

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	No software was used to collect data.
Data analysis	Software used for analyses are: Cellranger (v3.1); Seurat (v3.2); Harmony (v0.99.9); Scran (v1.12.1); ggplot2 (v3.2.1); clusterProfiler (v3.12); SCENIC (v1.2.4); CellChat (v0.5.5); R (v3.6.1); GraphPad Prism (v7); Flowjo 10.8.1

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 Portfolio [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 description of any restrictions on data availability
 - For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The scRNA-seq data used in this study have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0003064.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

This study has not involved sex and gender based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

This study has not involved race, ethnicity, or other socially relevant groupings based analysis.

Population characteristics

The clinical characteristics of 15 individuals are noted in Supplementary Table 1. For immunohistochemistry study, liver samples from patients with PBC were derived from ultrasound-guided needle liver biopsies and liver biopsies of liver transplantation recipients; normal liver tissues collected from 5 patients with hepatic hemangioma were used as the normal control group. The clinical characteristics of PBC biopsies used in correlation analysis of CD69+CD4+ T cells and clinical statistics were ALP (n=30, 486.4±937.5 U/L), GGT (n=30, 368.1±302.5 U/L), TBIL (n=30, 170.2±195.8 µmol/L), DBIL (n=30, 133.0±154.5 µmol/L), IgG (n=24, 1453.8±611.4 mg/dL), AMA-M2 (n=26, 2.3±1.6), fibrosis level (n=23, 2±1.2), and inflammation level (n=23, 2.6±0.6).

For flowcytometry 3 healthy background non-lesion liver tissue and 4 PBC liver tissue were acquired the same way as aforementioned. The clinical characteristics of healthy control were ALT (n=3, 20±8.5 U/L), AST (n=3, 21.7±6.7 U/L), ALP (n=3, 75.7±34.3 U/L), GGT (n=3, 32.3±32.9 U/L), TBIL (n=3, 5.6±2.1 µmol/L), DBIL (n=3, 2.5±0.90 µmol/L). 4 PBC liver tissue was obtained from patients (UDCA treatment-naïve, AMA-M2 positive, ANA negative, GP210 negative) were acquired the same way as aforementioned. The clinical characteristics of PBC were ALT (n=4, 47.8±32.8 U/L), AST (n=4, 45±23.2 U/L), ALP (n=4, 171.5±53.8 U/L), GGT (n=4, 107.8±113.4 U/L), TBIL (n=4, 14.2±7.7), DBIL (n=4, 5.5±1.80).

The upper limit of normal biochemical features is listed as below. ULN of ALT=50 U/L, ULN of AST=40 U/L, ULN of ALP=125 U/L, GGT=60 U/L, ULN of TBIL=26 µmol/L, ULN of DBIL=8 µmol/L, ULN of IgM=220 mg/dL, ULN of IgG=1740 mg/dL.

Recruitment

For scRNA-seq assay, healthy background non-lesion liver tissue (n=5) was obtained intraoperatively from patients undergoing surgical liver resection for hepatic hemangioma at the Hepatobiliary and Pancreatic Unit, Department of Clinical Surgery, the First Affiliated Hospital of Zhejiang University. 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.

PBC liver tissue (n=5) was obtained from patients (UDCA treatment-naïve, AMA-M2 positive, ANA negative, GP210 negative) undergoing diagnostic liver biopsy at the Department of infectious diseases, the First Affiliated Hospital of Zhejiang University. Blood from PBC patients was obtained before the diagnostic liver biopsy. All patients met the standard diagnostic criteria of PBC, as described elsewhere. Scheuer scoring system was used to evaluate fibrosis and inflammation of liver biopsies. Nakanuma staging system was used to determine the PBC stage.

Blood from healthy control donors (n=5) were obtained from medical examination center, the First Affiliated Hospital of Zhejiang University.

Ethics oversight

Local approval for procuring human liver tissue and blood samples for scRNA-seq and histological analysis was obtained from the First Affiliated Hospital of Zhejiang University Ethics Committee, following principles of the Declaration of Helsinki. All subjects provided written informed consent.

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

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

No statistical method was used to estimate sample size. For scRNA-seq, the sample size was based on published high quality scRNA-seq studies (see Referees: Poch, T. et al. J Hepatol. 2021 Aug;75(2):414-423; Ramachandran P, et al., Nature, 2019, 575, 512-518; Corridoni D, et al., Nat Med, 2020, 26, 1480-1490). According to the studies, we chosen 5 PBC samples and 5 control samples to perform scRNA-seq, which allowed us to perform statistical analysis on the obtained data. Other experiments were generally conducted with at least n = 3 biologically independent samples to guarantee reproducibility of the results. Sample size for each experiment is indicated in the figure legend.

Data exclusions

No data were excluded from analyses.

