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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 *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	Q-Exactive-plus mass spectrometer (Thermo Fisher Scientific) coupled with Esay-nLC 1200 (Thermo Fisher Scientific), and LTQ Obitrap ETD coupled with Esay-nLC 1000 (Thermo Fisher Scientific) were used to collect proteomics data. Ilumina Hiseq6000 platform (Novogene, Tianjing, China) was used to collect RNA-seq data; Reads were aligned to the human genome hg38. The IHC staining images were acquired with Leica AperioCS2.
Data analysis	Halo software (version 3.3.14) was used to analyze Images quantification of IHC. GraphPad Prism software (version 7.0) and R (version 4.2.2) were used for statistical analyses. Zeiss 800 confocal microscope (version ZEN 10.0) and Fiji-ImageJ software were used for visualization, presentation and analysis of fluorescence imaging. STAR (version 2.7.10b), RSEM (version 1.3.1) and GSEA (version 42.3) were used for presentation and analysis of RNA-seq. Pymol (version 4.60), AutoDock vina (version 1.1.2) and AutoDockTools (version 1.5.7) were used for visualization and analysis of molecular docking. Proteome Discoverer (version 2.1) was used for mass spectrometry proteomics data. Proteome Discoverer (version 1.4) was used for analysis of LC-MS/MS data of protein citrullination.

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

Mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium via the iProX repository with the data set identifier PXD046340 (https://www.iprox.cn//page/project.html?id=IPX0007354000). RNA-seq data supporting the findings of this study have been deposited into GEO and public with accession no. GSE233772 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE233772). 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	Sex and gender were not considered in the study design, nor were sex- and gender-based analyses performed. HCC occurs in both men and women, therefore the sex and gender of the patients used in our study were randomly selected.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	Formalin-fixed, paraffin-embedded primary HCC specimens were randomly selected from the archives of the First Affiliated Hospital of University of Science and Technology of China (Hefei, China). Of the 41 HCC patients, 4 were women and 37 were men, with 32 patients over the age of 50.
Recruitment	We used the samples of patients diagnosed with HCC for IHC experiments, which were mainly from 2017 to 2022.
Ethics oversight	Ethical approval for the studies was obtained from the Institutional Research Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China.

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	We determined the sample sizes based on preliminary studies in our laboratories or in similarly published research. The samples were enough to be detected and we observed statistical significant difference in replicated independent experiments. For animal experiments, we used 5-6 male BALB/c nude mice for each group and followed the 3 R's of animal research.
Data exclusions	No data were excluded from the data set.
Replication	We defined each sample in different groups by performing three independent biological experiments, and the results showed the same trend. For animal studies, we used 5-6 male BALB/c nude mice for each different group, and the statistical significance was shown in figures. We confirmed successful replication for our reported data.
Randomization	Mice were randomly allocated to control group or treatment groups. For in vitro experiments all samples were analyzed equally with no subsampling, and therefore there was no requirement for randomization.
Blinding	For in vitro experiments, the experimental conditions were not blinded since the comparisons were objective and quantitative. For in vivo experiments, investigators were blinded to group assignments. In the IHC analysis of HCC spacimens, the investigators were blinded for the clinical information of each sample prior to immunostaining 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

