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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	No software was used.
Data analysis	Statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, Inc.) or FlowJo, R/Bioconductor 2.13 open source software for bioinformatics.

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

All data underlying the findings reported in this manuscript are provided as part of the article. Source data are available online. Mouse and human microarray data are available at the Gene Expression Omnibus (GEO) GSE68161 and GSE116801. The raw data that support the findings of this study are available from the

corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to L.J.G.

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	No statistical method was used to predetermine animal's sample size. The sample size was determined based on experience with the used experimental models/setups.
Data exclusions	No samples were excluded from analyses.
Replication	No attempts at experimental replication failed. n values are reported throughout the manuscript.
Randomization	Mice were randomized in each study.
Blinding	The experiments were not conducted while blinded. Blinding was not possible due to the nature of interventions, in this case exercise.

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

Antibodies

Antibodies used

TGF-β2: Santa Cruz Biotechnology, Santa Cruz, CA, Catalog SC-90, Dilution 1:3000, rabbit polyclonal.
PPARa: Santa Cruz Biotechnology, Santa Cruz, CA, SC-9000, Dilution 1:3000, rabbit polyclonal.
SMAD2: Cell Signaling Technology, Beverly, MA, Catalog 5339, Dilution 1:1000, monoclonal.
pSMAD2: Cell Signaling Technology, Beverly, MA, Catalog 3101, Dilution 1:1000, rabbit polyclonal.
GAPDH: Cell Signaling Technology, Beverly, MA, 2118S, Dilution 1:5000, rabbit monoclonal.
CD45-PE-Cy7: eBiosciences, 25-0451-81, Clone 30-F11, Dilution 1:200, monoclonal.
F4/80-APC-Cy7: BioLegend, 123117, Clone BM8, Dilution 1:300, monoclonal.
CD206-Alex647: Serotec, Inc., MCA2235A647, Clone MR5D3, Dilution 1:200, monoclonal.
CD11c-PE: BD Pharmingen, 553802, Clone HL3, Dilution 1:200, monoclonal.
CD31-PE-Cy7: eBiosciences, 25-0311-82, Clone 390, Dilution 1:200, monoclonal.
F4/80-APC: eBiosciences, 50-112-9524, Clone BM8, Dilution 1:200, monoclonal.
Sca-1 FITC: eBiosciences, 50-112-9502, Clone D7, Dilution 1:200, monoclonal.

Validation

All the antibodies used in this study were validated by providers and previous reports.
TGF-β2: http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC6096141&blobtype=pdf
http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC5627931&blobtype=pdf
http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC4520553&blobtype=pdf
PPARa: https://www.scbt.com/scbt/product/pparalpha-antibody-h-98
SMAD2: https://www.cellsignal.com/products/primary-antibodies/smad2-d43b4-xp-rabbit-mab/5339
pSMAD2: https://www.cellsignal.com/products/primary-antibodies/phospho-smad2-ser465-467-antibody/3101
GAPDH: https://www.cellsignal.com/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118
CD45-PE-Cy7: https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/25-0451-81

F4/80-APC-Cy7: https://www.biolegend.com/en-us/products/apc-cy7-anti-mouse-f4-80-antibody-4072
CD206-Alex647: https://www.bio-rad-antibodies.com/monoclonal/mouse-cd206-antibody-mr5d3-mca2235.html?f=purified
CD11c-PE: http://www.bdbiosciences.com/ds/pm/tds/553802.pdf
CD31-PE-Cy7: https://www.thermofisher.com/antibody/product/CD31-PECAM-1-Antibody-clone-390-Monoclonal/25-0311-82
F4/80-APC: https://www.fishersci.com/shop/products/apc-anti-mouse-f4-80/501129524
Sca-1 FITC: https://www.fishersci.com/shop/products/anti-mouse-sca-1-fitc-100ug/501129502

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	C2C12, 3T3-L1 and WT-1 immortalized cell lines and human immortalized preadipocytes were used in this study. C2C12 and 3T3-L1 lines were purchased from American Type Culture Collection (ATCC). WT-1 cell line and human immortalized preadipocytes were previously generated in the laboratory of Dr. Tseng.
Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contaminantion.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the Joslin Diabetes Center and were in accordance with NIH guidelines. Nine-week-old male C57BL/6 mice (Charles River Laboratories) were used for exercise training studies and nine-week-old male ICR mice (TACONIC) were used for in vivo TGF-β2 treatment.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Human participants include healthy young (age 18 – 40 years) male subjects (Yfanti et al., Am. J. Physiol. Endocrinol. Metab, 2011) and healthy middle-aged (age 40-55 years) sedentary men (Montiani et al., Diabetes, Obes. Metab, 2017). Protocols were approved by local Ethical Committee of Copenhagen and Frederiksberg (KF 01 289434) and University of Turku ethic committees.
Recruitment	Subjects were recruited through newspaper advertisements. Blood and adipose tissue samples were obtained from participants recruited in previously studies (Yfanti et al., Am. J. Physiol. Endocrinol. Metab, 2011 and Montiani et al., Diabetes, Obes. Metab, 2017.

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	Cut fat tissue with scissors to small pieces of 2 mm and add 2 mg/ml collagenase (Sigma) in Hank's buffer salt solution (HBSS) with 2 % fetal bovine serum (FBS) directly to 50 ml tubes. Tubes were incubated at 37 degree water bath with shaking for 45 min. Add the 10% FBS-DMEM-Hi media and filer the fat through a cell strainer. Centrifuge the tubes at RT 1000 rpm for 5 min. The resulting suspension cells were lied with the addition of ACK lysis buffer. For M1 and M2 macrophage identification, cells were washed once and resuspended in FACS buffer with Fc blocking reagent. Cells were incubated with CD45-PE-Cy7 (eBiosciences), F4/80-APC-Cy7 (BioLegend), CD206-Alex647 (Serotec, Inc.) and CD11c-PE (BD Pharmingen) antibodies for 30 min in HBSS containing 2% FBS on ice and then washed and resuspended in solution with Sytox Blue (Thermo Scientific). For sorting out preadipocyte, endothelial cell and macrophage, cells were incubated with CD31-PE-Cy7 (eBiosciences), F4/80-APC

	(eBiosciences), and Sca-1 FITC (eBiosciences) antibodies for 30 min in HBSS containing 2% FBS on ice and then washed and resuspended in solution with Propidium Iodide Staining Solution (Sigma).
Instrument	FACSAria
Software	FlowJo
Cell population abundance	10000 cells were analyzed for fluorescent intensity in the defined gate.
Gating strategy	Starting cell population was gated by forward scatter and side scatter followed by exclusion of dead cells with Sytox Blue or Propidium Iodide staining, Positive/Negative populations were determined by FMO controls.

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