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Life Sciences Reporting Summary

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Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample sizes are biological replicates and were chosen based on preliminary data or previously published reports. We first performed normality tests using the Shapiro-Wilk test ($3 \le n \le 7$) or the D'Agostino–Pearson omnibus test ($n \ge 8$). Normally distributed data were further analyzed by parametric tests including a two-tailed Student's t-test for two-group comparisons or a one-way analysis of variance (ANOVA) for multiple comparisons involving one independent variable. When groups followed a non-normal distribution, Mann–Whitney U test was used.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   Data are representative of 2-4 independent experiments.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   For all animal experiments, the animals were randomly assigned at the time of purchase or weaning to minimize any possible bias. Age-matched mice were used for in vivo studies.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Histology was performed blinded. CLAMS were performed by a staff scientist at the University of Michigan Animal Phenotyping Core without in-depth knowledge about the scope of the current work. Other experiments were not blinded.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

All results are presented as mean ± s.e.m. and graphed using Prism 6. Images in Calcium Imaging experiments were processed with MetaFlour software (Olympus). Flow cytometry acquisition was performed on a 3-laser Fortessa flow cytometer (Becton Dickinson) and data were analyzed with FlowJo software (TreeStar). Image quantification for western blot was performed using ImageJ software. Adipocyte size was analyzed using ImageJ software (Adiposoft).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All the materials, cells, and animals used in the paper are available upon request under MTA with the University of Michigan.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

ChIP assay was performed with antibodies for PPARγ (#sc-7196, Santa Cruz) or IgG (#2729, Cell Signaling). Western blot was performed with following antibodies: from Abcam: UCP1 (ab10983), MitoProfile total OXPHOS (ab110413); from Cell Signaling: COXIV (#4850), phospho-PKA substrateS/T (#9621), phospho-CREBS133 (#9198), CREB (#9212), phospho-p38T180/Y182 (#9215), p38 (#9197) α-tubulin (#2144), β-actin (#8457), GAPDH (#5174), and HSP90 (#4874). Immunohistochemistry was performed with a rabbit polyclonal anti-UCP1 antibody (1:200; ab10983, Abcam). Immunofluorescence staining was performed with a rabbit polyclonal GFP antibody (A-6455, Life Technologies). Flow cytometric analysis was performed with the following antibodies for immune profiling: CD45 (30-F11, BioLegend), F4/80 (BM8, BioLegend), CD11b (M1/70, Biolegend), MHC Class II (M5/114.15.2, BioLegend), Siglec-F (E50-2440, BD Biosciences), CD11c (N418, BioLegend), CD206 (MR5D3, BioLegend), CD3 (17A2, BioLegend; 17A2, BioLegend), TCRγδ (UC7-13D5, BioLegend), TCRβ (H57-597, BioLegend), CD4 (GK1.5, BioLegend), CD8 (53-6.7, BioLegend), CD19 (1D3/CD19, BioLegend) and B220 (Ra3-6B2, BioLegend). Intracellular analysis of ChAT expression was performed with a rabbit monoclonal ChAT antibody (ab181023, Abcam) or isotype IgG control followed by BV421-conjugated anti-rabbit secondary antibody (BioLegend).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293 cells were used in the luciferase reporter assay and is from ATCC (CRL-1573).

b. Describe the method of cell line authentication used.

The HEK293 cells have not been authenticated by our lab independently of ATCC's authentication.

c. Report whether the cell lines were tested for mycoplasma contamination.

We confirmed no mycoplasma contamination in the cells using a commercially available kit.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

HEK293 cells were used in the luciferase reporter assay based on previous published studies using HEK293 cells for high transfection efficiency.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

The 129SVE and Balb/c mice were purchased from Taconic Farms Inc. The Ucp1-CRE transgenic mice, the Ai14 reporter mice, C57BL/6J, ob/ob, ChATBAC-eGFP, ChATfl/fl, Vav-iCre, B6.SJL and Chrna2 knockout mice were obtained from the Jackson Laboratory (Stocks #024670, #007914, #000664, #000632, #007902, #016920, #008610, #002014 and #005797 respectively). β-less mice were provided by Brad Lowell (Beth Israel Deaconess Medical Center, Boston). All animal studies were conducted according to the protocol reviewed and approved by the University Committee on Use and Care of Animals at the University of Michigan. All mice were housed under 12-h light/12-h dark cycle (6 a.m.–6 p.m.) with a standard rodent chow diet unless otherwise specified.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Only de-identified human adipose stromal cells isolated from subcutaneous and perirenal depots were used. All specimens were collected under the protocols reviewed and approved by the Western Institutional Review Board (Puyallup, WA) or the University of Michigan Medical School Institutional Review Board (IRBMED).