nature portfolio

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Last updated by author(s): Mar 27, 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	Xcalibur™ v4.4 Software (Thermo Scientific) was used for MS data collection. qPCR data was acquired by QuantStudio™ v1.4.3. Flir E6 was used to register thermal images.
Data analysis	PatternLab for Proteomics v4.1.1.25 (PatternLab) was used for MS data analysis. WebGestalt 2019 was used to perform pathway over-representation analyses. ImageJ v1.51h was used to analyze image data. FLIR Tools software was used to quantify thermal images. All plotted data were analyzed using GraphPad Prism 10.

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

For proteomic analysis Mus musculus proteome from UniProt (https://www.uniprot.org/uniprotkb?query=proteome:UP000000589) was used. The mass

spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD030485 (https://www.ebi.ac.uk/pride/archive/projects/PXD030485). Clinical trial registration and details can be found at the Australian New Zealand Clinical Trials Registry Anzctr.org.au, Registration number: ACTRN12622001519741. All the remaining data that supports the plots are available as part of supplementary/source information and all the experimental protocols that support the findings of this study are available from the corresponding author upon request. Leading contact: Carlos Escande, PhD. Email: escande@pasteur.edu.uy

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	For mouse experiments, only males are reported. Male mice were used based on the fact that the development of metabolic disease in response to diet-induced-obesity develops faster and male mice develop a stronger phenotype. Proof-of-concept experiments in female mice were also developed and results are available upon contact request to leading author. For clinical trial, human volunteers, both males and females were recruited and studied. Different ethnic groups were also included. The description of the characteristics of the different cohorts, including sex and ethnicity are available in extended data of the manuscript
Reporting on race, ethnicity, or other socially relevant groupings	Social variables were not considered in the study. The selection was based on physical and medical conditions. For the Single Ascending Dose, 47% of the participants were females and 53% males, with average age of 36 years old. Average BMI was 25. No obese volunteers were selected. For the Multiple Ascending Dose, 22% of volunteers were females while 78% were males. Average age was 35 years old and average BMI was 31. All detailed information is available in extended data of the manuscript.
Population characteristics	All detailed information is presented in extended data.
Recruitment	Volunteers were recruited by advertising in social and other communication media by the third-party CRO that run the trial.
Ethics oversight	Clinical study was performed in accordance with the World Medical Association Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP) E6 (R2), the applicable regulatory requirements, and the Australian National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research incorporating all updates. The clinical protocol was approved by the Bellberry Human Research Ethical Committee (Clinical protocol number MVD1-A1-001)

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. PMID: 26496606 PMID: 25053585 PMID: 31161155 PMID: 35150745
Data exclusions	Only data identified as outliers through the two-sides Grubb's test of the Graphpad sofware were excluded.
Replication	All experimental findings were replicate at least 3 times.
Randomization	For in vivo studies, mice were randomly asigned to treatment groups and randomization was performed by cages, with each cage housing 5 mice. Samples were processed in random order. For clinical studies, double blinded and randomization was performed by third party.
Blinding	The clinical trial was double-blinded. For the mouse experiments, there was no blinding. Experiments were carried out by different operators at different stages of the procedures.

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

Primary Antibodies:

Acetyl-CoA Carboxylase (1:1000) – Cell Signaling Technology (Cat# 3662, RRID: AB_2219400)
AMPKα (1:1000) – Cell Signaling Technology (Cat# 2532, RRID: AB_330331)
ATP5A (1:2000) – Abcam (Cat# ab14748, RRID: AB_301447)
Beta Actin (1:5000) – Abcam (Cat# ab8224, RRID: AB_449644)
Ckmt1 (1:1000) – Thermo Fisher Scientific (Cat# PA5-106347, RRID: AB_2854023)
Ckmt2 (1:1000) – Thermo Fisher Scientific (Cat# PA5-118822, RRID: AB_2903322)
GFP (Polyclonal) (1:500) – Thermo Fisher Scientific (Cat# A-11122, RRID: AB_221569)
p65 (1:1000) – Cell Signaling Technology (Cat# 8242, RRID: AB_10859369)
Phospho-Acetyl-CoA Carboxylase (Ser79) (1:1000) – Cell Signaling Technology (Cat# 3661,RRID: AB_330337)
Phospho-AMPKα (Thr172) (40H9) (1:1000) – Cell Signaling Technology (Cat# 2535, RRID:AB_331250)
UCP1 (1:1000) – Thermo Fisher Scientific (Cat# PA1-24894, RRID: AB_2241459)

Secondary Antibodies:

Alexa Fluor 488-conjugated (Anti-rabbit) (1:5000) – Thermo Fisher Scientific (Cat# A-11008,RRID: AB_143165)
HRP-conjugated (Anti-mouse) (1:10000) – Sigma-Aldrich (Cat# A9044, RRID: AB_258431)
HRP-conjugated (Anti-rabbit) (1:10000) – Sigma-Aldrich (Cat# A0545, RRID: AB_257896)

Validation

All antibodies were purchased directly from a commercial supplier. Validation data can be found on the manufactuer's website.

