;1:100; clone 2B8) and DAPI (in the same channel with Lineage staining), for live/dead cell detection.
For T cell analysis and PICs sorting, the following antibodies were used: PE-Dazzle-conjugated CD45 (1:1000; Biolegend, clone 30-F11) or FITC-conjugated CD45 (1:100; eBioscience, clone 30-F11), PE-Dazzle or PE-conjugated CD11c (Biolegend; 1:200; clone N418), FITC- or APC-Cy7 conjugated I-A/I-E (Biolegend; 1:150; clone M5/114.15.2), FITC-conjugated TCRbeta (Biolegend; 1:50; clone H57-597) or PE-conjugated TCRbeta (Biolegend; 1:200; clone H57-597), eFluor450-conjugated CD4 (eBioscience; 1:100;clone GK1.5), PE-Cy7-conjugated CD8 (Biolegend; 1:100; clone 53-6.7), PE-Cy5-conjugated CD62L (Biolegend; 1:100; clone DREG-56), APC-conjugated CD44 (Biolegend; 1:50; clone IM7).
For NKT cell analysis, FITC-conjugated CD45 (1:100; eBioscience, clone 30-F11), eFluor450-conjugated CD3 (eBioscience; 1:50; clone 17A2), PE-conjugated NK1.1 (eBioscience; 1:200; clone PK.126) were used. For monocyte analysis: FITC-conjugated CD45 (1:100; eBioscience, clone 30-F11), PerCP-Cy5.5 conjugated Ly6C (Biolegend; 1:200; clone HK1.4), and Brilliant Violet 650-conjugated CD11b (Biolegend; 1:200; clone M1/70).
For human blood DC analysis, the following antibodies were used: PerCP-Cy5.5-conjugated CD45 (1:20; Biolegend; clone H130), FITC-conjugated CD1c (1:20; Biolegend; clone L161), APC-conjugated CD11c (1:5; BD Bioscience; clone B-ly6), APC/Cy7-conjugated CD14 (1:20; BD Bioscience; clone MOPg), BV570-conjugated HLA-DR (1:20; Biolegend; clone L243), PE-Dazzle-conjugated CD141 (1:20; Biolegend; clone M80), PE/Cy7-conjugated CD19 (1:20; Beckman Coulter; clone J3-119).
For human DC sorting from the liver, the following antibodies were used: PE-conjugated CD45 (1:20; Biolegend; clone H130), FITC-conjugated Anti-Human Lineage Cocktail 3 (lin 3; including anti-CD3, anti-CD14, anti-CD19 and anti-CD20; BD Biosciences cat. 643510, according to the manufacturer's guidelines), BV421-conjugated CD11c (1:20; Biolegend clone 3.9) along with the live dead stain (Thermofischer, L34973).
For neutralization of XCL1, 50µg of anti-XCL1 antibodies (R&D systems MAB486) or of isotype-matched control antibodies (R&D systems MAB006) were injected i.p. twice a week for 4 weeks (total of 8 injections).

Validation

Validation of commercial antibodies was done by regular quality control of each lot by the manufacturer (e.g. Biolegend "The antibody was purified by affinity chromatography and conjugated with PE under optimal conditions.";"Each lot of this antibody is quality control tested by immunofluorescent stainign with flow cytometric analysis."; "Every lot of product is quality tested against the "gold standard" reference lot. A new lot is only released based on our defined QC specifications to ensure lot to lot reproducibility and reliability. Biolegend guarantees the stability and performance of all our products shipped at room temperature.")

Animals and other organisms

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

Laboratory animals

mice, both male and female, 8-24 weeks old. Strains used: C57bl/6, Ob/ob, Db/db, Xcr1Cre-mTFP147 and DTA+/+(B6.129P2-Gt(ROSA)26Sortm1(DTA)Lky/J).

Wild animals

study did not involve wild animals

Field-collected samples

study did not involve field collected samples

Ethics oversight	All experimental procedures were performed in accordance to Institutional Animal Care and Use Committee (IACUC) of the Weizmann Institute of Science and German Law (G141/19).

