

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                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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 | RNA-Seq was performed on a DNBSEQ-T7 platform (MGI). Proteomics analysis was performed on a Vanquish Neo UHPLC system (Thermo Fisher Scientific). Targeted metabolomics quantification was performed on an ACQUITY UPLC-Xevo TQ-S system (Waters). qRT-PCR was performed on an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific). Immunofluorescence imaging was performed on a Leica TCS SP5 confocal microscope (Leica). Flow cytometry was performed on a BD FACSCelesta™ instrument (BD Biosciences). ECAR was measured using an XFe96 Extracellular Flux Analyzer (Agilent Technologies). |
| Data analysis   | Statistical analyses were performed using GraphPad Prism (v8.0) and R (v4.3.1). RNA-seq data were analyzed using GSEA software (v3.0). Proteomic data were processed using DIA-NN (v1.8.1). Immunofluorescence images were processed using LAS X Life Science Microscope Software (v1.0.0). Metabolomics data were processed using TMBQ software (v1.0). ECAR was analyzed using Wave Desktop (v2.6.1). Flow cytometry data were analyzed using FlowJo (v10.6.2).                                                                                                                                     |

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD060315 (<https://www.ebi.ac.uk/pride/archive/projects/PXD060315>). RNA-seq data generated in this study are available in the NCBI Sequence Read Archive under BioProject accession PRJNA1203080 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1203080>). Metabolomics data have been deposited in the Metabolights under the accession number MTBLS12950 (<https://www.ebi.ac.uk/metabolights/MTBLS12950>). The single-cell RNA-seq data generated by mice in this study are available in the NCBI Sequence Read Archive under BioProject accession PRJNA1322306 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1322306>). The mRNA expression and clinicopathological data were downloaded from TCGA-LIHC dataset. GTEx data for normal liver tissues were downloaded from the GTEx website. Microarrays that contain gene expression data of HCC tissues and adjacent normal liver tissues were searched and downloaded from the GEO database (GSE14520, GSE76427, GSE138178, GSE105130, and GSE104310). 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                                        | The biological sex of all patients with HCC is reported in Supplementary Tables 1-3. The study was not designed to investigate sex-based differences, and no sex-specific analyses were conducted.                                       |
| Reporting on race, ethnicity, or other socially relevant groupings | The race, ethnicity and other socially relevant groupings of the patients were not considered in the study.                                                                                                                              |
| Population characteristics                                         | The HCC tissues used in this study were obtained from Zhongshan Hospital, Shanghai. The detailed clinical characteristics were shown in Supplementary Tables 1-3.                                                                        |
| Recruitment                                                        | Patient selection showed no evidence of bias. All samples were obtained from patients with immunohistochemically confirmed HCC, with matched histologically confirmed non-tumorous adjacent tissues collected from the same individuals. |
| Ethics oversight                                                   | The study involving human samples was approved by the Institutional Review Board of Zhongshan Hospital, Fudan University (Ethical number: B2021-081).                                                                                    |

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](https://www.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 predetermine sample size. sample sizes were chosen based on established practices in cell biological and animal experimentation. For animal studies, at least 3 mice were used per group.                            |
| Data exclusions | No data were excluded from the manuscript.                                                                                                                                                                                                             |
| Replication     | Biological replicates are defined for each experiment in the figure legends. In vitro experiments were repeated at least twice.                                                                                                                        |
| Randomization   | The allocation of mice was randomly. In vitro experiments using cell lines were not randomized since the studies were controlled by the same cell line receiving different treatments.                                                                 |
| Blinding        | The investigators who performed quantification of immunohistochemistry analysis were blinded to the patient information. The animal experiments were not performed in a blinded manner due to the practical constraints of the experimental procedure. |

## 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                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                                 |

## Methods

|                                     |                                                    |
|-------------------------------------|----------------------------------------------------|
| n/a                                 | Involved in the study                              |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

