EMBO reports

Mitophagy receptor FUNDC1 is regulated by PGC-1α/NRF1 to fine tune mitochondrial homeostasis

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DOI: 10.15252/embr.202050629

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Review Timeline:

Submission Date:	13th Apr 20
Editorial Decision:	29th May 20
Revision Received:	12th Oct 20
Editorial Decision:	19th Nov 20
Revision Received:	27th Nov 20
Accepted:	15th Dec 20

Editor: Deniz Senyilmaz Tiebe

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 29th May 2020

Dear Dr. Chen,

Thank you for the submission of your research manuscript to our journal, which was now seen by three referees, whose reports are copied below.

I apologize for the unusual delay in getting back to you. It took longer than anticipated to receive the referee reports.

We concur with the referees that the proposed role of FUNDC1 in adaptive thermogenesis is in principle very interesting. However, the referees also raise important concerns that need to be addressed prior to publication in this journal. In particular,

- more support into transcriptional regulation of FUNDC1 by NRF1 and/or PGC1 alpha (ref 1, points 1 and 4, ref 3 point 2) is required.
- mitochondrial biogenesis/mass, mitophagy and bioenergetics data should be strengthened (ref 1 point 5 and 7, ref 2, points 1 and 3, 4).
- Does the effect of cold exposure on mitophagy depend on a general effect on autophagy? (ref 2 point 2).

I find the reports informed and constructive, and believe that addressing the concerns raised will significantly strengthen the manuscript.

Given these positive requirements, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

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Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

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When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper. For more details on our Transparent Editorial Process, please visit our website:
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You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

4) a complete author checklist, which you can download from our author guidelines (<http://embor.embopress.org/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<http://embor.embopress.org/authorguide>).

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <http://embor.embopress.org/authorguide#expandedview>.

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) We would also encourage you to include the source data for figure panels that show essential data.

Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available <http://embor.embopress.org/authorguide#sourcedata>.

8) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <http://embor.embopress.org/authorguide#datacitation>.

9) Please make sure to include a Data Availability Section before submitting your revision - if it is not applicable, make a statement that no data were deposited in a public database. Primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see <http://embor.embopress.org/authorguide#dataavailability>).

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method) that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

10) Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates.
Please also include scale bars in all microscopy images.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

Yours sincerely,

Deniz Senyilmaz Tiebe

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Referee #1:

In this study the authors have analyzed the regulation of the mitophagy receptor FUNDC1 in brown adipocytes as well as the alterations driven by FUNDC1 deficiency in brown adipose tissue. The authors report that BAT FUNDC1 is induced by cold exposure, and that PGC1a and by NRF1 are involved in Fundc1 gene expression. In addition, the authors have phenotyped BAT-specific Fundc1 KO mice, and found that both mitochondrial biogenesis and mitochondrial function are altered upon cold exposure. The authors conclude that mitochondrial biogenesis factors such as PGC1a or NRF1 also regulate mitophagy through FUNDC1 induction. This may be the case but data shown in the present form of the manuscript are insufficient, and there are alternative options that are discussed below.

Major comments.
1. The authors document that PGC1a overexpression causes and enhanced FUNDC1 expression in brown pre-adipocytes, and that PGC1a deficiency reduces FUNDC1 expression. This latter studies should be performed in mature brown adipocytes otherwise the evidence remains unconvincing. It is also unclear what cells were used in panels 2D and 2E so please indicate.
2. NRF1 terminology induces to confusion so please indicate whether you are referring to the protein product of Nrf1 gene or instead of Nfe2l1 gene. In figure 3, authors do not specify the origin

of nuclear extracts used in ChIP analysis (panels E and F), and similarly do not specifiy the control and Findc1 deficient cells. The authors similarly to what mentioned in question 1 should document that NRF1 deficiency in brown adipocytes alters the expression of FUNDC1.
3. The data generated by the authors indicate that NRF1 or PGC1a deficiency cause FUNDC1 repression in cells (not indicated which ones). Based on this, the authors propose that NRF1 and PGC1a collaborate in Fundc1 transcription under specific conditions. However, an alternative possibility is that NRF1 deficiency represses PGC1a or viceversa that PGC1a deficiency represses NRF1. The authors should provide a response to this obvious possibility.
4. A more robust demonstration that NRF1 requires the putative NRF2 binding site is required. What is the effect of mutation of the binding site on promoter activity? And on NRF1-induced promoter activity?
5. The authors state that cold exposure induces mitophagy and the interaction between FUNDC1 and LC3 in BAT. This statement is not sufficiently supported by the data and mitophagy analysis requires some assessment of flux by using inhibitors of autophagy. In addition, an enhanced interaction of FUNDC1 and LC3 is proposed based on the observations that FUNDC1 immunoprecipitation carries more LC3 protein in BAT extracts obtained after cold exposure. This experiments has two weaknesses: a) the reverse experiment should be also performed (IP with an anti-LC3 antibody), and b) it seems that FUNDC1 interacts with LC3-I and not with LC3-II so how do the authors explain this as a mechanism of mitophagy acceleration?
6. The authors used two different mouse models to analyse the impact of FUNDC1 ablation in BAT but only characterize one of them. Please, provide documentation of appropriate depletion of FUNDC1 in adiponectin-Cre mice.
7. Interestingly, BAT-specific FUNDC1 KO mice showed not only an impaired mitochondrial quality but also reduced mitochondrial biogenesis (based on the reduced mtDNA). This is perhaps the explanation by which PGC1a induces FUNDC1, i.e., to permit an efficient mitochondrial biogenesis upon cold exposure. In this connection, the authors should measure the expression of different mitochondrial proteins in BAT from loxP and KO mice.

