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	SentrySuite software (v.3.20.8), Lunar Prodigy Advance Encore software (v.16), BioRad Image Lab (v.6.0.1), Oroboros Datlab (v.7.0), Zeiss Zen Black 2012, RNA-SeQC2 (v.2.3.4), DESeq2 (v.1.28.1).
Data analysis	Microsoft Excel 2016, Fuji ImageJ (v.2.7.0), BioRad Image Lab (v.6.0.1), GraphPad Prism (v.9.0), SigmaPlot (v.14.0), MaxQuant (v2.1.3.0), FastQC (v.0.11.9), Gencode (v.34), STAR (v.2.7.9a), GSEA tool (v.4.3.2), KEGG database gene set (v.2023.1), Uniprot Reference database (May 2021), R programming (v.3.6.2 and v.4.0.2) including LIMMA and ksea R packages.

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

RAW data and processed output tables have been deposited in the PRIDE proteomeXchange repository and can be accessed at https://www.ebi.ac.uk/pride/ with

the accession PXD045301, username: reviewer_pxd045301@ebi.ac.uk and password: HFFgPBlT. Raw RNA-sequencing reads produced in this work have been deposited to the EGA database, accession number EGAD50000000059. All other data that support the findings of this study are available from the corresponding author upon reasonable 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	Sex of the study participants was reported by self-identification. Both males and females study participants were recruited in the study with a similar proportion. Due to the low sample size in our data, we found it inappropriate to disaggregate our data for sex.
Reporting on race, ethnicity, or other socially relevant groupings	All study participants originated from the Greenlandic Inuit population and had an European genetic admixture of ~25-30%.
Population characteristics	We investigated a total of 16 study participants. Clinical characteristics of the study participants can be found in the Extended Data Table 1 and 2 of the manuscript.
Recruitment	All study participants were recruited from the Greenlandic cohort register called the Inuit Health in Transition (IHIT). Due to the low accessibility of the homozygous TBC1D4 variant carriers, all homozygous carriers who voluntary signed up, met the inclusion criteria and gave informed consent to participate were included in the study. Control subjects were recruited to the study to individually match the TBC1D4 variant carriers, according to age, self-reported sex, European genetic admixture, BMI and physical fitness. Inclusion criteria were: females and males, 25-70 years of age, BMI 20-35 kg/m2, no medical treatment for type 2 diabetes. Study participants were provided with financial compensation for their involvement in the research to offset any expenses incurred and to acknowledge their contribution to the study.
Ethics oversight	All study participants were provided oral and written study information. Written informed consent was obtained from all study participants before entering the study. The study was approved by the Commission for Scientific Research in Greenland and the Copenhagen Ethics Committee in Denmark as well as conformed to the declaration of Helsinki.

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 predetermine sample size. Sample size (number of study participants) were determined by the available number of study participants carrying the TBC1D4 variant.
Data exclusions	No data were excluded from the analyses.
Replication	Replication of the study results were not performed due to the strained logistics of recruiting and testing the study participants.
Randomization	N/A - all participants underwent the same experimental procedure.
Blinding	The investigators were not blinded to the group allocation (TBC1D4 variant vs. Control) because all study participants were genotyped before entering the study. Part of the data collection were performed blinded including blood analyses of hormones, enzyme activities, mitochondrial respirometry, GLUT4 imaging, RNA-sequencing and proteomics while other parts of the data collection were performed non-blinded including immunoblotting analyses. Investigators were blinded during data analysis but not blinded during the statistical analyses.

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 primary antibodies are listed with supplier name, catalog number, clone name / lot number, and dilution in that order (N/A, not applicable):
TBC1D4, Abcam, ab189890, GR320789-2, 0.5 µg/ml
TBC1D1, Proteintech, 22124-1-AP, N/A, 1:1000
TBC1D1, Abcam, ab229504, GR3248015-2, 1 µg/ml
GLUT4, Thermo Fisher, PA1-1065, SB242399, 1:1000
GLUT4, Thermo Fisher, PA5-23052, WJ3403509, 1:100
IRAP, Custom-made by prof. Susanne R. Keller, N/A, N/A, 1 µg/ml
GLUT1, Millipore, 07-1401, 3481410, 1 µg/ml
IR, Custom-made by prof. Ken Siddle, CT-21, N/A, 1:1000
IRS1, Upstate Biotechnology, 06-248, 31556, 0.75 µg/ml
Akt2, CST, 3063, 4, 1:1000
AMPKα2, SCBT, sc-19131, C0116, 0.2 µg/ml
mTOR, CST, 2972, 6, 1:250
p70 S6 kinase, CST, 9202, 20, 1:1000
HK2, SCBT, sc-130358, J3017, 1 µg/ml
GS, Custom-made by prof. Oluf Pedersen, N/A, N/A, 1:40000
CS, Abcam, ab96600, N/A, 1:1000
PDH E1α, Custom-made by prof. D.G. Hardie and prof. Henriette Pilegaard, N/A, N/A, 1 µg/ml
OXPHOS Complex 1, Abcam, ab110411, J5383, 1:10000
OXPHOS Complex 2, Abcam, ab110411, J5383, 1:10000
OXPHOS Complex 3, Abcam, ab110411, J5383, 1:10000
OXPHOS Complex 4, Abcam, ab110411, J5383, 1:10000
OXPHOS Complex 5, Abcam, ab110411, J5383, 1:10000
CD36, R&D Systems, AF2519, VYQOZ15041, 1:1000
FATP4, Abcam, ab200353, GR3267173-2, 1:1000
ATGL, CST, 2138, 4, 1:1000
Perilipin 3, Proscience Incorporated, 3881, N/A, 1:500
ACC, Jackson ImmunoResearch Labs, 016-030-084, 108001, 1:2000
p-Akt T308, CST, 9275, N/A, 1:1000
p-Akt S473, CST, 9271, 12, 1:1000
p-p70 S6K T389, CST, 9205, 21+22, 1:2000
p-TBC1D4 S318, CST, 8619, 1, 1:1000
p-TBC1D4 S341, Custom-made by prof. D.G. Hardie and prof. Carol MacKintosh, N/A, N/A, 4 µg/ml
p-TBC1D4 S588, CST, 8730, 1, 1:1000
p-TBC1D4 T642 (T649), CST, 8881, 3, 1:1000
p-TBC1D1 S596 (S590), CST, 6927, N/A, 1:1000
p-TBC1D4 S704 (S711), Custom-made by associate prof. Jonas T. Treebak, N/A, N/A, 1 µg/ml
p-GS site 2+2a, Custom-made by prof. D.G. Hardie and prof. Jørgen Wojtaszewski, N/A, N/A, 1.5 µg/ml
p-GS site 3a+3b, Custom-made by prof. D.G. Hardie and prof. Jørgen Wojtaszewski, N/A, N/A, 1 µg/ml
p-AMPKα T172, CST, 2531, 1b, 1:1000
p-ACC S221 (S212), CST, 3661, 10, 1:1000
p-P38 T180/Y182, CST, 9211, 23, 1:1000
p-TBC1D1 S237 (S231), Millipore, 07-2268, 2952313, 1:1000
p-PDH site 1, Custom-made by prof. D.G. Hardie and prof. Henriette Pilegaard, N/A, N/A, 1 µg/ml
p-PDH site 2, Custom-made by prof. D.G. Hardie and prof. Henriette Pilegaard, N/A, N/A, 1 µg/ml

The following secondary antibodies are listed with supplier name, catalog number, and dilution:
Goat-anti-rabbit-IgG-HRP, Jackson ImmunoResearch Labs, 111-035-045, 1:5000
Goat-anti-mouse-IgG-HRP, Jackson ImmunoResearch Labs, 115-035-062, 1:5000
Rabbit-anti-goat-IgG-HRP, Jackson ImmunoResearch Labs, 305-035-003, 1:5000
Rabbit-anti-sheep-IgG-HRP, Jackson ImmunoResearch Labs, 313-035-003, 1:5000

Validation

Some antibodies have previously been validated in KO cells/tissue. Other antibodies were validated by the manufacturer and confirmed with western blotting by their expected molecular weight or in OE/KO cells and tissue.
TBC1D4: validated (human) in the current study by Western blotting using muscle biopsy samples from homozygous TBC1D4 variant carriers that do not express TBC1D4 protein in skeletal muscle.