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	Blood samples were drawn from patients with chronic liver disease that were recruited at Chaim Sheba Tel-Hashomer Liver Diseases Center. The group included both male and female patients, average age was 50. Human liver tissue samples were collected from patients undergoing bariatric surgery at the Department of Surgery at Heidelberg University Hospital. The group included both male and female patients, average age was 50. Liver sections were stained for HE in the division of Chronic Inflammation and Cancer, DKFZ, and NAS-score was evaluated by an experienced liver pathologist (Prof. Dr. Achim Weber, University Zürich).
Recruitment	Patients with chronic liver disease registered at Chaim Sheba Tel-Hashomer Liver Diseases Center. The patients were recruited by the physicians in the Liver Disease Center In the Chaim Sheba Medical Center. Chaim Sheba is the largest governmental hospital in Israel. Therefore, patients from all over Israel can come to the Liver Disease Center. No self selection bias was taken into consideration. Prior to patient inclusion, written informed consent was obtained. The diagnosis of NAFLD was based on ultrasound evaluation. NASH diagnosis was based on the presence of ballooning, steatohepatitis and fibrosis on liver biopsy, when it was available, or on the presence of fibrosis by noninvasive liver assessment. Liver biopsies were collected from patients undergoing bariatric surgery at the Department of Surgery at Heidelberg University Hospital, and the selection of the two groups was performed later, on the basis of the liver histology (NAS-score) diagnosed by an experienced pathologist independently on the BMI. We did not considered any other biases (e.g. age, sex, co-morbidities...) that would affect the results in the selection of the two groups.
Ethics oversight	For the human blood analysis, the protocol was approved by the institutional review board of the Chaim Sheba Medical Center. For the human liver study institutional review board approval was obtained from the Medical Ethics Committee of Heidelberg University (reference number S-629/2013) headed by Prof. Thomas Strowitzki (full committee http://www.medizinische-fakultaet-hd.uni-heidelberg.de/Mitglieder.108623.0.html). All patients had previously signed the corresponding institutional review board-approved consent forms.

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 axis 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	Mice were fasted for 14h and anaesthetized with an intraperitoneal injection of a ketamine (100mg kg−1) and xylazine (10 mg kg−1) mixture. Liver leukocytes were isolated by a modification of the two-step collagenase perfusion method of Seglen Digestion using Liberase™ TL (cat. no. 5401020001; Roche) for scRNA-seq, or using The Liver Dissociation Kit (Miltenyi Biotec, cat. no. 130-105-807) according to the manufacturer's guidelines for FACS analyses. Bone marrow was isolated by flushing from tibia and femur. Cervical, celiac and portal lymph nodes were processed for recovery of DC-T PICs and single DC and T cells as previously described48. Briefly, lymph nodes were digested in Isocove's modified Dulbecco's medium (Sigma-Aldrich) medium supplemented with Liberase-TL (100µgml−1, Roche) and DNase I (100µgml−1, Roche) at 37°C for 20min and dissociated with 18G and 21G needle. Blood was collected from the heart. Red cell lysis was performed on bone marrow and blood samples (RBC Lysis Buffer, Invitrogen). Isolated leukocytes stained cell surface markers for flow cytometry analysis and sorting. Cell populations were sorted using either SORP-aria (BD Biosciences) or ARIA-III instrument (BD Biosciences), and analyzed using BD FACSDIVA software (BD Biosciences) and FlowJo software. Immune cells in Fig.1 were sorted as Cells -> singlets -> DAPI- ->CD45+ cDC in Extended data Fig.3a were sorted as Cells-> singlets -> ->CD45+ ->DAPI-Lin- ->MCH-IIhiCD11chi, cDC1 were gated as Cells-> singlets -> ->CD45+ ->DAPI-Lin- -> MCH-IIhiCD11chi -> CD103+XCR1+CD11b-, cDC2 were gated as Cells-> singlets -> ->CD45+ ->DAPI-Lin- -> MCH-IIhiCD11chi -> CD103-XCR1-CD11b+. mDC were gated as Cells-> singlets -> ->CD45+ ->DAPI-Lin- -> MCH-IIhiCD11chi -> CD86+/CCR7+ CDP (Fig.4, Extended data fig.6a) were selected as: cells -> singlets -> CD45+ -> Lin− -> CD135+CSF−1R+ -> CD117int -> BrdU+ preDC (Fig.4, Extended data fig.6a) were selected as: cells -> singlets -> CD45+ -> Lin− -> CD135+CD11c+ -> CD172- -> BrdU+ T cells (Fig 5, Extended data fig.7) were selected as: cells -> CD45+ -> Lin− -> CD11c-MHC-II- -> TCRb+ PICS (Fig 5, Extended data fig.7) were selected as: cells -> CD45+ -> Lin− -> CD11c+MHC-II+ -> TCRb+ CD8+ T cell subsets (Fig 6, Extended data fig.10) were selected as: cells -> singlets -> CD45+ -> Lin− -> CD11c+MHC-II+ -> CD8 + -> CD44+CD62L+(central memory)/ CD44+CD62L-(effector memory)/ CD44-CD62L+(naive)

CD4+ T cell subsets (Fig 6, Extended data fig.10) were selected as: cells -> singlets -> CD45+ -> Lin− -> CD11c+MHC-II+ -> CD4 + -> CD44+CD62L+(central memory)/ CD44+CD62L-(effector memory)/ CD44-CD62L+(naive)
Monocytes (Fig 6, Extended data fig.10) were selected as: cells -> singlets -> CD45+ -> CD11b+Ly6c+
NK (Fig 6, Extended data fig.10) were selected as: cells -> singlets -> CD45+ -> TCRb+-> NK1.1+