### Antibodies used

Rabbit monoclonal anti-PON1, Abcam, 1:1000, Cat: ab92466  
 Rabbit monoclonal anti-PON1, Abcam, 1:100, Cat: ab126597  
 Rabbit monoclonal anti-PON1, Abcam, 1:100, Cat: ab248121  
 Rabbit Polyclonal anti-HIF1 alpha, HUABIO, 1:1000, Cat: ER1802-41  
 Rabbit Polyclonal anti-HIF1 alpha, AiFang, 1:50, Cat: AF02369  
 Mouse monoclonal anti-VHL, Santa cruz, 1:500, Cat: sc-514041  
 Mouse monoclonal anti-Ubiquitin, 1:500, Cat: sc-8017  
 Mouse monoclonal anti-ACTIN, 1:5000, Cat: 30103ES60  
 Mouse monoclonal anti-Histone H3, 1:500, cat: sc-517576  
 Mouse monoclonal anti-Tubulin, 1:1000, cat: sc-69971  
 Pacific Blue anti-mouse CD45, Biolegend, 103126, 1:100  
 PE anti-mouse CD4, Biolegend, 100408, 1:100  
 Brilliant Violet 510 anti-mouse CD8a, Biolegend, 100751, 1:100  
 PE-cy7 anti-mouse PD-1, Biolegend, 135215, 1:100  
 PE anti-mouse LAG-3, Ebioscience, 12-2239-421, 1:100  
 PE anti-mouse Tim3, Biolegend, 134003, 1:100  
 APC anti-mouse IFN-g, Biolegend, 506510, 1:50  
 PE anti-mouse TNF- $\alpha$ , Biolegend, 506305, 1:50  
 Alexa Fluor 647 anti-mouse FOXP3, Biolegend, 126407, 1:50  
 Fixable Viability Dye eFluor 506, Ebioscience, 65-0866-14, 1:200  
 APC anti-human CD45, Biolegend, 304011, 1:100  
 Brilliant Violet 421 anti-human CD3, Biolegend, 317343, 1:100  
 PE anti-human CD4, Biolegend, 980804, 1:100  
 FITC anti-human CD8, Biolegend, 344703, 1:100  
 PE/Cyanine7 anti-human CD11b, Biolegend, 982608, 1:100  
 PE/Dazzle 594 anti-human CD11c, Biolegend, 301642, 1:100  
 Brilliant Violet 510 anti-human CD16, Biolegend, 302047, 1:100  
 Alexa Fluor 700 anti-human CD56, Biolegend, 362521, 1:100  
 Brilliant Violet 785 anti-human CD68, Biolegend, 333825, 1:100  
 APC/Cyanine7 anti-mouse/human B220, Biolegend, 103223, 1:100  
 PE-cy7 anti-mouse CD8, Ebioscience, 25-0088-42, 1:100  
 APC anti-mouse CD8, Ebioscience, MA5-28718, 1:100  
 PE anti-mouse PD-1, Ebioscience, 12-9969-42, 1:100  
 APC/Fire™ 750 anti-mouse LAG-3, Biolegend, 125239, 1:100  
 APC anti-mouse Tim3, Ebioscience, 17-3109-42, 1:100  
 eFluor 450 anti-mouse IFN-g, Ebioscience, 48-7311-82, 1:50  
 Brilliant Violet 421 anti-mouse TNF- $\alpha$ , Ebioscience, 404-7321-82, 1:50  
 APC anti-mouse Annexin V, Biolegend, 640919, 1:50  
 FITC anti-mouse Ki-67, Biolegend, 1:50  
 CD16/CD32 Monoclonal Antibody, Ebioscience, 14-0161-86, 1:200

### Validation

All antibodies were purchased from reputable commercial vendors and applicable citations are available on product listings for all antibodies.

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

### Cell line source(s)

Hepa1-6 (mouse hepatoma) cell line was purchased from the National Collection of Authenticated Cell Cultures. Hep-53.4 (mouse hepatoma) cell line was purchased from EFE Biotechnology Co., Ltd (Shanghai, China). T cells were isolated from the

spleens of 6-8 week-old male C57BL/6 mice.

Authentication

Hepa1-6 and Hep-53.4 cell lines were authenticated by short tandem repeat profiling prior to use.

Mycoplasma contamination

All cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines  
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

## 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

C57BL/6 mice were purchased from GemPharmatech Co., Ltd (Nanjing, China). Pon1-knockout (Pon1-KO) mice on a C57BL/6 background were generated and provided by Cyagen Biosciences (Guangzhou, China). Rag1<sup>-/-</sup> mice were provided by Professor Yiwei Chu. All mice were male, 6- to 8-weeks old, and in a specific pathogen-free condition, temperature- and humidity-controlled vivarium with a 12-hour light/dark cycle at the Fudan University animal facility (Shanghai, China).

Wild animals

This study did not involve wild animals.

Reporting on sex

Sex-based analysis was not performed in this study. To ensure experimental consistency and comparability, 6-8-week-old male mice were used in this study.

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight

All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Welfare and Ethics Subcommittee of the Department of Laboratory Animal Science, Fudan University (Ethical number: 202309031S).

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

## Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

## 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

Cells isolated from human and mouse tissues were incubated with an anti-mouse CD16/32 antibody for 15 min on ice to block nonspecific Fc receptor binding. The cells were then stained with cell surface markers. After 30min surface antibody incubation at 4 °C, cells were washed twice. Intracellular molecular staining was performed using the FOXP3-Transcription Factor Staining Buffer Set according to the manufacturer's protocol. For intracellular cytokine detection, cells were stimulated in vitro with cell stimulation cocktail (phorbol myristate acetate, ionomycin, monensin, and brefeldin A) for 5 h. The cells were fixed, permeabilized, and stained with the indicated monoclonal antibodies. Fluorescence was measured using a FACS flow cytometer (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), and data were analyzed using FlowJo software (Tree Star, Ashland, USA).

Instrument

BD FACSCelesta

|                           |                                                                                                                                                                                                               |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Software                  | Flowjo v10.6.2                                                                                                                                                                                                |
| Cell population abundance | CD4+ T cell purity were identified by MACS, and the average purity of CD4+ T cell populations was greater than 98%.                                                                                           |
| Gating strategy           | Cell debris were removed by FSC/SSC gating, then the sticky cells were excluded by trigger pulse width gating. Gating strategies of immune cells are shown in Supplementary Fig. 2a and Supplementary Fig. 7. |

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