nature portfolio

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Last updated by author(s): Feb 13, 2025

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	BAT-12 microprobe thermometer (Physitemp) OneTouch UltraVue automatic glucometers (Johnson & Johnson) Zeiss Axio Scan7 slide scanner (ZEISS) Oxymax/CLAMS metabolic cage system (Columbus Instruments), Metascreen software (version 2.2.15.12) Orbitrap Astral (Thermo Fisher) Zeiss LSM980+ Airyscan2 (ZEISS) Epson Scan software (version 3.9.3.4) Peaks Studio Xpro software (version 10.6) Proteome Discoverer (version 2.2)
Data analysis	Prism 8.0 (GraphPad software, version 8) Image J software (version 1.8.0 National Institute of Health Freeware) ZEISS ZEN 3.8 (Zeiss software, version 3.8.99.01000) VENNY 2.1 website (https://bioinfogp.cnb.csic.es/tools/venny/) Adobe Illustrator 2025 (version 29.1)

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

All the cell lines, plasmids and mouse model generated in this study will be made available on request, but we may require a payment or a completed materials transfer agreement if there is potential for commercial application.
All the mass spectrometry proteomics data in this study have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD072406, PXD072318 and PXD072319.
Original western blot images and statistics source data can be found in Extended data, and are publicly available as of the date of publication.
Microscopy data reported in this paper will be shared by the lead contact upon request

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications. N=3 mice were used to perform unbiased quantitative phosphoproteomic analysis; n=5 mice were used to evaluate the cold tolerance, oxygen consumption and other metabolic test; n=6 mice were used to evaluate the BAT lipolysis activity. Measurements for all cell-based assays were based on 3 to 5 technical replicates (wells) within a single experiment.
Data exclusions	No data were excluded.
Replication	All experimental findings were repeated at least three times, as stated in figure legends, and all additional replication attempts were successful.
Randomization	Randomization was applied wherever possible. For mice experiment, wild-type mice used in quantitative phosphoproteomic analysis and *Kat5*$^{flox/flox}$ used for AAV injection were randomly divided into two groups. For cell experiments, cells were seeded in parallel and randomly assigned to different treatments. Otherwise, randomization was not performed. Mice were assigned to groups according to genotype (wild-type as control, knockout as experimental) in a non-randomized design. Other experimental procedures were randomized where possible.
Blinding	Blinding was applied wherever possible. For example, samples, cages or vials during sample collection and processing were labelled as code names by the individual who picked and treated animals or cells but did not participate in processing and experimental determinations, and the samples were revealed after completion of the experiment. Similarly, during microscopy data collection and statistical analyses, the fields

of view were chosen on a random basis, and are often performed by different operators, preventing potentially biased selection for desired phenotypes.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing	N/A
Data exclusions	N/A
Non-participation	N/A
Randomization	N/A

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	N/A
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☒ No

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

The following antibodies were purchased from Cell Signaling Technology.
rabbit UCP1 Polyclonal antibody (cat. 72298S, 1:1,000 for IB)
CREB Rabbit Monoclonal Antibody (cat. 9197T, 1:1,000 for IB)
GSK-3 alpha Rabbit Monoclonal Antibody (cat. 4337S, 1:1,000 for IB)
GSK-3 beta Rabbit Monoclonal Antibody (cat. 9043, 1:1,000 for IB)
Phospho-GSK-3 beta (Ser9) Rabbit Monoclonal Antibody (cat. 9323S, 1:1,000 for IB)
HSL Antibody (cat. 4107S, 1:1,000 for IB)
Phospho-HSL (Ser563) Antibody (cat. 4139S, 1:1,000 for IB)
Phospho-HSL (Ser660) Antibody (cat. 45804S, 1:1,000 for IB)
Perilipin-1 Rabbit Monoclonal Antibody (cat. 9349S, 1:1,000 for IB)
Mouse Anti-rabbit IgG (Conformation Specific) Monoclonal Antibody (cat. 5127S, 1:2,000 for IB)
Rabbit Monoclonal Antibody IgG Isotype Control (cat. 3900S)

The following antibodies were purchased from Proteintech.
TIP60/KAT5 Polyclonal antibody (cat. 10827-1-AP, 1:1,000 for IB)
Beta Actin antibody (cat. 66009-1-Ig, 1:5,000 for IB)
PRKACA Polyclonal antibody (cat. 27398-1-AP, 1:1,000 for IB)
Phospho-CREB1 (Ser133) Polyclonal antibody (cat. 28792-1-AP, 1:1,000 for IB)
MYC-tag antibody (cat. 16286-1-Ig, 1:1,000 for IB)
HA-tag antibody (cat. 51064-2-AP, 1:1,000 for IB)
HSL Polyclonal antibody (cat. 17333-1-AP, 1:200 for IF)

The following antibodies were purchased from Thermo Fisher Scientific.
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (cat. A-11012, 1:1,000 for IF)
Goat anti-Mouse IgG (H+L) Secondary Antibody (cat. 31430, 1:5,000 for IB)
Goat anti-Rabbit IgG (H+L) Secondary Antibody (cat. 31460, 1:5,000 for IB)

Rabbit anti-AcK577-HSL (1:1,000 for IB) was generated by our laboratory. KAT5 / Tip60 (phospho S86) antibody (cat. Ab73207, 1:1,000 for IB) was purchased from Abcam. HA-tag antibody (cat. Sc-7392, 1:100 for IP) was purchased from Santa Cruz Biotechnology. Anti-Acetyllysine Rabbit mAb (cat. PTM105RM, 1:1,000 for IB) was purchased from PTM Bio. ANTI-FLAG(R) antibody produced in rabbit was purchased from Sigma-Aldrich (cat. F7425, 1:1,000 for IB).

Validation

The following commercially available antibodies were validated by the company, as well as other researchers (as the information collected by the RRID database).
Rabbit UCP1 Polyclonal antibody (cat. 72298S, 1:1,000 for IB), RRID: AB_2936479
CREB Rabbit Monoclonal Antibody (cat. 9197T, 1:1,000 for IB), RRID: AB_331277
GSK-3 alpha Rabbit Monoclonal Antibody (cat. 4337S, 1:1,000 for IB), RRID: AB_10859910. (validated in Fig. 2h)
GSK-3 beta Rabbit Monoclonal Antibody (cat. 9043, 1:1,000 for IB), RRID: AB_2636978. (validated in Fig. 2h)
Phospho-GSK-3 beta (Ser9) Rabbit Monoclonal Antibody (cat. 9323S, 1:1,000 for IB), RRID: AB_2115201
HSL Antibody (cat. 4107S, 1:1,000 for IB), RRID: AB_2296900. (validated in Fig. 6d)
Phospho-HSL (Ser563) Antibody (cat. 4139S, 1:1,000 for IB), RRID: AB_2135495
Phospho-HSL (Ser660) Antibody (cat. 45804S, 1:1,000 for IB), RRID: AB_2893315
Perilipin-1 Rabbit Monoclonal Antibody (cat. 9349S, 1:1,000 for IB), RRID: AB_10829911
Mouse Anti-rabbit IgG (Conformation Specific) Monoclonal Antibody (cat. 5127S, 1:2,000 for IB), RRID: AB_10892860
Rabbit Monoclonal Antibody IgG Isotype Control (cat. 3900S), RRID: AB_1550038
TIP60/KAT5 Polyclonal antibody (cat. 10827-1-AP, 1:1,000 for IB), RRID: AB_2128431. (validated in Fig. 1d)
Beta Actin antibody (cat. 66009-1-Ig, 1:5,000 for IB), RRID: AB_2687938
PRKACA Polyclonal antibody (cat. 27398-1-AP, 1:1,000 for IB), RRID: AB_2880861. (validated in Fig. 2e)
Phospho-CREB1 (Ser133) Polyclonal antibody (cat. 28792-1-AP, 1:1,000 for IB), RRID: AB_2918203
MYC-tag antibody (cat. 16286-1-Ig, 1:1,000 for IB), RRID: AB_11182162. (validated in Extend data Fig. 4b)
HA-tag antibody (cat. 51064-2-AP, 1:1,000 for IB), RRID: AB_11042321. (validated in Fig. 4c)
HSL Polyclonal antibody (cat. 17333-1-AP, 1:200 for IF), RRID: AB_2878386.
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594 (cat. A-11012, 1:1,000 for IF), RRID: AB_2534079
Goat anti-Mouse IgG (H+L) Secondary Antibody (cat. 31430, 1:5,000 for IB), RRID: AB_228307
Goat anti-Rabbit IgG (H+L) Secondary Antibody (cat. 31460, 1:5,000 for IB), RRID: AB_228341
KAT5 / Tip60 (phospho S86) antibody (cat. Ab73207, 1:1,000 for IB), RRID: AB_1523845. (validated in Fig. 4b)
HA-tag antibody (cat. Sc-7392, 1:100 for IP), RRID: AB_627809.
Anti-Acetyllysine Rabbit mAb (cat. PTM105RM, 1:1,000 for IB), RRID: AB_3099509. (validated in Fig. 4c)
ANTI-FLAG(R) antibody (cat. F7425, 1:1,000 for IB), RRID: AB_439687. (validated in Fig. 4c)