TBC1D1 (Proteintech): https://www.ptglab.com/products/TBC1D1-Antibody-22124-1-AP.htm
TBC1D1 (Abcam): https://www.abcam.com/en-dk/products/primary-antibodies/tbc1d1-antibody-ab229504
GLUT4: https://www.thermofisher.com/antibody/product/GLUT4-Antibody-Polyclonal/PA1-1065
GLUT4: https://www.thermofisher.com/antibody/product/GLUT4-Antibody-Polyclonal/PA5-23052
IRAP: validated in PMID: 8621739
GLUT1: https://www.merckmillipore.com/DK/en/product/Anti-GLUT-1-Antibody-CT,MM_NF-07-1401?bd=1
IR: https://absoluteantibody.com/product/anti-hinsr-c-terminus-ir-ct-21/
IRS1: https://www.emdmillipore.com/US/en/product/Anti-IRS1-Antibody,MM_NF-06-248
Akt2: https://www.cellsignal.com/products/primary-antibodies/akt2-d6g4-rabbit-mab/3063
AMPKα2: Validated in PMID: 31010958
mTOR: https://www.cellsignal.com/products/primary-antibodies/mtor-antibody/2972
p70 S6 kinase: https://www.cellsignal.com/products/primary-antibodies/p70-s6-kinase-antibody/9202
HK2: https://www.scbt.com/p/hxk-ii-antibody-1a7
GS: Validated in PMID: 17928598
CS: https://www.abcam.com/en-dk/products/primary-antibodies/citrate-synthetase-antibody-ab96600#
PDH E1α: validated in PMID: 17957032
OXPHOS Complex 1: https://www.abcam.com/en-dk/products/panels/total-oxphos-human-wb-antibody-cocktail-ab110411
OXPHOS Complex 2: https://www.abcam.com/en-dk/products/panels/total-oxphos-human-wb-antibody-cocktail-ab110411
OXPHOS Complex 3: https://www.abcam.com/en-dk/products/panels/total-oxphos-human-wb-antibody-cocktail-ab110411
OXPHOS Complex 4: https://www.abcam.com/en-dk/products/panels/total-oxphos-human-wb-antibody-cocktail-ab110411
OXPHOS Complex 5: https://www.abcam.com/en-dk/products/panels/total-oxphos-human-wb-antibody-cocktail-ab110411
CD36: https://www.rndsystems.com/products/mouse-cd36-sr-b3-antibody_af2519
FATP4: https://www.abcam.com/en-dk/products/primary-antibodies/slc27a4-fatp4-antibody-epr17319-26-ab200353
ATGL: https://www.cellsignal.com/products/primary-antibodies/atgl-antibody/2138
Perilipin 3: https://www.prosci-inc.com/product/tip47-antibody-3881/
p-Akt T308: https://www.cellsignal.com/products/primary-antibodies/phospho-akt-thr308-antibody/9275
p-Akt S473: validated in PMID: 31444134
p-p70 S6K T389: https://www.cellsignal.com/products/primary-antibodies/phospho-p70-s6-kinase-thr389-antibody/9205
p-TBC1D4 S318: validated (human) in the current study by Western blotting using muscle biopsy samples from homozygous TBC1D4 variant carriers that do not express TBC1D4 protein in skeletal muscle.
p-TBC1D4 S341: validated (human) in the current study by Western blotting using muscle biopsy samples from homozygous TBC1D4 variant carriers that do not express TBC1D4 protein in skeletal muscle.
p-TBC1D4 S588: validated (human) in the current study by Western blotting using muscle biopsy samples from homozygous TBC1D4 variant carriers that do not express TBC1D4 protein in skeletal muscle.
p-TBC1D4 T642 (T649): validated (human) in the current study by Western blotting using muscle biopsy samples from homozygous TBC1D4 variant carriers that do not express TBC1D4 protein in skeletal muscle.
p-TBC1D1 S596 (S590): https://www.cellsignal.com/product/productDetail.jsp?productId=6927
p-TBC1D4 S704 (S711): Validated in PMID: 37074686
p-GS site 2+2a: validated in PMID: 1983
p-GS site 3a+3b: validated in PMID: 32504885
p-AMPKα T172: validated in PMID: 32504885
p-ACC S221 (S212): validated in PMID: 24185692
p-P38 T180/Y182: https://www.cellsignal.com/products/primary-antibodies/phospho-p38-mapk-thr180-tyr182-antibody/9211
p-TBC1D1 S237 (S231): Validated in PMID: 31010958
p-PDH site 1: validated in PMID: 17957032
p-PDH site 2: validated in PMID: 17957032

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	NCT04170972
Study protocol	The study protocol is provided with the article
Data collection	Pre-screening and clinical tests in Greenland were conducted at a clinical laboratory at Queen Ingrid's Hospital in Nuuk from November 2017 to August 2018. The main experimental test was performed at the Department of Nutrition, Exercise and Sports in Copenhagen, Denmark from May 2018 to February 2019.
Outcomes	Primary and secondary outcome measures have been thoroughly pre-defined in the clinical trial registration. The primary outcome measures include changes in leg glucose uptake and whole-body insulin sensitivity that have been assessed during a hyperinsulinemic-euglycemic clamp (gold-standard). Also, global unbiased transcriptomic (RNA-sequencing) and proteomic (Mass spectrometry) muscle analyses as well as the molecular signaling signature (immonoblotting) of skeletal muscle following exercise and insulin stimulation were part of the primary outcome measures.

Plants

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