nature portfolio

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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	1: Western blot: ChemiDoc™ MP Imaging System (Bio-Rad). 2: Histology imaging: Leica Biosystems. 3: Mouse liver Proteomics: Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer coupled with Ulimate-3000 nano RSLC (Thermo Fisher Scientific), a trap column (PepMap C18 100 µm x 2 cm, 5 µm particle size, 100 Å pore size; ThermoFisher Scientific) and an analytical column (PepMap C18 analytical column 75 µm x 50 cm, 2 µm particle size, 100 Å pore size; ThermoFisher Scientific). 4: Rat liver and Human Cell Line SNU398 phosphoproteomics: Dionex UltiMate 3000 RSLCnano system equipped with a Dionex UltiMate 3000 RS autosampler, an Acclaim PepMap RSLC analytical column (75 µm x 50 cm, nanoViper, C18, 2 µm, 100Å; Thermo Scientific) and an Acclaim PepMap 100 trap column (100 µm x 2 cm, nanoViper, C18, 5 µm, 100Å; Thermo Scientific). 5: Serum amino acid and acylcarnitine: Electrospray ionization tandem mass spectrometry (ESI-MS/MS), using a Waters Xevo TQD triple quadrupole mass spectrometer (Waters GmbH, Eschborn, Germany) equipped with an electrospray ion source.
Data analysis	1. Statistics and graphing: SigmaPlot v.14 software (Systat Software GmbH, DEU) and GraphPad Prism 9.4 (GraphPad Software, LLC). 2. Proteomics LC-MS/MS analysis: Spectronaut (Version 16.3) and Perseus (Version 1.6.15). 3. Phosphoproteomics analysis: MaxQuant software suite (Version 1.6.5.0), Perseus (Version 1.6.2.3) and Phospho-Analyst (https://analyst-suites.org/apps/phospho-analyst/). 4. Amino acid and acylcarnitine analysis: MassLynx software (Waters GmbH, Eschborn, Germany).

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 of the raw data used to make the figures are included in the source data file. Processed proteomics data can be found within Supplementary Files 1-3. Raw proteomics data has been uploaded to the PRIDE/ProteomeXchange database (Rat liver: PXD049186, Human hepatoma: PXD052303, Mouse liver SEC22B IP: PXD049203).

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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 did not conduct statistical analyses to determine sample sizes in advance of conducting experiments. The sample sizes for each experiment are specified.
Data exclusions	N/A
Replication	The experimental design incorporated a minimum of three biological replicates, outlined in figure legends and methods. This approach ensured consistent data, supported by significant results unless stated otherwise.
Randomization	In the in vivo study, mice and rats were age and weight-matched to ensure a similar starting point between groups . Random allocation then assigned them to experimental groups.
Blinding	We conducted blinding for liver histological scoring. However, blinding was not feasible in the mouse studies due to the distinct treatments

Blinding involving injections.

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

Vinculin Mouse monoclonal primiary antibody (Sigma-Aldrich, Cat#V9131)
SEC22B Mouse monoclonal primiary antibody (SantaCruz, Cat#sc-101267)
GFP Mouse monoclonal primiary antibody (SantaCruz, Cat#sc-9996)
FLAG Rabbit polyclonal primary antibody (Sigma-Aldrich, Cat#F7425)
Phospho-PKA Substrate (RRXS*/T*) Rabbit monoclonal primiary antibody (Cell Signaling Technology, Cat#9624)
Phospho-CREB (Ser133) Rabbit monoclonal primiary antibody (Cell Signaling Technology, Cat#9198)
SQSTM1/p62 Rabbit polyclonal primary antibody (Cell Signaling Technology, cat #5114)
LC3B Rabbit polyclonal antibody (Cell Signaling Technology, cat #2775),

Validation

Vinculin Mouse monoclonal primiary antibody (Sigma-Aldrich, Cat#V9131); Manufacture's website: https://www.sigmaaldrich.com/AU/en/product/sigma/v9131
SEC22B Mouse monoclonal primiary antibody (SantaCruz, Cat#sc-101267); Manufacture's website: https://www.scbt.com/p/sec22b-antibody-29-f7
GFP Mouse monoclonal primiary antibody (SantaCruz, Cat#sc-9996); Manufacture's website: https://www.scbt.com/p/gfp-antibody-b-2
FLAG Rabbit polyclonal primary antibody (Sigma-Aldrich, Cat#F7425); Manufacture's website: https://www.sigmaaldrich.com/AU/en/product/sigma/f7425
Phospho-PKA Substrate (RRXS*/T*) Rabbit monoclonal primiary antibody (Cell Signaling Technology, Cat#9624); Manufacture's website: https://www.cellsignal.com/products/primary-antibodies/phospho-pka-substrate-rrxs-t-100g7e-rabbit-mab/9624
Phospho-CREB (Ser133) Rabbit monoclonal primiary antibody (Cell Signaling Technology, Cat#9198); Manufacture's website: https://www.cellsignal.com/products/primary-antibodies/phospho-creb-ser133-87g3-rabbit-mab/9198
SQSTM1/p62 Rabbit polyclonal primary antibody (Cell Signaling Technology, cat #5114); https://www.cellsignal.com/products/primary-antibodies/sqstm1-p62-antibody/5114
LC3B Rabbit polyclonal antibody (Cell Signaling Technology, cat #2775),Manufacture's website: https://www.cellsignal.com/products/primary-antibodies/lc3b-antibody/2775

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) SNU398 hepatoma cells

Authentication The SNU398 cell line was obtained from Prof. M. Celeste Simon (University of Pennsylvania). The authenticity of the cell line is based on the provider's validation.

Mycoplasma contamination Negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register) N/A

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 Male Sprague-Dawley rats (Animal Resources Centre, Perth, Australia) or male C57Bl/6N mice (Monash Animal Research Platform, Clayton, Australia) were used for experiments.Unless stated otherwise, male mice aged 7-8 weeks upon arrival, were acclimatized to

	the local housing facility (12-12h light-dark cycle, 22-24°C) for one week prior to experimentation and were fed a standard rodent chow diet (8720610, Barastoc, Australia).Male Sprague-Dawley rats were used for the in situ liver glucagon treatment studies. Each weighed ~160 grams and were not subjected to fasting.
Wild animals	The study did not involve the use of wild animals.
Reporting on sex	The entirety of experimental data from mice and rats were sourced exclusively from male subjects.
Field-collected samples	The study did not utilize samples collected in the field.
Ethics oversight	All mouse experiments were performed in accordance with the NHMRC Australian Code of Practice for the Care and Use of Animals was approved by the MARP-2 Animal Ethics Committee of Monash University (Approval 27814) and the University of New South Wales Animal Ethics committee (Approval AEC1836B).

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.*