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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

qPCR data were acquired on a Light cycler 480 II (Roche). Western blots were imaged on a Bio-rad Chemidoc. Flow cytometry data were acquired on a LSRII or Aria (BD), using Diva software (v8.0.1). Body composition data were acquired with Echo-MRI (Echo Medical Systems). Sequencing data were obtained using HiSeq3000 for single cell (Illumina) or HiSeq2500 for bulk RNA-seq. Metabolic cages data were acquired from TSE PhenoMaster System (V3.0.3) Indirect Calorimetry System. Keyence microscope was used to image histology samples. Fecal bomb calorimetry was performed on Parr 6200 Isoperibol Calorimeter. cFOS mapping was performed using light-sheet microscope (Miltenyi Blaze). Temperature mapping with BIRDS was performed on a 9.4T Bruker scanner. EPR spectra were recorded in a quartz flat cell using an X-band EMX plus EPR Spectroscope. Metabolomics analyses were performed with orbitrap-type MS (Q-Exactive focus; Thermo Fisher Scientific) connected to a high-performance ion-chromatography system (ICS-5000+, Thermo Fisher Scientific), LCMS-8060 triple-quadrupole mass spectrometer (Shimadzu corporation), MALDI MS linear ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific). Lipidomics data were obtained with Lipidome Q-Exactive focus orbitrap mass spectrometer (Thermo Fisher Scientific) connected to an HPLC system (Ultimate3000, Thermo Fisher Scientific).

Data analysis	Data were analyzed using GraphPad (version 9.4.0) or R (v3.4.2). Images were processed through ImageJ (v1.53q). Cytometry data were analyzed with FlowJo (v10.3). For single cell analysis: Sample demultiplexing, barcode processing, and single-cell 3' counting was performed using the Cell Ranger Single-Cell Software Suite (10x Genomics). The Seurat package in R (v3.4.2) was used for subsequent analysis. For bulk RNA-seq, quality was assessed with FastQC and mapped using STAR aligner. Gene differential expression was calculated using DESeq2 (v1.8.1). Pathway analysis was done using fgsea (fast GSEA) R-package and the Canonical Pathways from the MSigDB C2 pathway set [MSigDB1, MSigDB2], v6.1. For lipidomics, the acquired data were analyzed using LipidSearch software (Mitsui Knowledge Industry). For MALDI-MS, on images were reconstructed using ImageQuest 1.1.0 software (Thermo Fisher Scientific). For cFos brain mapping, images were reconstructed with ClearMap software (Reinier et al, 2016) for quantification or Imaris 10.1 for visualization.

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

Sequencing data are accessible on GEO repository. Murine bulk RNA-seq data of the adipose depots after cysteine restriction: GSE292788. Single-cell RNA-seq of the adipose stromal vascular fraction: GSE293660. Human RNA-seq data from the CALERIE-II study were published previously1.

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	Both men and women have been included in the study.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	Men were aged 21 to 50 years old and females were 21 to 47 years old, with a BMI between 22 and 28. The full trial protocol is available: https://clinicaltrials.gov/study/NCT00427193
Recruitment	Non-obese healthy individuals that fulfill the inclusion criteria detailed on https://clinicaltrials.gov/study/NCT00427193, were recruited for the study. Participants were randomly allocated to caloric restriction or control group.
Ethics oversight	The full trial protocol is available: https://clinicaltrials.gov/study/NCT00427193

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	Sample size was decided based on power calculation from previous experiments and experiences.
Data exclusions	No data were excluded from the study.
Replication	Each experiment was repeated at least 2 times. All repeats were successful. Sequencing, metabolomics analyses were performed once, with at least n=4 biological replicates per group. When feasible, data were replicated with other experimental approaches in independent cohorts.
Randomization	Allocation to group was performed according to the genotype of the animals when comparing two genotypes. Otherwise, when working with the same genotype, mice were randomly allocated to groups.
Blinding	Investigators were blinded for brain mapping, sequencing and metabolomics analyses. Otherwise, investigators were not blinded due to the visible difference between the diet and the body weight of the mice.

