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# 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 (*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<br>*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<br>*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 | No specific software was used for data collection. The datasets were processed and analyzed using the open source software listed below. |
| Data analysis | Celescope (v.1.15.0) was used to align and quantify scRNA-seq data to obtain a matrix for analysis.<br>Space Ranger (v.3.1.1) was used to process spatial transcriptomics data to obtain a matrix for analysis.<br>milo (v.2.0.0) was used to test for the differential abundance of cellls within defined neighborhoods.<br>Scirpy (v.0.19.1) was used for scVDJ data analysis.<br>Scanpy (v.1.10.3) was used for the downstream analysis after object creation and clustering the scRNA-seq datasets.<br>flowsig (v.0.1.2) was used to identify differentially flowing signal variables.<br>CellPhoneDB (v.5.0.0) and CellChat (v.2.1.2) was used to identify cell-cell interactions.<br>CCplotR (v.0.99.3) was used to visualize significant ligand-receptor pairs.<br>commot (v.0.0.3) was used to assess ligand-receptor interaction intensities within Visium sections.<br>STAligner (v.1.0.0) was used for batch effect correction in Visium ST datasets.<br>Cell2location (v.0.1.3) was used for the spots' deconvolution of Visium ST datasets.<br>pyscenic (v.0.12.1) was used to infer gene regulatory networks from scRNA-seq datsets.<br>igraph (v.2.0.3) and ggraph (v.2.1.0) were used for the visualization of gene regulatory networks.<br>decoupler (v.1.6.0) was used for the enrichment of GO and KEGG terms.<br>sctm (v.0.1.3) was used for the identification of spatial gene topics.<br>Hotspot (v.1.1.1) was used to identify and display metabolic programs.<br>STalign (v.1.0) was used to integrate spatial transcriptomics with spatial metabolomics.<br>GraphPad Prism software (v.10.4.2) was used for experimental data statistical analysis and visualization. |

The code required to reproduce the analyses in this paper is available through GitHub (github.com/OMIC-coding/Spatial_multiomics_analysis_MASLD) and Zenodo (doi.org/10.5281/zenodo.17192831).

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

All raw data for scRNA-seq and 10x Visium generated in this study are deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA007511) that are available under controlled access at https://ngdc.cncb.ac.cn/gsa-human; The spatial metabolomic data have been deposited in the OMIX, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession no.OMIX009098). The processed data of scRNA-seq and Visium ST are available from the OMIX with the accession number OMIX010136. The spatial proteomic data is also available from the OMIX under accession number OMIX009117. All processed data can be visualized and downloaded on the HMSMA website (https://db.genomics.cn/stomics/hmsma). Source data are provided with this paper.

## 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 | Information on sex is summarized in Supplementary Table 1. Sex and gender were not considered in the study design. |
| Reporting on race, ethnicity, or other socially relevant groupings | All 61 participants are Asian. Race, ethnicity, or other socially relevant groupings were not considered in study design. |
| Population characteristics | Control liver samples were procured from ten patients at the Institute of Hepatobiliary Surgery of Southwest Hospital, none of whom showed evidence of fatty liver disease, primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), liver cancer, viral hepatitis, or other metabolic liver diseases. Samples from patients with MASLD were obtained through biopsy or surgical procedures. The diagnosis of MASLD was based on integrating clinical features, laboratory tests, and pathological diagnosis while excluding other known hepatic conditions. |
| Recruitment | The participants were diagnosed at the First Affiliated Hospital of Third Military Medical University and the First Affiliated Hospital of Wenzhou Medical University, fulfilling our pre-established inclusion criteria, voluntarily participating in the study. Prior to the commencement of the study, written informed consent was obtained from all participants. |
| Ethics oversight | The study protocol was reviewed and approved by the Ethics Committees of the First Affiliated Hospital (Southwest Hospital) of Army Medical University [approval nos. (A)KY2021061 and (B)KY202350] and the First Affiliated Hospital of Wenzhou Medical University [approval no. 2016-246]. |

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.

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|---|---|
| Sample size | A total of 29 liver samples were subjected to single-cell sequencing, including 6 controls, 7 MASL samples, 16 MASH samples.<br>Liver tissue samples for spatial transcriptomics were collected from 35 donors, including 7 controls, 10 MASL samples, 18 MASH samples.<br>Liver tissue samples for spatial metabolomics were collected from 27 donors, including 6 controls, 9 MASL samples, and 12 MASH samples.<br>A total of 14 liver samples were subjected to spatial proteomic profiling, including 4 controls, 3 MASL samples, 7 MASH samples.<br>No statistical method was used to predetermine sample size. |
| Data exclusions | No data were excluded from the analyses. |
| Replication | All in vitro experiments were repeated independently at least three times. |

| | |
|---|---|
| Randomization | No randomization was used in the study because the patient data is observational. |
| Blinding | The investigators were not blinded to allocation during experiments and outcome assessment. |

# 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

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|---|---|
| Antibodies used | All antibodies used in the study are listed in the Supplementary Table 3.<br>TREM2, Cell Signaling, Danvers, MA/91068<br>MITF, Abcam, Cambridge, MA/ab303530<br>CYP3A4, Santa Cruz, Santa Cruz, CA/sc-53850<br>EPCAM, Cell Signaling, Danvers, MA/14452<br>HNF4a, Cell signaling, Danvers, MA/3113<br>Ki67, Abcam, Cambridge, MA/ab16667<br>MET, Cell signaling, Danvers, MA/8198S<br>Mouse IgG, Absin, Shanghai, China/abs50012<br>Rabbit IgG, Absin, Shanghai, China/abs50012 |
| Validation | All antibodies used in the study are commercially available with validation procedures described on the sites of the manufacture.<br>TREM2, https://www.cellsignal.cn/products/primary-antibodies/trem2-d8i4c-rabbit-mab/91068<br>MITF, https://www.abcam.cn/products/primary-antibodies/mitf-antibody-epr26363-10-ab303530.html<br>CYP3A4, http://www.scbt.com/p/cyp3a4-antibody-hl3<br>EPCAM, http://www.cellsignal.cn/products/primary-antibodies/epcam-d9s3p-rabbit-mab/14452<br>HNF4a, http://www.cellsignal.cn/products/primary-antibodies/hnf4a-c11f12-rabbit-mab/3113<br>MET, http://www.cellsignal.cn/products/primary-antibodies/met-d1c2-xp-rabbit-mab/8198<br>Ki67, http://www.abcam.cn/products/primary-antibodies/ki67-antibody-sp6-ab16667.html<br>Mouse IgG, https://www.absin.cn/four-color-multi-label-immunofluorescence-kit/abs50012.html<br>Rabbit IgG, https://www.absin.cn/four-color-multi-label-immunofluorescence-kit/abs50012.html |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

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|---|---|
| Cell line source(s) | THP-1 and HepG2 cell lines were purchased from Hunan Fenghui Biotechnology Co., Ltd, China. |
| Authentication | Cell line authentication was performed for THP-1 and HepG2 via Short Tandem Repeat (SRT) profiling prior to experimentation. Each profile matched the known reference standard. |
| Mycoplasma contamination | All cell lines were tested for Mycoplasma by PCR, and the results were negative for Mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used in this study. |

## Plants

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|---|---|
| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |