

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <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	Molecular affinity measurements were performed via biolayer interferometry (BLI) using a Octet RED96 System (ForteBio, Fremont, USA). Transthoracic echocardiography was conducted using a VINNO 6 LAB Ultrasound Imaging System (VINNO Technology, Suzhou, China) to assess systolic and diastolic function in mice. Quality assessment of the RNA samples was carried out using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). Subsequent steps included the construction of a cDNA library and sequencing on the BGISEQ-500 platform (Beijing Genomics Institute, Shenzhen, China). Raw data of scRNA-seq analysis were processed using the Cell Ranger 7.2.0 sequence workflow with default and suggested settings. Mass spectrometry data were collected by timsTOF Pro mass spectrometer (Bruker Daltonics, Billerica, USA).
Data analysis	ImageJ analysis software version 1.8.0 172 (NIH, Bethesda, MD, USA); GraphPad Prism 10.0 (GraphPad, San Diego, CA); Bowtie2 (v2.2.5) ; Hisat2 (v2.0.4); RSEM (v1.2.12); DESEQ2 (version 1.4.5); R packages "Clusterprofile"; R package Seurat (v4.1.1) . The Mass spectrometry raw data was processed using MaxQuant with integrated Andromeda search engine (v.1.5.2.8).

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 raw bulk RNA sequencing data have been deposited in the Sequence Read Archive (SRA) under BioProject accession number PRJNA1223556. The single-cell RNA sequencing datasets are available in the Gene Expression Omnibus (GEO) database under accession code GSE290932. The raw data of mass spectrometry is available at the PRIDE database under accession code PXD061479. All processed data are included within the article or Supplementary Information or are available from the authors upon reasonable request. Source Data are provided with this manuscript.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

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 against OTUD1 (#bs-17563R) were purchased from Bioss (Beijing, China). Antibodies against α -actinin (#ab137346), Vimentin (#ab8978), SDHA (#ab14715), UQCRC2 (#ab14745), MTCO1 (#ab14705) and ATP5A (#ab14748) were purchased from Abcam (Cambridge, UK). Antibodies against Cy3-conjugated goat anti-rabbit IgG (#GB21303) and Alexa Fluor® 488-conjugated goat anti-rabbit IgG (#GB25303) were purchased from Servicebio (Wuhan, China). Antibodies against AMPK α 2 (#MA5-37501) were purchased from Invitrogen (CA, America). Antibodies against Flag (#20543-1-AP), HK-1 (#19662-1-AP), Camk2 (#11549-1-AP), LKB1(#10746-1-AP), GAPDH (#60004-1-Ig), HA-tag (#51064-2-AP) and MYC-tag (#16286-1-AP) were purchased from Proteintech(Wuhan, China). Antibodies against pT172-AMPK (#50081), p-ACC (#3661S), ACC (#3662S) and AMPK (#2532S) were purchased from Cell Signaling Technology (MA, USA). Antibodies against Grim19 (#sc-365978) were purchased from SCBT (Santa, USA). Antibodies against mTOR (#F0169), NRF1 (#F11099) and VDAC1 (#F2882) were purchased from Selleck (TX, USA).

Validation

Antibodies were previously validated for immunoblot or immunofluorescence of rat or mouse sample either in the laboratories of ourselves or commercial suppliers, see below for details:

Anti - OTUD1 (Rabbit polyclonal antibody, #bs-17563R,) has been quality validated in immunoblot by previous study (PubMed: 37334346) and also stated in the manufacture's website.

Anti - α -actinin (Rabbit polyclonal antibody, #ab137346) has been quality validated in immunofluorescence by previous study (PubMed: 37051312) and also stated in the manufacture's website.

Anti - Vimentin (Mouse monoclonal [RV202], #ab8978) has been quality validated in immunofluorescence by previous study (PubMed: 37853303) and also stated in the manufacture's website.

Anti - SDHA (Mouse monoclonal [2E3GC12FB2AE2], #ab14715) has been quality validated in immunoblot by previous study (PubMed: 37860849) and also stated in the manufacture's website.

Anti - UQCRC2 (Mouse monoclonal [13G12AF12BB11], #ab14745) has been quality validated in immunoblot by previous study (PubMed: 37989448) and also stated in the manufacture's website.

Anti - MTCO1 (Mouse monoclonal [1D6E1A8], #ab14705) has been quality validated in immunoblot by previous study (PubMed: 35084690) and also stated in the manufacture's website.

Anti - ATP5A (Mouse monoclonal [15H4C4], #ab14748) has been quality validated in immunoblot by previous study (PubMed: 38123683) and also stated in the manufacture's website.

Anti - Cy3-conjugated goat anti-rabbit IgG (#GB21303) has been quality validated in immunofluorescence by previous study (PubMed: 35197442) and also stated in the manufacture's website.

Anti - Alexa Fluor® 488-conjugated goat anti-rabbit IgG (#GB25303) has been quality validated in immunofluorescence by previous study (PubMed: 38882001) and also stated in the manufacture's website.

Anti - AMPK α 2 (Mouse monoclonal [206CT5.4.2], #MA5-37501) has been quality validated in immunofluorescence stated in the manufacture's website.

Anti - Flag-Tag (Rabbit polyclonal antibody, #20543-1-AP) has been quality validated in immunoblot by previous study (PubMed: 38011644) and also stated in the manufacture's website.

Anti - HK-1 (Rabbit polyclonal antibody, #19662-1-AP) has been quality validated in immunoblot by previous study (PubMed: 39577415) and also stated in the manufacture's website.

Anti - Camk2 (Rabbit polyclonal antibody, #11549-1-AP) has been quality validated in immunoblot by previous study (PubMed: 28723898) and also stated in the manufacture's website.

Anti - LKB1(Rabbit polyclonal antibody, #10746-1-AP) has been quality validated in immunoblot by previous study (PubMed: 37950995) and also stated in the manufacture's website.

Anti - GAPDH (Mouse monoclonal [1E6D9], #60004-1-Ig) has been quality validated in immunoblot by previous study (PubMed: 36477534) and also stated in the manufacture's website.

Anti - HA-tag (Rabbit polyclonal antibody, #51064-2-AP) has been quality validated in immunoblot by previous study (PubMed: 38297130) and also stated in the manufacture's website.

Anti - MYC-tag (Rabbit polyclonal antibody, #16286-1-AP) has been quality validated in immunoblot by previous study (PubMed: 36823147) and also stated in the manufacture's website.

Anti - pT172-AMPK (Rabbit monoclonal [D4D6D], #50081) has been quality validated in immunoblot by previous study (PubMed: 39736512) and also stated in the manufacture's website.

Anti - p-ACC (Rabbit polyclonal antibody, #3661S) has been quality validated in immunoblot by previous study (PubMed: 39814550) and also stated in the manufacture's website.

Anti - ACC (Rabbit polyclonal antibody, #3662S) has been quality validated in immunoblot by previous study (PubMed: 39949396) and also stated in the manufacture's website.

Anti - AMPK (Rabbit polyclonal antibody, #2532S) has been quality validated in immunoblot by previous study (PubMed: 39819643) and also stated in the manufacture's website.

Anti - Grim19 (Mouse monoclonal [F-10], #sc-365978) has been quality validated in immunoblot by previous study (PubMed: 36835152) and also stated in the manufacture's website.

Anti - mTOR (Rabbit monoclonal antibody, clone name not provided, #F0169) has been quality validated in immunoblot by previous study (PubMed: 32386617) and also stated in the manufacture's website.

Anti - NRF1 (Rabbit monoclonal antibody, clone name not provided, #F1099) has been quality validated in immunoblot by previous study (PubMed:29149604) and also stated in the manufacture's website.
Anti- VDAC1 (Rabbit monoclonal antibody, clone name not provided, #F2882) has been quality validated in immunoblot by previous study (PubMed: 28713289) and also stated in the manufacture's website.

Eukaryotic cell lines

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

Cell line source(s)	HEK293T cells (derived from female) and HeLa cells (derived from female) were sourced from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). AMPK α 2-/- H9C2 cells (sex unknown, derived from rat embryonic cardiomyocytes) were provided by PTM BIO (Hangzhou, China).
Authentication	Cell lines were authenticated by STR.
Mycoplasma contamination	Cell lines were tested to be mycoplasma-negative by the standard PCR method.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell line was 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	Mice with cardiac-specific deletion of Otud1, generated by crossing Otud1 floxed mice (Otud1fl/fl, 8-week-old, strain No. T005045) with α -myosin heavy chain promoter-driven heterozygous Cre (Myh6-Cre, strain No. T004713) mice, were purchased from Gempharmatech (Nanjing, China). The db/db mice (8-week-old, C57BLKS/J background) were obtained from the Laboratory Animal Center of Hangzhou Medical College (Hangzhou, China) .
Wild animals	The study did not involve wild animals.
Reporting on sex	All animal experiments were conducted with male mice to eliminate the interference of estrogen.
Field-collected samples	Study did not involve samples collected from the field.
Ethics oversight	All animal experiments were conducted with the approval of the Animal Care and Ethics Committee at the Zhejiang Center of Laboratory Animals, adhering to the Guide for the Care and Use of Laboratory Animals (ZICLA-IACUC-20030091).

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

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

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

Primary cardiomyocytes were seeded on 6-well plates. After pre-treatment, cells were collected in a 15-mL centrifugal tube and incubated with MitoSOX reagent for 30 min at 37 °C. Then, the cells were washed with cold PBS and analyzed immediately using flow cytometry.

Instrument

FACSCelesta flow cytometer (BD, Franklin Lakes, USA).

Software

Data were analyzed using FlowJo V10 software (FlowJo, Ashland, USA).

Cell population abundance

The flow cytometry experimental procedure in this study mainly measured the fluorescence intensity of mitochondrial ROS and did not involve cell population abundance analysis.

Gating strategy

The flow cytometry process in this study mainly measured the fluorescence intensity of mitochondrial ROS and did not require a gating strategy to differentiate between subpopulations of cells with different phenotypes or states.

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