Minor comments.
1. The Introduction section is excessively long. Please shorten.

Referee #2:

In the manuscript by Lui et al., the authors investigate the role of FUNDC1 on brown fat mitochondrial homeostasis during cold stress - a protein previously shown to be involved in mitophagy. The authors find that cold stress leads to a PGC1a-dependent upregulation of FUNDC1 and propose that PGC1a co-ordinately regulates both mitochondrial biogenesis and mitophagy. Loss of FUNDC1 in BAT, blocks mitophagy, impairs mitochondrial function and results in an impaired thermogenic response to cold. This is certainly an interesting paper and builds on work directly linking mitochondrial biogenesis and mitophagy, as well as adding a role for FUNDC1-dependent mitophagy in BAT. I do have some points though that will help address some concerns with the current data.

Main points:
1) The mitochondrial biogenesis data in Figure 1 would be strengthen by probing for other critical pathway proteins, such as TFAM. Additionally, as mitochondrial mass is increasing, then surely FUNDC1 (a mitochondrial protein) should also increase in-line with this. How then does this lead to mitophagy? I am not quite sure what the authors are proposing here with respect to PGC1a and

FUNDC1. Are the authors saying that PGC1a induces more FUNDC1 compared to the other proteins that are induced (thus even though there are more mitochondria, each mitochondrion has more PGC1a than normal)? Or, are they saying that PGC1a induces additional changes to FUNDC1 (eg PTMs)? Or do they not know? I think a little clarification as the results are being described would help. For the former, could the authors plot FUNDC1 (and other induced proteins) levels relative to mitochondria/mtDNA?
2) In Figure 4, the authors show an increase in autophagy proteins upon cold exposure. Is this increase dependent on FUNDC1, i.e. could a loss of autophagy in-general be responsible for less mitophagy observed in Figure 5?
3) In Figure 5, the low level of mtDNA upon loss of FUNDC1 during the cold is a little puzzling. If mtDNA is a readout of mitochondrial mass, then surely it should be increasing if mitophagy is blocked? Can the authors probe for another measure of mitochondrial mass under these conditions, such as citrate synthase activity/mtDNA/western blot? How does total mtKeima signal change?
4) The bioenergetics data in Figure 5 needs to be better explained. (a) Figure 5G is unnecessary as the same information is in figure 5H with stats. (b) The authors mention in the methods that they perform a classical seahorse-type experiment, perhaps these measurements should be included - they observe no decrease in presence of oligomycin in their basal measurements. This suggests that most of the respiration recorded represents proton leak in all the groups, and that ATP-linked respiration is negligible. The mitochondria might be already in an uncoupled state in BAT due to the UCPs. The FCCP measurement would help explain this. (c) If there is still mitophagy going on upon loss of FUNDC1 in the Cold+Cre+ condition (Fig 5C-D suggests a small amount), then there might be less mitochondria as well. The authors should provide a measurement of mtDNA (mitochondrial mass) as this could explain the decreased OCR values compared to Cold+Cre-.
5) The statistical tests used in the article are not appropriate - the authors mention in the Material and Methods section that they used t-tests only. These should only be used with 2 groups. However, most of the graphs displayed have at least 3 groups. This will lead to an increased Type I error and consequently, some conclusions might be erroneous. One/two-way ANOVA/repeated measure ANOVAs are more appropriate.

Other points
6) In relation to figure 2A and 2B, the authors mention "however, the mRNA level of NRF1 was unchanged, which suggests that the stability of NRF1 protein is increased by cold exposure (Figure 2C)." This might be due to the timepoint chosen. Mitochondrial biogenesis protein mRNA expression is very dynamic, and it is possible that the increase of NRF1 mRNA simply occurred at an earlier timepoint and as there is no need to keep it "up" constantly after the adaptations, the mRNA levels might already be back to normal. This should be briefly mentioned as a possibility.
7) In figure 2F and 2G, is it a double knockdown of PGC1b and PGC1a, as suggested in figure 2F, or is this a typo and it is only PGC1b?
8) In relation to figure 3H, the authors mention "the induction of FUNDC1 by PGC1a was suppressed by knockdown of NRF1". This claim may not be so straightforward as Figure 3H shows an increased FUNDC1 with PGC1a NRF1 KD compared to NRF1 KD alone. This is further confirmed by the increase in another mitochondrial protein (CYCS). This result suggests that NRF1 is not the sole interactor with the FUNDC1 promoter (possibly NRF2 and/or TFAM).
9) The Material and Methods section concerning Western Blotting mentions the use of a Myc antibody, but there is however no mention of it in the article.

Referee #3:

The current manuscript presents an investigation into the coordinated mitochondrial biogenesis and mitophagy in brown adipose tissue in response to thermogenic stress. The thermogenic stress in BAT prompts PGC-1/NRF-mediated mitochondrial biogenesis is an established mechanism. The role of mitophagy to maintain the quality of the expanded mitochondrial pool during thermogenesis is less well resolved. The current paper provides compelling evidence to indicate that PGC1/NRF upregulate the mitophagy adaptor FUNDC1 to promote mitophagy, thereby revealing a new level of coordination of mitochondrial homeostasis following thermogenesis. The paper is well-written and logical, The data presented is largely well-controlled, convincing and appropriately interpreted. There are some points that should be addressed although I am also mindful of potential limitations on lab work during the current COVID-19 restrictions.

1. The authors indicate that they do not detect Parkin expression at the protein or mRNA level. However, Lu et al (Scientific Reports 2018) and Cairo et al (EmboJ 2019) have reported that Parkin is expressed in BAT at the protein and mRNA level. Can the authors explain this discordance? Additionally, Lu et al found that Parkin protein levels in BAT increase. The cold-stress induced mitophagy was PINK1 mediated suggesting that Parkin is the mitophagy effector in BAT following cold stress. This included Pink1-/- had increased defective mitochondria (abnormal cristae) in mitophagosomes similar to the observation here in the absence of FUNDC1. Could there be redundant mechanisms in cold stressed BAT?

2. Figure 3- EMSA assay, the whole gel should be shown to show free probe in each lane and also the mutated oligo probe in 3B should serve as a negative control for NRF binding. Fig 3D- Super shift should ideally be tested with the PGC1 Ab to confirm complex formation and also a negative control Ab.

3. The authors report that FUNDC1 deletion in BAT inhibited the cold-induced increase in mtDNA copy number suggesting positive feedback between mitophagy and biogenesis. Although the mechanism is unresolved, the authors comment that PGC1 is reduced in cold-treated FUNDc1-deficient BAT. I agree that this is an interesting observation I recommend the authors include this data as it highlights the interplay between the two mitochondrial mechanisms.

4. For the broader readership, I would recommend including schematic of the pathway model.

Specific comments
1. Page 13: For clarity I would reword the 2nd sentence as follows: "We found that the OCRs of Fundc1-deficient BAT did not significantly increase upon cold exposure…
2. Page 13, first paragraph, last sentence should be amended to clarify " …FUNDC1-dependent mitophagy is essential for mitochondrial turnover and quality control in response to cold stress>"
3. 3C- Typo "Positve"
4. In 4F- Although there is an increase in the input fractions in the cold treated sample, the IP of FUNDC1 is the same irrespective of treatment?
5. The "blank" UCP1 blots in S3 appear to be identical.

Point-by point response to the reviewers:

Reviewer #1

In this study the authors have analyzed the regulation of the mitophagy receptor FUNDC1 in brown adipocytes as well as the alterations driven by FUNDC1 deficiency in brown adipose tissue. The authors report that BAT FUNDC1 is induced by cold exposure, and that PGC1a and by NRF1 are involved in Fundc1 gene expression. In addition, the authors have phenotyped BAT-specific Fundc1 KO mice, and found that both mitochondrial biogenesis and mitochondrial function are altered upon cold exposure. The authors conclude that mitochondrial biogenesis factors such as PGC1a or NRF1 also regulate mitophagy through FUNDC1 induction. This may be the case but data shown in the present form of the manuscript are insufficient, and there are alternative options that are discussed below.

Response 1: We appreciate the candid opinions from the reviewer. As seen below, we have performed all the experiments suggested to address the concerns of the reviewer, and we wish that it is satisfactory to the reviewer.

Major comments.

1. The authors document that PGC1a overexpression causes and enhanced FUNDC1 expression in brown pre-adipocytes, and that PGC1a deficiency reduces FUNDC1 expression. This latter studies should be performed in mature brown adipocytes otherwise the evidence remains unconvincing. It is also unclear what cells were used in panels 2D and 2E so please indicate.

Response 2: We agree with the reviewer and have performed the experiments as suggested. We now showed that knockdown of PGC-1α also reduces the expression of Fundc1 in mature brown adipocytes (Figure 2G and H). The cells used in panels 2D and 2E were brown preadipocytes as indicated (see page 34, line 24)

2. NRF1 terminology induces to confusion so please indicate whether you are referring to the protein product of Nrf1 gene or instead of Nfe2l1 gene. In figure 3, authors do not specify the origin of nuclear extracts used in ChIP analysis (panels E and F), and similarly

do not specifiy the control and Findc1 deficient cells. The authors similarly to what mentioned in question 1 should document that NRF1 deficiency in brown adipocytes alters the expression of FUNDC1.

Response 3: Many thanks for the suggestion. The full name of NRF1 genes in our paper is Nuclear Respiratory Factor 1 and we have clarified it in the abstract. The ChIP analysis was performed in HeLa cells and we have included it in the main text (see page 10, line 12). The control and *Fundc1* deficient cells were brown preadipocytes and we have indicated it in the main text (see page 13, line 22). We have performed the experiments in mature brown adipocytes as suggested and found that the expression of Fundc1 is reduced when NRF1 was knockdown (Figure EV2D and E).

3. The data generated by the authors indicate that NRF1 or PGC1a deficiency cause FUNDC1 repression in cells (not indicated which ones). Based on this, the authors propose that NRF1 and PGC1a collaborate in Fundc1 transcription under specific conditions. However, an alternative possibility is that NRF1 deficiency represses PGC1a or vice versa that PGC1a deficiency represses NRF1. The authors should provide a response to this obvious possibility.

Response 4: This is a good suggestion. We have followed the reviewer's suggestion and performed new experiments. Our new data showed that the expression of NRF1 was reduced when PGC-1α was deficient and the expression of PGC-1α was unaffected when NRF1 was knockdown in mature brown adipocytes (Figure 2G and H and Figure EV2D and E). Our data were consistent with a prior report (Uldry *et al*, 2006), which showed that PGC-1α dramatically induced mRNA of NRF1.

4. A more robust demonstration that NRF1 requires the putative NRF2 binding site is required. What is the effect of mutation of the binding site on promoter activity? And on NRF1-induced promoter activity?

Response 5: Thanks for the suggestion. The consensus of NRF2 binding site is "TGCTGAGTCA", which is distinct from that of NRF1, and we have searched the sequence of Fundc1 promoter and we failed to find NRF2 binding site.

5. The authors state that cold exposure induces mitophagy and the interaction between FUNDC1 and LC3 in BAT. This statement is not sufficiently supported by the data and mitophagy analysis requires some assessment of flux by using inhibitors of autophagy. In addition, an enhanced interaction of FUNDC1 and LC3 is proposed based on the observations that FUNDC1 immunoprecipitation carries more LC3 protein in BAT extracts obtained after cold exposure. This experiments has two weaknesses: a) the reverse experiment should be also performed (IP with an anti-LC3 antibody), and b) it seems that FUNDC1 interacts with LC3-I and not with LC3-II so how do the authors explain this as a mechanism of mitophagy acceleration?

Response 6: Following the suggestion from the reviewer, we treated the mice with chloroquine when mice were exposed to cold and found that the levels of LC3-II in this group were significantly higher than chloroquine alone treated group, indicating that the autophagic flux was promoted in BAT by cold exposure (Figure EV2G and H). We also performed reverse IP between LC3 and FUNDC1 and found that more FUNDC1 were immunoprecipited by LC3 upon cold exposure (Figure 4G). We also have optimized our experimental condition and redone the immunoprecipitation by FUNDC1 antibody. Our new data showed that more LC3-II was also immunoprecipited by FUNDC1 antibody upon cold exposure (Figure 4F).

6. The authors used two different mouse models to analyse the impact of FUNDC1 ablation in BAT but only characterize one of them. Please, provide documentation of appropriate depletion of FUNDC1 in adiponectin-Cre mice.

Response 7: We have checked the expression of Fundc1 in BAT and WAT and other tissues from adiponectin cre- and cre+ mice and provide the data in Figure EV3D.

7. Interestingly, BAT-specific FUNDC1 KO mice showed not only an impaired mitochondrial quality but also reduced mitochondrial biogenesis (based on the reduced mtDNA). This is perhaps the explanation by which PGC1a induces FUNDC1, i.e., to permit an efficient mitochondrial biogenesis upon cold exposure. In this connection, the authors should measure the expression of different mitochondrial proteins in BAT from loxP and KO mice.

Response 8: This is a good question. We also noticed that mitochondrial

biogenesis is also inhibited by *Fundc*1 KO and confirmed that by measuring the expression of mitochondrial proteins (Figure EV5C and D). Interestingly, the induction of PGC-1α was also blunted in *Fundc1* dedicient BAT (Figure EV5C and D), the underlying mechanism remains to be fully elucidated in our future study.

Minor comments.

1. The Introduction section is excessively long. Please shorten.

Response 9: We have shortened the introduction section from 1309 words to 1187 words.

Referee #2:

In the manuscript by Lui et al., the authors investigate the role of FUNDC1 on brown fat mitochondrial homeostasis during cold stress - a protein previously shown to be involved in mitophagy. The authors find that cold stress leads to a PGC1a-dependent upregulation of FUNDC1 and propose that PGC1a co-ordinately regulates both mitochondrial biogenesis and mitophagy. Loss of FUNDC1 in BAT, blocks mitophagy, impairs mitochondrial function and results in an impaired thermogenic response to cold. This is certainly an interesting paper and builds on work directly linking mitochondrial biogenesis and mitophagy, as well as adding a role for FUNDC1-dependent mitophagy in BAT. I do have some points though that will help address some concerns with the current data.

Response 10: We wish to thank the reviewer for his positive comments and appreciation of the significance of our work.

Main points:

1) The mitochondrial biogenesis data in Figure 1 would be strengthen by probing for other critical pathway proteins, such as TFAM. Additionally, as mitochondrial mass is increasing, then surely FUNDC1 (a mitochondrial protein) should also increase in-line with this. How then does this lead to mitophagy? I am not quite sure what the authors are proposing here with respect to PGC1a and FUNDC1. Are the authors saying that PGC1a induces more FUNDC1 compared to the other proteins that are induced (thus even though there are more mitochondria, each mitochondrion has more PGC1a than normal)? Or, are they

saying that PGC1a induces additional changes to FUNDC1 (eg PTMs)? Or do they not know? I think a little clarification as the results are being described would help. For the former, could the authors plot FUNDC1 (and other induced proteins) levels relative to mitochondria/mtDNA?

Response 11: Thanks for the reviewer's suggestion. We have detected the expression of TFAM in normal and cold exposed BAT and found that TFAM was also induced by cold exposure (Figure 1A). Following the reviewer's advice, we have plotted FUNDC1 and other mitochondrial protein levels relative to mtDNA and found that more FUNDC1 and other PGC-1α regulated proteins (CYCS, UCP1,TFAM) compared to other mitochondrial proteins (Tim23, HSP60 and VDAC1) were induced upon cold exposure (Figure 1E). Our data indicate that PGC-1α induces more FUNDC1 compared to the some other mitochondrial proteins upon cold exposure and we have clarified this in results section. (see page 7, line 25). In addition to FUNDC1 protein level, we also found the interaction of FUNDC1 and LC3 was enhanced upon cold exposure (Figure 4F and G). We argue that the induction of FUNDC1 by PGC-1α/NRF1 has set a new mitophagy threshold for increased mitophagy during cold stimulation.

2) In Figure 4, the authors show an increase in autophagy proteins upon cold exposure. Is this increase dependent on FUNDC1, i.e. could a loss of autophagy in-general be responsible for less mitophagy observed in Figure 5?

Response 12: Following the reviewer's suggestion, we have checked the expression of autophagy related proteins in control and *Fundc1* KO BAT in normal and cold conditions (Figure EV5A and B). Our new data showed that the increase in autophagy proteins upon cold exposure was unaffected by deficiency of *Fundc1,* which indicated that FUNDC1 was mainly responsible for mitophagy in BAT upon cold exposure.

3) In Figure 5, the low level of mtDNA upon loss of FUNDC1 during the cold is a little puzzling. If mtDNA is a readout of mitochondrial mass, then surely it should be increasing if mitophagy is blocked? Can the authors probe for another measure of mitochondrial

mass under these conditions, such as citrate synthase activity/mtDNA/western blot? How does total mtKeima signal change?

Response 13: This question was also raised by other reviewers and we have checked the expression of mitochondrial proteins and confirmed that mitochondrial biogenesis was also compromised by *Fundc*1 deficiency (Figure EV5C and D). We also found that the induction of PGC-1α was also blunted in *Fundc1* deficient BATs (Figure EV5C and D), however the underlying mechanism remains to be fully elucidated in our future study. We have quantified the total mtKeima signal (green signal) and found that the increase of mtKeima signals upon cold exposure was also suppressed in *Fundc1* deficient BAT (Figure 5F).

4) The bioenergetics data in Figure 5 needs to be better explained. (a) Figure 5G is unnecessary as the same information is in figure 5H with stats. (b) The authors mention in the methods that they perform a classical seahorse-type experiment, perhaps these measurements should be included - they observe no decrease in presence of oligomycin in their basal measurements. This suggests that most of the respiration recorded represents proton leak in all the groups, and that ATP-linked respiration is negligible. The mitochondria might be already in an uncoupled state in BAT due to the UCPs. The FCCP measurement would help explain this. (c) If there is still mitophagy going on upon loss of FUNDC1 in the Cold+Cre+ condition (Fig 5C-D suggests a small amount), then there might be less mitochondria as well. The authors should provide a measurement of mtDNA (mitochondrial mass) as this could explain the decreased OCR values compared to Cold+Cre-.

Response 14: Following the reviewer's suggestion, we have omitted Figure 5G and added FCCP to stimulate maximal OCR in OCR measurement (Figure 5H). Our new data showed that the OCR levels were not significantly elevated upon FCCP treatment, which indicates that the mitochondria was in an uncoupled state in BAT as the reviewer mentioned (Figure 5H). We also checked mtDNA and found that mtDNA is also decreased in *Fundc1* deficient BAT upon cold exposure (Figure 5E). Our data showed that both mitochondrial quality and mitochondrial biogenesis were impaired in *Fundc1* deficient BAT, which are responsible for the decreased

OCR values.

5) The statistical tests used in the article are not appropriate - the authors mention in the Material and Methods section that they used t-tests only. These should only be used with 2 groups. However, most of the graphs displayed have at least 3 groups. This will lead to an increased Type I error and consequently, some conclusions might be erroneous. One/two-way ANOVA/repeated measure ANOVAs are more appropriate.

Response 15: Thanks the reviewer for the suggestion and we have tested our data by using one-way ANOVA to determine whether there are any statistically significant differences between more than 2 groups.

Other points

6) In relation to figure 2A and 2B, the authors mention "however, the mRNA level of NRF1 was unchanged, which suggests that the stability of NRF1 protein is increased by cold exposure (Figure 2C)." This might be due to the timepoint chosen. Mitochondrial biogenesis protein mRNA expression is very dynamic, and it is possible that the increase of NRF1 mRNA simply occurred at an earlier timepoint and as there is no need to keep it "up" constantly after the adaptations, the mRNA levels might already be back to normal. This should be briefly mentioned as a possibility.

Response 16: We have amended the sentence to “however, the mRNA level of NRF1 was unchanged, which suggests that the stability of NRF1 protein is increased by cold exposure or the increase of NRF1 mRNA occurs at an earlier time point”, (see page 8, line 19).

7) In figure 2F and 2G, is it a double knockdown of PGC1b and PGC1a, as suggested in figure 2F, or is this a typo and it is only PGC1b?

Response 17: We have moved figure 2F and G to Figure EV2A and B and corrected the typo.

8) In relation to figure 3H, the authors mention "the induction of FUNDC1 by PGC1a was suppressed by knockdown of NRF1". This claim may not be so straightforward as Figure 3H shows an increased FUNDC1 with PGC1a NRF1 KD compared to NRF1 KD alone. This is further confirmed by the increase in another mitochondrial protein (CYCS). This

result suggests that NRF1 is not the sole interactor with the FUNDC1 promoter (possibly NRF2 and/or TFAM).

Response 18: We have corrected the sentence to "the induction of FUNDC1 by PGC1a was partially suppressed by knockdown of NRF1" (see page 10, line 21).

9) The Material and Methods section concerning Western Blotting mentions the use of a Myc antibody, but there is however no mention of it in the article.

Response 19: We are sorry for the mistake and we have omitted it.

Referee #3:

The current manuscript presents an investigation into the coordinated mitochondrial biogenesis and mitophagy in brown adipose tissue in response to thermogenic stress. The thermogenic stress in BAT prompts PGC-1/NRF-mediated mitochondrial biogenesis is an established mechanism. The role of mitophagy to maintain the quality of the expanded mitochondrial pool during thermogenesis is less well resolved. The current paper provides compelling evidence to indicate that PGC1/NRF upregulate the mitophagy adaptor FUNDC1 to promote mitophagy, thereby revealing a new level of coordination of mitochondrial homeostasis following thermogenesis. The paper is well-written and logical, The data presented is largely well-controlled, convincing and appropriately interpreted. There are some points that should be addressed although I am also mindful of potential limitations on lab work during the current COVID-19 restrictions.

Response 20: We wish to thank the reviewer for his positive comments and appreciation of the significance of our work

1. The authors indicate that they do not detect Parkin expression at the protein or mRNA level. However, Lu et al (Scientific Reports 2018) and Cairo et al (EmboJ 2019) have reported that Parkin is expressed in BAT at the protein and mRNA level. Can the authors explain this discordance? Additionally, Lu et al found that Parkin protein levels in BAT increase. The cold-stress induced mitophagy was PINK1 mediated suggesting that Parkin is the mitophagy effector in BAT following cold stress. This included Pink1-/- had

increased defective mitochondria (abnormal cristae) in mitophagosomes similar to the observation here in the absence of FUNDC1. Could there be redundant mechanisms in cold stressed BAT?

Response 21: We have re-checked the expression of Parkin by using the new antibody as mentioned in Cairo et al.'s paper (#2132, CST) and the new primers. We found that the protein and mRNA levels of Parkin were drastically reduced in BAT upon cold exposure (Figure 1A-C), which was consisted with Cairo et al.'s report (Cairo et al, 2019). We also have checked the expression of PINK1 and found the the protein level of PINK1 was increased although the mRNA of PINK1 was decreased upon cold exposure (Figure 1A-C), consistent with Cairo et al.'s report (Cairo et al, 2019). It is possible that FUNDC1-mediated mitophagy and Parkin-mediated mitophagy play distinct roles upon acclimation or deacclimation, to maintain mitochondrial homeostasis (see the details in discussion section, page 18, line 22). As the mitochondrial quality control is essential for the function of BAT, we are fully agreed that redundant mitophagy mechanisms should be existed in cold stressed BAT, which required further investigation.

2. Figure 3- EMSA assay, the whole gel should be shown to show free probe in each lane and also the mutated oligo probe in 3B should serve as a negative control for NRF binding. Fig 3D- Super shift should ideally be tested with the PGC1 Ab to confirm complex formation and also a negative control Ab.

Response 22: We have shown the free probes in each lane and we have cropped some blank part of the images due to the space limitation. We have redone the NRF1 EMSA experiment and added IgG control and Fundc1 mutant panels （Figure 3C and D）. We also have tried many times to detect the super shift by adding PGC1α antibody, unfortunately, we could not get the positive results. It has been well demonstrated that the formation of PGC1α-NRF1 complex(Wu *et al*, 1999)，we speculated that, as a transcriptional cofactor, PGC-1α does not bind DNA directly，the sensitivity of EMSA assay is not enough to detect the binding of PGC1α with *Fundc1* promoter *in vitro*.

3. The authors report that FUNDC1 deletion in BAT inhibited the cold-induced increase in

mtDNA copy number suggesting positive feedback between mitophagy and biogenesis. Although the mechanism is unresolved, the authors comment that PGC1 is reduced in cold-treated FUNDc1-deficient BAT. I agree that this is an interesting observation I recommend the authors include this data as it highlights the interplay between the two mitochondrial mechanisms.

Response 23: We have now included this data (Figure EV5C and D).

4. For the broader readership, I would recommend including schematic of the pathway model.

Response 24: We have include a scheme of the pathway model in Figure 6H.

Specific comments

1. Page 13: For clarity I would reword the 2nd sentence as follows: "We found that the OCRs of Fundc1-deficient BAT did not significantly increase upon cold exposure...

Response 25: Thanks the reviewer for the advice and we have amended it (see page 13, line 14).

2. Page 13, first paragraph, last sentence should be amended to clarify " ...FUNDC1-dependent mitophagy is essential for mitochondrial turnover and quality control in response to cold stress>"

Response 26: Thanks the reviewer for the advice and we have amended it (see page 13, line 24).

3. 3C- Typo "Positve"

Response 27: Thanks the reviewer for the advice and we have corrected it.

4. In 4F- Although there is an increase in the input fractions in the cold treated sample, the IP of FUNDC1 is the same irrespective of treatment?

Response 28: In order to detect the endogenous interaction of FUNDC1 and LC3, we have used excessive BAT protein lysates and the same IP of FUNDC1 was due to the equal amount of FUNDC1 antibodies were used for FUNDC1 precipitation.

5. The "blank" UCP1 blots in S3 appear to be identical.

Response 29: Because UCP1 protein is specifically expressed in BAT, we can not detect any signals of UCP1 proteins in other tissues, which makes the “blank” UCP1 blots in S3 appear to be identical. The original images of UCP1 and tubulin

were listed below. [Figure for referees not shown.]

References

1 Cairo M, Campderros L, Gavalda-Navarro A, Cereijo R, Delgado-Angles A, Quesada-Lopez T, Giralt M, Villarroya J, Villarroya F (2019) Parkin controls brown adipose tissue plasticity in response to adaptive thermogenesis. *EMBO Rep* 20

2 Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM (2006) Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab* 3: 333-341

3 Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC *et al* (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115-124

1st Revision - Editorial Decision 19th Nov 2020

Dear Dr. Liu,

Thank you for submitting your revised manuscript. It has now been seen by all of the original referees.

