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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   The sample size was determined based on our experience with the experimental models, anticipated biological variables, previous literatures, and also by a statistical power calculation (α=0.05).

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All the experiments were replicated at least twice. For example, metabolic characterization of Prdm16 Tg x Ucp1-/- mice, such as body-weight and glucose tolerance under a high-fat diet, were repeated in 3 independent cohorts under ambient temperature and in 2 independent cohorts under thermoneutrality. Tissue and cell respiration assays were performed, at least, in two independent experiments. RNA-sequencing and metabolomics were performed once but three independent mice were analyzed and further validated by alternative approaches, such as qRT-PCR. Western blotting data were confirmed by two or three independent samples.

4. Randomization

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

   Mice were randomly assigned at the time of purchase or weaning to minimize any possible bias.

5. Blinding

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

   RNA-sequencing, metabolomics, and histology (electron microscopy) were performed blinded. CLAMS were performed by a staff scientist without in-depth knowledge about the scope of the current work. Other experiments were not blinded.

Note: all studies involving animals and/or human research participants must disclose 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals 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

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.	For RNA-sequencing data analysis, TopHat (http://ccb.jhu.edu/software/tophat/) and Cufflinks (http://cufflinks.cbcb.umd.edu) were used. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA).

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 for-profit company.	All the materials, cells, and animals used in the paper are available upon request under MTA with UCSF.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The following antibodies were used: UCP1 antibodies (abcam, ab10983 , 1:1000 for mouse UCP1 protein and Sigma, U6382, 1:1,000 for human UCP1 protein), SERCA2 (Thermo Fisher, 2A7-A1, 1:200 for immunoprecipitation, 1:100 for immunostaining), β–actin (Sigma, A3854, 1:10,000 for immunoblot), Calnexin (Santa Cruz, sc11397, 1:100 for immunostaining), RyR2 polyclonal antibody (Thermo Fisher, PA5-36121, 1:200), MitoProfile® total OXPHOS rodent antibody cocktail (abcam, ab110413, 1:1000), Rabbit polyclonal PRDM16 antibody was originally developed in the lab and reported in Seale et al. JCI 2011 (1:1000 for immunoblot).

10. Eukaryotic cell lines

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

Immortalized preadipocytes were isolated from the iBAT or the inguinal WAT of Ucp1-/- mice and Prdm16 Tg x Ucp1-/- mice by infecting retrovirus expressing SV-Large T antigen and subsequently selected by puromycin. Immortalized SVF cells from the subcutaneous WAT of neonatal pigs are established by the same approach. Clonal human beige adipocytes were previously reported by our group (Shinoda et al. Nature Medicine 2015).

b. Describe the method of cell line authentication used.

Immortalized mouse Ucp1-/- brown and beige adipocyte lines and immortalized human beige adipocyte line were established by us. We confirmed the authentication (their origins and cell types) by global expression analyses, such as RNA-sequencing and protein expression.

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.

No commercial misidentified cells were used.

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

Male mice in C57BL6J background were used in the study. Prdm16 Tg x Ucp1-/- mice were backcrossed to the Bl6 background for more than eight generations. For high fat diet feeding experiments, male mice at 6 weeks old were fed 60% fat diet for 12-24 weeks.

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.

No human research participants are involved in the current study.