Rabbit anti-AcK577-HSL was validated in Fig. 3I.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	SVF cells were isolated from the interscapular BAT of *Kat5*$^{flox/flox}$ mice (including both male and female mice) as described previously (Ref 35, 36). HEK293T cells (cat. CRL-3216) cells were purchased from ATCC. Mouse BAC cells were provided by Professor Junli Liu from Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China). *Kat5* knockout BAC cells, *Hsl* knockout BAC cells, *Prkaca* knockout BAC cells and *Plin1* knockout BAC cells were generated by our laboratory.
Authentication	HEK293T cells and BAC cells were authenticated by STR sequencing performed by Immocell Biotechnology Corporation (Xiamen, China).
Mycoplasma contamination	The cell lines were routinely tested negative for mycoplasma contamination in our laboratory.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Palaeontology and Archaeology

Specimen provenance	N/A
Specimen deposition	N/A
Dating methods	N/A

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	N/A

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

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 C57BL/6J mice (8 weeks old) were obtained from the Xiamen University Laboratory Animal Center. *Kat5*$^{flox/flox}$ mice were generously provided by Professor Jianfeng Wu from Xiamen University. *Ucp1-Cre* mice were acquired from the Jackson Laboratory (Cat 024670; RRID: IMSR_JAX:024670). Mice were housed with free access to water and a standard diet (65% carbohydrate, 11% fat, 24% protein; cat. XTI01WC-010, Xietong biology) under specific pathogen-free (SPF) conditions. The light was on from 7:00 to 19:00, with the temperature kept at 20–23°C and humidity at 40–70%.
Wild animals	The study did not involve wild animals.
Reporting on sex	Both male and female animals were used in this study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Protocols for all animal experiments were approved by the Institutional Animal Care and the Animal Committee of Xiamen University (XMULAC20220050).

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	N/A
Study protocol	N/A
Data collection	N/A
Outcomes	N/A

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	N/A
Files in database submission	N/A
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N/A
Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A
Software	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	N/A
Instrument	N/A
Software	N/A
Cell population abundance	N/A
Gating strategy	N/A

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

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s) N/A

Field strength N/A

Sequence & imaging parameters N/A

Area of acquisition N/A

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software N/A

Normalization N/A

Normalization template N/A

Noise and artifact removal N/A

Volume censoring N/A

Statistical modeling & inference

Model type and settings N/A

Effect(s) tested N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference N/A

(See Eklund et al. 2016)

Correction N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis