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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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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

- [ ] [x] The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] [x] A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [ ] [x] 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.
- [ ] [x] A description of all covariates tested
- [ ] [x] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [ ] [x] 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)
- [ ] [x] 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.
- [x] [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [x] [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [x] [ ] 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: No software was used for data collection.

Data analysis:
1. MS proteomics analysis was completed using Patternlab 4.1.0.17 (http://www.patternlabforproteomics.org/Download.html).
2. Ingenuity Pathway Analysis (Qiagen, Germantown, MD, USA) was used to identify the canonical pathways. Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used for gene ontology analysis. This analysis was performed online. No version number is available.
3. Metabolomics were analyzed using TraceFinder 4.1 (ThermoFisher Scientific).
4. The percentage of CD31-positive areas was calculated using ImageJ 1.53t.
5. The protein bands of interest were analyzed using the Image Studio Software 4.0.21 (Licor, Lincoln, NE).
6. Muscle fiber size and type in sections of TA, gastrocnemius, and soleus were quantified using MyoVision 2.0.
7. FlowJoTM 10.8 was used to analyze the flow cytometry data.
8. Statistical analysis was performed using GraphPad Prism (Version 8.0) statistical software.

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

Mass spectrometry raw data is available in ProteomeXchange member repository for protein mass spectrometry data (https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=pxd030565; Username: reviewer_pxd030565@ebi.ac.uk; Password: aoJ8wv2F). Metabolomics original data is available on Metabolomics Workbench (https://www.metabolomicsworkbench.org/data/DRCCMetadata.php?Mode=Study&StudyID=ST002204). Source data are provided with this paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 determined after performing a power analysis with the following considerations: detection of a 25% difference between groups, assuming a standard deviation (SD) of 25% from the mean of each group, $\alpha = 0.05$, and $\beta = 0.80$.
Data exclusions	For some experiments, outlying data was excluded using the Identify Outliers of Graphpad Prism at parameter of Q=1%.
Replication	All experiments were replicated at least twice. Each experiment was conducted independently at different times.
Randomization	Male mice of same age were randomly assigned into different groups. For in vitro experiments, samples were randomly assigned as control group or treatment groups.
Blinding	Hyperinsulinemic-euglycemic clamps, indirect calorimetry, proteomics, metabolomics, and some immunohistochemistry experiments were done by operators in a blinded fashion. Analysis of these data was completed by laboratory members after decoding animal identity, therefore, the analysis was not blinded. All other experiments were done by laboratory members in our laboratory. Therefore, these experiments were not performed in a non-blinded manner.

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

Methods

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

Antibodies

Antibodies used

SIRT1 antibody (sc-15404, 1:500, Santa Cruz Biotech, Dallas, TX), von Willebrand Factor antibody (ab11713, Lot# GR189147-10, 1:200, Abcam), fluorescent secondary antibodies goat anti-rabbit IgG(H+L) Alexa fluor 647 (A32733, ThermoFisher scientific), donkey anti-sheep IgG (H+L) Alexa fluor 568(A-21099, ThermoFisher Scientific), goat anti-rabbit IgG(H+L) Alexa fluor 647 (A32733, ThermoFisher Scientific),anti-CD31-PE (102407, Lot# B224809, 1:200, Biolegend),anti-Akt (9272, Lot# 9,27, 1:1000, Cell Signaling,anti-Akt s473 (9271, Lot#13, 14, 1:1000, Cell Signaling), anti-Akt t308 (9275, Lot# 19,21, 1:1000, Cell Signaling), anti-GAPDH (2275-pc-100, Lot# 20601, 1.1000 Trevigen), anti-GSK 3β s9 (9331, Lot#20, 1:1000, Cell Signaling), anti-GSK 3α/β(sc-7292, Lot# E1513, 1:1000, Santa Cruz), anti-GFP (55494, Lot#1, 1:1000, Cell Signaling) anti-Tβ4 (AB6019 1:500, Millipore-Sigma), anti-flag (A8592, Lot# 087K6011, 1:500, Sigma), Phospho-Insulin/IGF Receptor Antibody Sampler Kit (8338, 1:1000, Cell Signaling), anti- NF-kB p65 (6956, Lot#7,1:1000,Cell Signaling,), anti-NF-kB p65 S536 (3036, Lot#:11,1:1000, Cell Signaling), anti-Atg7(8558, Lot:4,1:1000, Cell Signaling), anti-LC3A/B(L8918,1:1000, Sigma), anti-p62 (5114s, Lot# 6, 1:1000, Cell Signaling,), anti-HA-tag (sc-53516, 1:1000, Santa Cruz), anti-ILK (3862, Lot# 4, 1:1000, Cell Signaling), anti-mouse IgG(H+L) (Dylight 800 4X PEG conjugate) (5257, Lot#: 10, 1: 10000, Cell Signaling), anti-rabbit IgG(H+L) (Dylight 800 4X PEG conjugate) (5151, Lot:15, 1: 10000, Cell Signaling), anti-mouse IgG(H+L) (Dylight 680 conjugate) (5470, Lot:13, 1: 10000, Cell Signaling), anti-rabbit IgG(H+L) (Dylight 680 conjugate) (5366, Lot:13,1: 10000, Cell Signaling),CD31 antibody (550274, Lot# 1025824, 1:200; BD Bioscience, San Diego, CA, USA), anti-myosin BA-F8 IgG2b (1:250, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA), anti-myosin BF-F3 IgM (1:250, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA), anti-myosin BF-F35 IgG1 (1:250, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA), rabbit anti-mouse Laminin antibody (L9393,1:500, Sigma), donkey anti-rat IgG(H+L) Alex647(A-21247, Lot# 2420724, 1:200, ThermoFisher Scientific),goat anti-rabbit Alex IgG(H+L)568 (A-11011, Lot#:1670154, 1:200, ThermoFisher Scientific), goat anti-rabbit Alex IgG(H+L)488 (ab150077, 1:200, Abcam), goat anti-rabbit Alex IgG(H + L)405 (A31556B, 1:200, ThermoFisher Scientific), anti-mouse 647 IgG2B (A21242,1:200, ThermoFisher scientific), anti-mouse 555IgG1 (A21127, 1:500, ThermoFisher scientific), anti-mouse 488 IgM (A21042, 1:200, ThermoFisher Scientific). CD161-BUV563 (748971,1:12, BD Biosciences), LY6G-PE (127607, 1:100, Biolegend), F4/80-PE-Cy5 (123111, 1:100, Biolegend), CD163-Brilliant Violet 421 (155309, 1:25, Biolegend), CD4-Alexa Fluor 488 (100425, 1:1000, Biolegend), CD8-APC-Cy7 (155015, 1:200, Biolegend), CD3-PE-Cy7 (100219, 1:200, Biolegend), CD11c-Brilliant Violet 605 (117333, 1:25, Biolegend), CD19-Brilliant Violet 785 (115543, 1:20, Biolegend), CD45-PerCP-Cy5.5 (157611, 1:100, Biolegend).

Validation

The primary antibodies used in the study have been validated by the commercial vendors. There are many citations using these antibodies. SIRT1 antibody (sc-15404) has been cited in 79 research papers; von Willebrand Factor antibody (ab11713) in 105, anti-CD31-PE (102407) in 61; anti-Akt (9272) in 23,006; anti-Akt s473 (9271) in 26,526; anti-Akt t308 (9275) in 1, 338; anti-GAPDH (2275-pc-100) in 79; anti-GSK 3β s9 (9331) in 518; anti-GSK 3α/β (sc-7291) in 255; Tβ4 antibody (AB6019) in 3; anti-flag (A8592) in 1,605; Phospho-Insulin/IGF Receptor Antibody Sampler Kit (8338) in 1. Moreover, single antibodies in the sampler kit have been cited many times in other research papers; anti- NF-kB p65 (6956) in 431; anti-NF-kB p65 S536 (3036) in 326; anti-Atg7 (8558) in 575; anti-LC3A/B (L8918) in 301; anti-p62 (5114) in 1,231; anti-HA-tag (sc-53516) in 21; anti-ILK (3862) in 56; CD31 antibody (550274) in 7; anti-myosin BA-F8 IgG2b (Developmental Studies Hybridoma Bank, University of Iowa) in 38 ; anti-myosin BF-F3 IgM (Developmental Studies Hybridoma Bank, University of Iowa) in 49; anti-myosin SC-71 MIgG1 (Developmental Studies Hybridoma Bank, University of Iowa) in 90; rabbit anti-mouse Laminin antibody (L9393) in 1,448; CD161-BUV563 (748971) in 6; LY6G-PE (127607) in 201; F4/80-PE-Cy5 (123111) in 28; CD163-Brilliant Violet 421 (155309) in 2; CD4-Alexa Fluor 488 (100425) in 33; CD8-APC-Cy7 (155015) in 1; CD3-PE-Cy7 (100219) in 100; CD11c-Brilliant Violet 605 (117333) in 78; CD19-Brilliant Violet 785 (115543) in 30; and CD45-PerCP-Cy5.5 (157611) in 9, respectively.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (C2519A); Mouse C2C12 myoblasts were from ATCC (CRL1772, ATCC). No commonly misidentified cells lines were in this study.

Authentication

These two cell lines have been authenticated by their suppliers. The methods involve morphology, quantitative real-time PCR, immunoblotting, etc.

Mycoplasma contamination

HUVECs were negative for Mycoplasma, bacteria, yeast, and fungi when shipped by supplier. C2C12 cell line was not tested for Mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

n/a

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

Endothelium–specific Sirt1 knockout mice (E-SIRT1 KO) in a C57BL/6 background were generated by crossing Sirt1flox/flox (WT) mice (purchased from Jackson Labs) with mice expressing Cre recombinase under the control of the endothelial Cadherin 5 promoter (Jackson Labs). Sirt1 flox/flox mice were used as wild-type controls. Male mice of age of 8-30 weeks were used for the metabolic and glucose homeostasis studies. Female mice were used for breeding and vascular reactivity studies.

Wild animals

This study did not involve wild animals.

Reporting on sex

Metabolic and glucose homeostasis studies were done in male mice on the C57BL6/J background because there are significant differences in these parameters between male and females.

Field-collected samples

This study did not invole samples collected from the field.

Ethics oversight

All protocols were approved by the University of Iowa Institutional Animal Care and Use Committee, and all experiments were carried out according to the National Institutes of Health (NIH) guidelines.

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	Two drops of blood were collected into an EDTA-coated blood collection tube (Ram Scientific, Inc, Nashville, TN37308) from the confluence of the facial vein and submandibular vein at the rear end of the mandibular bone. Red blood cells (RBCs) were lysed in an ACK lysing buffer (Gibco, A10492-01) according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) were re-suspended in the flow buffer (PBS supplied with 2% FBS). PBMCs were stained for 30 min at 4 °C using 100 uL of antibody mixture composed of CD161-BUV563 (1:12, BD Biosciences, 748971), LY6G-PE (1:100, Biolegend,127607), F4/80-PE-Cy5 (1:100, Biolegend,1231111), CD163-Brilliant Violet 421 (1:25, Biolegend,155309), CD4-Alexa Fluor 488 (1:1000, Biolegend,100425), CD8-APC-Cy7 (1:200, Biolegend,155015), CD3-PE-Cy7 (1:200, Biolegend,100219), CD11c-Brilliant Violet 605 (1:25, Biolegend,117333), CD19-Brilliant Violet 785 (1:20, Biolegend, 115543), and CD45-PerCP-Cy5.5 (1:100, Biolegend, 157611). After washing with the flow buffer 2 times, cells were fixed with 100 uL of BD stabilizing fixative (BD, 554665) for 30 min at 4 °C in the dark. After washing with 200 uL of the flow buffer, cells were re- suspended in 300 uL of the flow buffer and acquired on a Cytek Aurora/Cytek Aurora 2 within 24 h.
Instrument	Cytek Aurora/Cytek Aurora 2 was used to perform flow cytometry.
Software	FlowJoTM 10.8
Cell population abundance	100,000 CD45+ cells was created.
Gating strategy	All live PBMCs were first gated on the basis of FSC-A (forward scatter) and SSC-A (side scatter), and then single cell is gated on the basis of FSC-S (forward scatter) and FSC-W (forward scatter width). CD45-positive cells are gated on the basis of the surface expression of CD45, a white blood cell marker. The t-distributed stochastic neighbor embedding (t-SNE) tool is used to analyze the sub-populations of CD45 positive cells on the basis of surface expression markers, and t-SNE map of 100,000 CD45+ cells was created.

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