Peripheral blood from human patients was collected to EDTA-containing tubes (Becton Dickinson). Each sample was diluted 1:1 in ice cold FACS buffer. Mononuclear cell separation was performed by density centrifugation media (Ficoll-Paque; GE Healthcare Life Sciences) in a 1:1 ratio with diluted blood cells. Centrifugation (460g, 25min) was performed at 10°C, and the mononuclear cells were carefully aspirated and washed with ice-cold FACS buffer. After red blood cell lysis (Sigma-Aldrich) for 5min at 4°C and washing cells were frozen in 10% DMSO in FBS and stored at -80°C until further processing. On the day of the analysis, the cells were washed twice with FACS buffer, stained and analyzed using BD FACSDIVA software (BD Bioscience) and FlowJo software (FlowJo, LLC).
cDCs in human blood (Fig.3f-i, Extended data fig.5f) Cells -> single -> CD45+ -> Lin- -> CD11c+HLA-DR+. cDC1 were selected as CD1c-CD141+. cDC2 were selected as CD1c+CD141-.
Liver biopsies from bariatric surgery patients were collected in RPMI (Supplemented with 10%FBS) and immediately transported on ice to the laboratory. A section of tissue was formalin fixed for histopathological evaluation; however, the bulk of the tissue was digested using Miltenyi Tumor dissociation Kit (cat no. 130-095-929) as per the manufacturer's instructions. Single cell suspensions thus generated were stored in Liquid nitrogen in FBS+20% DMSO until further processing. For sorting and single cell RNAseq of DCs obtained from control and NASH livers, the stored cells were thawed in a 37oC water bath, washed once with complete media and once with FACS buffer (PBS+2% FBS). After FC blocking, cells were stained in dark for 30 min and washed once with FACS buffer and once with sorting buffer (PBS+0.5mM EDTA).
cDCs in human liver (Fig.3a,b, Extended data fig.6a) were gated Cells -> single -> live -> CD45+ -> Lin- -> CD11c+
Mice were fasted for 14h and anaesthetized with an intraperitoneal injection of a ketamine (100mg kg−1) and xylazine (10 mg kg−1) mixture. Liver leukocytes were isolated by a modification of the two-step collagenase perfusion method of Seglen Digestion using Liberase™ TL (cat. no. 5401020001; Roche) for scRNA-seq, or using The Liver Dissociation Kit (Miltenyi Biotec, cat. no. 130-105-807) according to the manufacturer's guidelines for FACS analyses. Bone marrow was isolated by flushing from tibia and femur. Cervical, celiac and portal lymph nodes were processed for recovery of DC-T PICs and single DC and T cells as previously described48. Briefly, lymph nodes were digested in Isocove's modified Dulbecco's medium (Sigma-Aldrich) medium supplemented with Liberase-TL (100µgml−1, Roche) and DNase I (100µgml−1, Roche) at 37°C for 20min and dissociated with 18G and 21G needle. Blood was collected from the heart. Red cell lysis was performed on bone marrow and blood samples (RBC Lysis Buffer, Invitrogen). Isolated leukocytes stained cell surface markers for flow cytometry analysis and sorting. Cell populations were sorted using either SORP-aria (BD Biosciences) or ARIA-III instrument (BD Biosciences), and analyzed using BD FACSDIVA software (BD Biosciences) and FlowJo software.
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preDC (Fig.4, Extended data fig.6a) were selected as: cells -> singlets -> CD45+ -> Lin− -> CD135+CD11c+ -> CD172- -> BrdU+
T cells (Fig 5, Extended data fig.7) were selected as: cells -> CD45+ -> Lin− -> CD11c-MHC-II- -> TCRb+
PICS (Fig 5, Extended data fig.7) were selected as: cells -> CD45+ -> Lin− -> CD11c+MHC-II+ -> TCRb+
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cDCs in human liver (Fig.3a,b, Extended data fig.6a) were gated Cells -> single -> live -> CD45+ -> Lin- -> CD11c+

Instrument

SORP-FACSAria or FACS-AriaIII sorter

Software

BD FACSDIVA V.9.0 software (BD Biosciences) and FlowJo software V.10.7.1.

Cell population abundance

scRNA-seq with index sorting was used to determine population purity in Fig.2,4,5,6. In fig 2,6, IHC was used to validate FACS data.

Gating strategy

Gating strategies for each FACS experiment are indicated in main and/or supplementary figures. Briefly:
Immune cells in Fig.1 were sorted as Cells -> singlets -> DAPI- ->CD45+
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☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.