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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	For RNA sequencing, reads were processed and mapped to the mouse genome mm10 using the Bcbio-nextgen framework version 0.9.0. The aligner used was STAR 2.14.1d and alignment quality assessed with QualiMap v.2.1.1. Differentially expressed genes were identified using DESeq2.1.1.
Data analysis	Ingenuity Pathway Analysis (Qiagen Bioinformatics) was used to analyse differentially expressed genes. Gene ontology (GO) enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) to find Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched within differentially expressed genes. Microsoft Excel v15.25 for Mac and GraphPad Prism v7 were used for all other data analysis. For oxygen consumption in mice, Oxymax v4.4 (Columbus Instruments) was used for data analysis. Opticon Monitor v3.1 was used for analysis of RT-PCR data. LAS AF v. 2.7.3.9723 software was used for SP5 confocal microscopy. R v3.2.0 was used for DSeq2 normalisation of RNA sequencing data.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

RNA sequence data is available from GEO (accession number: GSE120429). All other datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	For in vivo studies, in most cases a minimum of 6 mice per condition was used. Based on our own previous experience, as well as results from other similar published studies, this sample size provides sufficient power to detect physiologically relevant changes. No Power calculations were performed for ex vivo studies (e.g. western blotting, RT-PCR). Sample size was between 3-6 independent samples and was based on availability of samples.
Data exclusions	In one case, a mouse was excluded from the in vivo oxygen consumption determination because the readings were abnormally low. The reason for this was later identified as an error with the flow rate/oxygen calibration system for that specific cage unit. This error was not predetermined.
Replication	In vivo experiments were carried out on multiple independent cohorts of animals with similar results. Oxygen consumption in adipose tissue measured ex vivo was performed on tissue from individual mice and repeated on different days, yielding similar results (significant differences between wild type and gain-of-function AMPK mice).
Randomization	Mice were not randomized, but experiments were performed on cohorts of age and sex matched mice with the appropriate genotype.
Blinding	Investigators were not blinded during the in vivo studies (in most cases, the bodyweight difference between genotypes is obvious).

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Unique materials are available upon request, and following completion of a material transfer agreement.

Antibodies

Antibodies used	All antibodies were used according to the supplier's instructions. Vinculin (Sigma V9131), 1:1000 dilution; PGC1a (Abcam ab54481), 1:1000; Total OXPHOS monoclonal cocktail (Abcam ab110413), 1:1000; UCP1 (Abcam ab10983), 1:1000; Atp2a1 (Abcam ab109899), 1:1000; Atp2a2 (Invitrogen MA3-919), 1:1000; Ckmt2 (abcam ab55963), 1:1000; FLAG (Cell Signaling, 14793), 1:1000; TOM-20 (SantaCruz, sc-11415), 1:200.
Validation	Vinculin: The antibody reacts with the 116 kDa vinculin band in immunoblotting. The product reacts with vinculin of many species. Good reactivity is obtained with human, bovine, chicken, dog, rat, mouse, turkey, and Xenopus. The antibody shows cross reactivity with smooth muscle metavinculin. Citeab lists 635 citations. PGC1a: Raised to synthetic peptide corresponding to Human PGC1 alpha aa 777-797. Validated for western blotting using mouse BAT. Referenced 150 times on Abcam website. Total OXPHOS: The monoclonal antibodies in the cocktail were chosen because they are against a subunit that is labile when its complex is not assembled. Moreover, the combination is readily resolved in SDS-PAGE when the appropriate gel conditions are used. Referenced 279 times on Abcam website. UCP1: Raised to synthetic peptide corresponding to Human UCP1 aa 145-159 conjugated to keyhole limpet haemocyanin. Validated for western blotting using rat BAT. Referenced 246 times on Abcam website. Atp2a1: Raised against partial sequence of human Atp2a1 (amino acids 522-612). Antibody reactive against recombinant protein No longer available from Abcam, but now available from Novus Biologicals (H00000487-M01). No references available due to change in supplier/catalogue reference. Atp2a2: MA3-919 has been successfully used in Western blot, immunocytochemistry and immunoprecipitation procedures. By Western blot, this antibody detects an ~110 kDa protein representing SERCA2 ATPase in rat cardiac tissue. Referenced 173 times on Sigma website. Ckmt2: Raised against synthetic peptide corresponding to N terminal amino acids 37-86 of Human CKMT2. Positive control: Jurkat cell lysate Human skeletal muscle tissue. Referenced 3 times on Abcam website. FLAG: Monoclonal antibody produced by immunizing animals with a synthetic DYKDDDDK peptide. Recognises FLAG epitope fused to either the amino-terminus or carboxy-terminus of the target protein. Referenced 48 times on Cell Signaling website. TOM-20: Detection of Tom20 of mouse, rat, human and avian origin by WB, IP, IF and IHC(P); also reactive with additional species, including and equine, canine, bovine, porcine and avian. This clone has been discontinued. Referenced 80 times on Santa Cruz website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293 cells were obtained from the American Type Tissue Collection.
Authentication	HEK293 cells were not authenticated.
Mycoplasma contamination	HEK293 cells were tested using MycoAlert and cells tested were negative.
Commonly misidentified lines (See ICLAC register)	None were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	C57BL/6J mice harbouring either wild type AMPK gamma1 transgene (in the ROSA26 locus) or AMPK gamma1 with mutation of aspartic acid residue 316 to alanine, were used in this study. These mice were crossed with mice expressing cre-recombinase under the control of specific promoters, as described in the study. Male and female mice, aged between 8 - 30 weeks were used for studies.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field-collected samples were used in the study.