ACC - https://www.cellsignal.com/products/primary-antibodies/acetyl-coa-carboxylase-antibody/3662?srsltid=AfmBOoo2X5smrqSyPmocdQKbk6IYQkGJpivJLflmFAxi5UoaSZ290NL9

AMPKα - https://www.cellsignal.com/products/primary-antibodies/ampka-antibody/2532?srsltid=AfmBOoqt9o0N-d0_vm5U3vUdKae2isj0PBTHnRfz7x9-mFRggPPd67el

ATP5A - https://www.abcam.com/en-us/products/primary-antibodies/atp5a-antibody-15h4c4-mitochondrial-marker-ab14748?srsltid=AfmBOorNaDRP-mBHOqozWNi4LfvWc77tHrtZ8SpxBG-w5HNRrslVwbG8

Actin - https://www.abcam.com/en-us/products/primary-antibodies/beta-actin-antibody-mabcam-8224-loading-control-ab8224?srsltid=AfmBOoqUhgCFqpGvLhixi1LlUWa8ht560evTof529_aXHuRQL9U3PgTK

Ckmt1 - https://www.thermofisher.com/antibody/product/CKMT1A-Antibody-Polyclonal/PA5-106347

Ckmt2 - https://www.thermofisher.com/antibody/product/CKMT2-Antibody-Polyclonal/PA5-118822

GFP - https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-11122

p65 - https://www.cellsignal.com/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242?srsltid=AfmBOor8AYCdLiaP8SbEDQfeMnH_idRuC8u6AE6U40O7HWSo0HzwbZFl

pACC - https://www.cellsignal.com/products/primary-antibodies/phospho-acetyl-coa-carboxylase-ser79-antibody/3661?srsltid=AfmBOopC28M1tD2IO0fSrjGPt6oxwy5cVOPVgJEnCu3c97nrj2F1qIgM

pAMPK - https://www.cellsignal.com/products/primary-antibodies/phospho-ampka-thr172-40h9-rabbit-mab/2535?srsltid=AfmBOopSuLmONTniR4P2oXN1FUd-5Nvdq4dqnx6tsx7kXvpi4dxsL6O5

Ucp1 - https://www.thermofisher.com/antibody/product/UCP1-Antibody-Polyclonal/PA1-24894

Alexa Fluor 488-conjugated (Anti-rabbit) -https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008

HRP-conjugated (Anti-mouse) -https://www.sigmaaldrich.com/UY/en/product/sigma/a9044?srsltid=AfmBOopqo3SwMHroeWw1lxOqRpu9HcSmOQQR1KvddXCoq9qXrGh4FjKA

HRP-conjugated (Anti-rabbit) -https://www.sigmaaldrich.com/UY/en/product/sigma/a0545?srsltid=AfmBOorFnbNNqX7OTN1V16gjrrqdxnOnElT5r7dCo-N8xAHum4Fe6iaT

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	C2C12 cells – ATCC (CRL-1772) SH-SY5Y cells – ATCC (CRL-2266) TERT-WmA cells (Supplied by Peter Breding – Department of Biomedicine, Aarhus University, Denmark) THP-1 cells – ATCC (TIB-202)
Authentication	Authenticated cell lines obtained from ATCC were used at a low passage number, as recommended by the supplier. TERT-WmA cells have been authenticated by Markussen (PMID: 28957413). Adipocyte progenitors were validated by us based on their capacity to differentiate into lipid-laden fat cells.
Mycoplasma contamination	All cell lines were tested for mycoplasma contamination by PCR, being negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	C57BL/6J (Jackson Labs, USA) mice and zebrafish (Danio rerio) were used. AMPK, CKMT1 and UCP1 KO mice were also used. Experiments were performed on young male mice (10-12 weeks of age). Animals were housed with a 12-hour light/dark cycle. Relative humidity was maintained between 30% and 70%. Food and water were provided ad libitum.
Wild animals	The study did not involve the use of wild animals.
Reporting on sex	For mice experiments, male mice were used due to development of a stronger and faster DIO phenotype. Proof-of-concept experiments in females were performed and are available upon request.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	For the majority of the experiments, the protocol was approved by the Institutional Animal Care and Use Committee of the Institut Pasteur de Montevideo (CEUA, Protocols 003-19 and 006-19). Procedures involving UCP1 KO mice were approved by the Ethics Committee for Animal Use at the Institute of Biomedical Sciences, University of São Paulo (CEUA #6071181120, ICB/USP). CKMT1 KO mice were maintained at the University of Guelph, with experiments approved by its Animal Care Committee (AUP #5071) following the Canadian Council on Animal Care guidelines. AMPK KO mice were bred and maintained at the Henry Ford Health animal facility, with all experiments approved by its Animal Care Committee (IACUC #1530). For the CLAMS studies, C57BL/6J mice were purchased from Jackson Laboratory, and the experiments were approved by the Institutional Animal Care and Use Committees of West Virginia University and Mayo Clinic (Jacksonville, USA) (IACUC #A00003888-18-R24).

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	Trial registration and details can be found at the Australian New Zealand Clinical Trials Registry (Anzctr.org.au, Registration number: ACTRN12622001519741, date submitted: 11/21/2022, date registered: 12/07/2022, last update: 08/11/2024, secondary ID: MVD1-AU-001).
Study protocol	Trial study details can be found at the Australian New Zealand Clinical Trials Registry (Anzctr.org.au, Registration number: ACTRN12622001519741
Data collection	Participants were required to fast overnight for at least 8 hours prior to the screening visit and prior to admission to the clinical facility on Day -1. On all dosing days (Day 1 to Day 15), participants were required to fast for at least 2 hours prior to administration of each dose of study drug and until at least 2 hours post-dose. A final overnight fast of at least 8 hours was required on Day 15 prior to final PD measurements on Day 16. During all fasting periods in Part A (SAD) and Part B (MAD), no food or beverages were to be consumed by the study participants except for water.

Outcomes	Primary outcomes Pk and Pd profile, body fasting glucose and insulin, fructosamine, any kind of adverse events at the clinical and para clinical levels.

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.*