Antibodies for western blot:
Mouse monoclonal anti-Citrulline (2D3.1); Invitrogen; Cat:MA5-27573; lot:WG3323401; 1:1000
Mouse purified anti-HIF-1α; BD Biosciences; Cat:610959; lot:9049717; 1:1000
Rabbit polyclonal anti-HIF-1αR698 citrullination; This study (Abclonal); Cat:WG-00942P; 1:1000
Rabbit polyclonal anti-PADI4; Abclonal; Cat:A1906; lot:3560845004; 1:1000
Mouse monoclonal anti-His-tag ; Proteintech; Cat:66005-1-Ig; lot:10004365; 1:5000
Mouse monoclonal anti-GST-tag; Proteintech; Cat:66001-1-Ig; lot:10005463; 1:5000
Mouse monoclonal anti-HA-tag (6E2) (HRP Conjugate); Cell Signaling Technology; Cat:2999s; lot:4; 1:1000
Mouse monoclonal anti-Flag-tag; Proteintech; Cat:66008-3-Ig; lot:10016566; 1:5000
Mouse monoclonal anti-Beta Actin; Proteintech; Cat:66009-1-Ig; lot:10004156; 1:5000
Rabbit polyclonal anti-LDHA; Proteintech; Cat:21799-1-AP; lot: 00051270; 1:5000
Rabbit polyclonal anti-PDK1; Proteintech; Cat:18262-1-AP; 1:5000
Rabbit monoclonal anti-Hydroxy-HIF (Pro564) (D43B5); Cell Signaling Technology; Cat:3434s; lot:8; 1:1000
Mouse monoclonal anti-VHL; Santa Cruz Biotechnology; Cat:sc-135657; lot:K02222; 1:2000
Rabbit polyclonal anti-Histone-H3; Proteintech; Cat:17168-1-AP; lot:000945157; 1:8000
Rabbit recombinant anti-Histone H3 (citrulline R2+R8+R17); Abcam; Cat:ab281584; lot:1071842-21; 1:1000
Antibodies for Immunofluorescence:
Rabbit polyclonal anti-HIF-1α (c-Term); Cayman; Cat:10006421; lot:0608579-1; 1:100
Mouse monoclonal anti-HIF-1α; Novus Biologicals; Cat:NB100-105SS; lot:BU; 1:100
Rabbit polyclonal anti-HIF-1αR698 citrullination; This study (Abclonal); Cat:WG-00942P; 1:100
Mouse monoclonal anti-PADI4; Abcam; Cat:ab128086; lot:GR3266397.3; 1:200
Goat polyclonal anti-Mouse IgG (CoraLite594-conjugated); Proteintech; Cat:SA00013-3; lot:20000128; 1:200
Goat polyclonal anti-Mouse IgG (CoraLite488-conjugated); Proteintech; Cat:SA00013-1; lot:20000422; 1:200
Goat polyclonal anti-Rabbit IgG (CoraLite594-conjugated); Proteintech; Cat:SA00013-4; lot:20000314; 1:200
Goat polyclonal anti-Rabbit IgG (CoraLite488-conjugated); Proteintech; Cat:SA00013-2; lot:20000450; 1:200
Antibodies for immunohystochemistry:
Rabbit polyclonal anti-HIF-1α (c-Term); Cayman; Cat:10006421; lot:0608579-1; 1:200
Rabbit polyclonal anti-HIF-1αR698 citrullination; This study (Abclonal); Cat:WG-00942P; 1:200
Mouse monoclonal anti-PADI4; Abcam; Cat:ab128086; lot:GR3266397.3; IHC 1:500

Validation

All antibodies were purchased from commercial suppliers including Invitrogen, BD Biosciences, Abclonal, Proteintech, Abcam, Santa Cruze Biotechnology, Cayman, Novus Biologicals, and Cell Signaling Technology with validation data,statement and applicable citations available on product listings for all antibodies (see individual catalog numbers). Additionally, all antibody were titrated for optimal dilution in the assay. We also validated the antibodies by over-expressing vectors or specific targeting shRNAs through western blot.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	human HEK293T, Hep3B, HepG2, RCC10, RCC90, THLE3 cells and mouse Hepa1-6 cells were purchased from ATCC.
Authentication	Cell line identities were confirmed by STR profiling.
Mycoplasma contamination	All cell lines were tested routinely to make sure they are negative for mycoplasma contamination by Mycoplasma PCR detecting method.
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	5-week-old male mice (BALB/c nude; SJA Laboratory Animal Company of China) All animals were housed at a suitable temperature (22–24 °C) and humidity (40–70%) under a 12/12-h light/dark cycle with unrestricted access to food and water for the duration of the experiment.
Wild animals	Not used.
Reporting on sex	There were no sex-based analyses in our study, and only male mice were used to perform experiments.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal studies were conducted with approval from the Animal Research Ethics Committee of South China University of Technology.

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

Plants

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.*