Replication

Experiments were conducted at least 3 times. Exact n-values are given in the figure legends.

Randomization

No randomization was used in the study due to patient data is observational.

Blinding

Scoring of murine PBC hepatic inflammation or PBC patients' liver histopathology was performed on coded H&E-stained sections of liver by

“blinded” pathologists during data collection and analysis.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

All antibodies used in the study are noted in methods.

Antibodies used in human Multiplex immunochemistry staining:

anti-CD69 (ab233396, Abcam)

anti-CXCR6 (ab273116, Abcam)

anti-IFN γ (ab231036, Abcam) , anti-TNF α (ab270264, Abcam), anti-CK19 (ab7754, Abcam), anti-GDF15 (ab206414, Abcam), antiCCL21 (ab23116, Abcam)

anti- α SMA (ab124964, Abcam)

anti-CLEC4C (ab239078, Abcam)

anti-Secretin (bs-0088R, Bioss).

Antibodies used for the murine multiplex immunochemistry staining:

Ki67(ab15580, Abcam)

anti-Phospho-Stat1 Ser727 (8826, CST)

CD3 (ab16669, Abcam)

anti-CD4 (ab183685, Abcam)

anti-CD8 (ab217344, Abcam)

Antibodies used for murine flow cytometry analysis :

BV510 Hamster Anti-Mouse CD45 (563891, BD)

BV786 Hamster Anti-Mouse CD3e (564379, BD)

BV786 Hamster Anti-Mouse TCRb (742484, BD)

BUV395 Rat Anti-Mouse CD4 (563790, BD)

APC Rat Anti-Mouse CD8a (553035, BD)

BV650 Rat Anti-Mouse CD44 (740455, BD)

PE-Cy[™]7 Rat Anti-Mouse CD62L (560516, BD)

FITC Hamster Anti-Mouse CD69 (104505, Biolegend)

PE Hamster Anti-Mouse CD103 (121406, Biolegend)

FITC Rat Anti-Mouse IFN- γ (554411, BD)

BV605 Mouse Anti-Mouse Tbet (644817, BD) .

Antibodies used for human flow cytometry analysis :

APC-H7 Mouse anti-Human CD45(560178,BD)

BUV395 Mouse Anti-Human CD3(564001,BD)

FITC Mouse Anti-Human TCR $\alpha\beta$ (555547,BD)

BV480 Mouse Anti-Human CD4(566104,BD)

BUV737 Mouse Anti-Human CD25(612806,BD)

Alexa Fluor[®] 647 Mouse anti-Human FoxP3(560045,BD)

PE-Cy[™]7 Mouse Anti-Human CD69 (557745,BD)

PE anti-human CD186 (CXCR6) Antibody(356003,Biolegend)

Alexa Fluor[®] 700 Mouse Anti-Human CD8 (557945,BD)

PE-CF594 Mouse Anti-Human Perforin(563763;BD).

Validation

IHC and flow cytometry validation statements on manufactures' website.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Adult male C57BL/6JCrI mice aged 8-10 weeks were purchased from Charles River (Shanghai). Mice were housed under specific pathogen-free conditions at the Zhejiang University for the establishment of murine PBC model.

Wild animals

The study did not involve wild animals.

Reporting on sex

Only female mice was used for the establishment of PBC model as the former research recommended.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All experimental protocols were approved by the the First Affiliated Hospital of Zhejiang University Ethics Committee(2020917).

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

Liver tissue was obtained from liver fine needle aspiration biopsy of patients with PBC. Control human liver tissue was the non-involved surrounding tissue obtained from patients undergoing partial hepatectomy for hepatic hemangioma. Samples were collected from the First Affiliated Hospital of Zhejiang University. The freshly human livers were kept on ice in RPMI1640 +10%FBS solution. Single-cell suspension was obtained by grinding the tissue and sequentially digested with 37°C pre-warmed solution containing 10mM HEPES (Sigma) and 400µg/mL Liberase TM (Roche). Samples were enzymatically dissociated for 10 minutes at 37°C in a water bath, then put on ice with EDTA addition to a final concentration of 10mM to stop Liberase digestion. After centrifuging at 800g for 5min, the supernatant was decanted, and the pellet was resuspended in ACK lysis buffer to lyse the red blood cells. Cells were washed twice in PBS+2% FBS and filtered through 40µm mesh. PBMCs were isolated from blood using human lymphocyte separation medium.

Instrument

Stained cells were assessed on BD Fortessa and CytoFLEX LX(Beckman) depends on different assays' needs .

Software

FlowJo software (Tree Star, Ashland, OR) was used for flow analysis.

Cell population abundance

Around 1×10^6 /ml cells was washed and suspended in PBS supplemented with 1.5%FBS.

Gating strategy

Gating strategy is described in manuscript.

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