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 used in this study:
Flow cytometry
- CD45-BV711 (Biolegend, 103147, dilution 1:200, clone 30-F11)
- CD45 PE Cy7 (Biolegend, 103114, dilution 1:200, clone 30-F11)
- CD3 PerCP Cy5.5 (Biolegend, 100327, dilution 1:200, clone 145-2C11)
- B220 AF488 (Biolegend, 103225, dilution 1:200, clone RA3-6B2)
- CD11b BV711 (Biolegend, 101241, dilution 1:200, clone M1/70)
- F4/80 BV421 (Biolegend, 123132, dilution 1:200, clone BM8)
- Ly6G AF700 (Biolegend, 127622, dilution 1:200, clone 1A8)
- SiglecF AF647 (BD, 562680, dilution 1:200, clone E50-2440)
- CD24-PerCP-Cy5.5 (eBioscience, 45-0242-80, dilution 1:200, clone M1/69)
- F3-PE (R&D, FAB3178P, dilution 1:200)
- CD31-PE-Cy7 (eBioscience, 25-0311-81, dilution 1:200, clone 390)
- Pdgfra-BV605 (Biolegend, 135916, dilution 1:200, clone APA5
- Dpp4-FITC (Biolegend, 137805, dilution 1:200, clone H194-112)
- CD9-APC (Biolegend, 124813, dilution 1:200, clone MZ3)
Immunostaining
- ATGL (Cell signaling, 2439S, dilution 1:1000, clone 30A4)
- UCP1 (abcam, ab10983, dilution 1:1000)
- ACTIN (cell Signaling, 4967, dilution 1:1000)
- CHOP (Cell Signaling, 2895, dilution 1:1000, clone L63F7)
- Calnexin (Novus, NB300-518, dilution 1:1000, clone AF18)
- IRE1a (Cell Signaling, 3294, dilution 1:1000, clone 14C10)
- BiP (Cell Signaling, 3177, dilution 1:1000 clone C50B12)
- pHSL (Ser660) (Cell Signaling, 4126, dilution 1:1000)
- HSL (Cell Signaling, 4107, dilution 1:1000)
- CTH (Novus Bio, H00001491-M03, dilution 1:1000, clone S51)
- Anti-puromycin (Sigma Aldrich, MABE343, dilution clone 12D10)
- HSP90 (Cell Signaling, 4874, dilution 1:1000)
- TH (Cell Signaling, 2792, dilution 1:1000)
- TUBULIN (Abcam, ab7291, dilution 1:1000, clone DM1A)
- HSP40 (Cell Signaling, 4868, dilution 1:1000)
- cFOS (Sysy antibodies, 226008, clone Rb108B5)
Secondary antibodies
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (ThermoFisher, A-21235)
- Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling, #7074, dilution 1:5000)
- Peroxidase AffiniPure™ Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, #111-035-003, dilution 1:5000)

Validation

All antibodies are commercially available and have been validated by the manufacturers and previous publications. All information can be found on the manufacturers' website.
- CD45-BV711: https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd45-antibody-10439
- CD45 PE Cy7: https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd45-antibody-1903
- CD3 Percp cy5.5: https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd3epsilon-antibody-4191
- B220 AF488: https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-human-cd45r-b220-antibody-2707
- CD11b BV711: https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-human-cd11b-antibody-7927
- F4/80 BV421: https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-f4-80-antibody-7199
- Ly6G AF700: https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-ly-6g-antibody-6754
- SiglecF AF647: https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-647-rat-anti-mouse-siglec-f.562680

- CD24-PerCP-Cy5.5: https://www.thermofisher.com/antibody/product/CD24-Antibody-clone-M1-69-Monoclonal/45-0242-82
- F3-PE: https://www.rndsystems.com/products/mouse-coagulation-factor-iii-tissue-factor-pe-conjugated-antibody_fab3178p
- CD31-PE-Cy7: https://www.thermofisher.com/antibody/product/CD31-PECAM-1-Antibody-clone-390-Monoclonal/25-0311-81
- Pdgfra-BV605: https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd140a-antibody-15109
- Dpp4-FITC: https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd26-dpp-4-antibody-6946
- CD9-APC: https://www.biolegend.com/en-us/products/apcfire-750-anti-mouse-cd9-antibody-16761

Immunostaining
- ATGL: https://www.cellsignal.com/products/primary-antibodies/atgl-30a4-rabbit-mab/2439
- UCP1: https://www.abcam.com/en-us/products/primary-antibodies/ucp1-antibody-ab10983
- ACTIN: https://www.cellsignal.com/products/primary-antibodies/b-actin-antibody/4967
- CHOP: https://www.cellsignal.com/products/primary-antibodies/chop-l63f7-mouse-mab/2895
- Calnexin: https://www.novusbio.com/products/calnexin-antibody-af18_nb300-518
- IRE1a: https://www.cellsignal.com/products/primary-antibodies/ire1a-14c10-rabbit-mab/3294
- BiP: https://www.cellsignal.com/products/primary-antibodies/bip-c50b12-rabbit-mab/3177
- pHSL (Ser660): https://www.cellsignal.com/products/primary-antibodies/phospho-hsl-ser660-antibody/4126
- HSL: https://www.cellsignal.com/products/primary-antibodies/hsl-antibody/4107
- CTH: https://www.novusbio.com/products/cystathionase-antibody-s51_h00001491-m03
- Anti-puromycin: https://www.sigmaaldrich.com/US/en/product/mm/mabe343
- HSP90: https://www.cellsignal.com/products/primary-antibodies/hsp90-antibody/4874
- TH: https://www.cellsignal.com/products/primary-antibodies/tyrosine-hydroxylase-antibody/2792
- TUBULIN: https://www.abcam.com/en-us/products/primary-antibodies/alpha-tubulin-antibody-dm1a-loading-control-ab7291
-HSP40: https://www.cellsignal.com/products/primary-antibodies/hsp40-antibody/4868
- cFOS: https://www.sysy.com/product/226008

Secondary antibodies
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235
- Anti-rabbit IgG, HRP-linked Antibody: https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
- Peroxidase AffiniPure™ Goat Anti-Rabbit IgG (H+L): https://www.jacksonimmuno.com/catalog/products/111-035-003

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	All mice were on the C57BL/6J (B6) genetic background. Cth-/- mice (C57BL/6NTac-Cthtm1a(EUCOMM)Hmgu/leg) were purchased from the European Mouse Mutant Cell Repository. Breeding these mice to Flipase transgenic mice from Jackson Laboratories generated Cthfl/fl mice which were crossed to Adipoq-cre and Albumin-cre, purchased from Jackson Laboratories. Ucp1-/- and CHOP-/- mice were purchased from Jackson laboratories and crossed to Cth-/- mice. Fgf21-/- mice were kindly provided by Dr. Steven Kliewer (UT Southwestern) and crossed to Cth-/- mice. All mice used in this study were housed in specific pathogen-free facilities in ventilated cage racks that deliver HEPA-filtered air to each cage with free access to sterile water through a Hydropac system at Yale School of Medicine. Mice were fed a standard vivarium chow (Harlan 2018s) unless special diet was provided and housed under 12 h light/dark cycles. All mice were aged 3 to 5 months old when starting the experiment.
Wild animals	This study did not involve any wild animals.
Reporting on sex	Unless mentioned, male mice were used for the experiments. No sexual dimorphism was observed.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal experimentations were performed under the approval of the Institutional Animal Care and Use Committee (IACUC) of Yale School of Medicine.

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	The participants in this study were part of the CALERIE Phase 2 study which was a multi-center, parallel-group, randomized controlled trial by recruitment of non-obese healthy individuals. The trial registration number is NCT00427193.
Study protocol	The full trial protocol is available: https://clinicaltrials.gov/study/NCT00427193
Data collection	Participants were randomly assigned to of 25% caloric restriction (CR) or ad libitum caloric intake for two years. CR group participants actually reached 14% of CR. Men were between 20 and 50 years old and women were between 20 and 47 years old. Their body mass index (BMI) was between 22.0 and 27.9 kg/m2 at the initial visit. Samples for abdominal subcutaneous adipose tissue biopsy were collected at baseline, 1 year, and 2 years of intervention.

Outcomes	Abdominal subcutaneous adipose tissue biopsy was performed on a portion of CR group participants and used for RNA-sequencing and metabolomics in this study.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	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	Adipose tissue biopsies were minced and then digested at 37°C in HBSS (Life Technologies) + 0.1% collagenase I or II (Worthington Biochemicals). The stromal vascular fraction was collected by centrifugation, washed and filtered using 100um and 70um strainers. Cells were stained with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) and then for surface markers including CD45, CD3, B220, CD11b, F4/80, Ly6G, Siglec F, CD24, F3, CD31, Pdgfra, Dpp4, and CD9. All antibodies were purchased from eBioscience, Biolegend, BD or R&D. Cells were fixed in 2% PFA.
Instrument	Data were acquired on LSR II (BD) or FACSAria (BD) for cell sorting.
Software	Data were acquired using DIVA software and then analyzed with FlowJo v10.8.1.
Cell population abundance	Adipose tissue macrophages (F4/80+ Cd11b+) represent approximately 25-40% of the hematopoietic fraction of the adipose tissue. Endothelial and progenitors represent approximately 30% and 40% of the live CD45- cells of the adipose tissue.
Gating strategy	For adipose tissue macrophages sorting, cells were first gated on FSC, SSC and singlets, then live and CD45+ cells. Non lymphocytes (CD3- B220-) cells were gated on SiglecF and Ly6G to exclude eosinophils and neutrophils. Then macrophages are CD11b+ F4/80+ cells. For stromal cells: singlet cells are gated initially on FSC, SSC, then live CD45- cells. CD31+ cells are endothelial cells and Pdgfra + cells are progenitors. F3+, CD9+, DDP4+ and CD24+ cells are gated onto the progenitor population.

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