As you can see, the referees find that the study is significantly improved during revision and recommend publication. Before I can accept the manuscript, I need you to address some minor points below:

• Please address the remaining minor issues of referee #2.
• Please provide 3-5 keywords for your study. These will be visible in the html version of the paper and on PubMed and will help increase the discoverability of your work.
• Please add 'Author Contributions' and 'Conflict of Interest' sections.
• We note the phrase 'data not shown' on the page 14, which is not allowed as per journal policy.
• We note that funding information is currently missing. Please make sure that the funding information is complete in both the manuscript and the manuscript submission system.
• Papers published in EMBO Reports include a 'synopsis' and 'bullet points' to further enhance discoverability. Both are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst summarizing the study in 1 or 2 sentences that summarize the paper and are provided by the authors and streamlined by the handling editor. I would therefore ask you to include your synopsis blurb and 3-5 bullet points listing the key experimental findings.
• In addition, please provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.
• Our production/data editors have asked you to clarify several points in the figure legends (see attached document). Please incorporate these changes in the attached word document and return it with track changes activated.

Thank you again for giving us to consider your manuscript for EMBO Reports, I look forward to your minor revision.

Kind regards,

Deniz Senyilmaz Tiebe

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Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Referee #1:

The authors have adequately answered my prior questions, and I think it is now suitbale for its publication.

Referee #2:

This is a re-review of the revised manuscript by Liu et al. The authors have done a very good job in addressing the concerns raised. I just have a point about statistics.
The authors should confirm that they are using the right tests:
- Figure 5 B, D, E, F, G, H, and I should be analysed with a two-way Anova as there are two factors (Genotype and temperature)
- Figure 6 A and B should be analysed with a repeated measure Anova
- Figure 6C, E , and F should be analysed with a two-way Anova as there are two factors (Genotype and temperature)
- Figure EV3H should be analysed with a two-way Anova as there are two factors (Temperature and +/-chloroquine)
There also seem to be a mistake in the legend of figure EV3H as cold+CQ is listed twice.
- Figure EV5B and EV5D should be analysed with a two-way Anova as there are two factors (Genotype and temperature).

Referee #3:

The authors have addressed my concerns adequately.

EMBO Report

November 25th, 2020

Dear Tiebe

Thank you for sending our revised manuscript to original referees and we are very pleased that our manuscript has been significantly improved with the help of the reviewers and editors. The minor concerns of the reviewer and editors are addressed point by point as listed below.

Yours sincerely,

Lei Liu, Ph. D

Please address the remaining minor issues of referee #2.

We have re-analyzed our data following the referee#2's advice.

• Please provide 3-5 keywords for your study. These will be visible in the html version of the paper and on PubMed and will help increase the discoverability of your work.

We have provided keywords in manuscripts.

• Please add 'Author Contributions' and 'Conflict of Interest' sections.

We have added these two sections in manuscripts.

• We note the phrase 'data not shown' on the page 14, which is not allowed as per journal policy.

We have provided the data in figure EV5E.

• We note that funding information is currently missing. Please make sure that the funding information is complete in both the manuscript and the manuscript submission system.

We have provided the funding information in acknowledge section in manuscripts.

• Papers published in EMBO Reports include a 'synopsis' and 'bullet points' to further enhance discoverability. Both are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short standfirst summarizing the study in 1 or 2 sentences that summarize the paper and are provided by the authors and

streamlined by the handling editor. I would therefore ask you to include your synopsis blurb and 3-5 bullet points listing the key experimental findings.

We have provided synopsis in manuscripts.

• In addition, please provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.

We have provided an image for synopsis.

• Our production/data editors have asked you to clarify several points in the figure legends (see attached document). Please incorporate these changes in the attached word document and return it with track changes activated.

We have incorporated these changes in this submission.

2nd Revision - Editorial Decision 15th Dec 2020

Dear Dr. Liu,

Thank you for submitting your revised manuscript. I have now looked at everything and all is fine. Therefore I am very pleased to accept your manuscript for publication in EMBO Reports.

Congratulations on a nice study!

While performing the final checks on the manuscript, I have noticed that the synopsis image size is currently 550 pixels (width) and 182 pixels (height). As per our format requirements the height of the synopsis image must be minimum 300 pixels. You can send the synopsis image to me per email, after that we can transfer your manuscript to our production team.

Kind regards,

Deniz Senyilmaz Tiebe

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Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports
--
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Corresponding Author Name: Quan Chen
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2020-50629

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size calculations were based on literatures
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No statistical methods were used to predetermine sample size. For animal experiments at least 3 animals were used per group.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluede from the analyisis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	Mice were randomly divided into the distinct groups.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	Data collection and analysis were not performed blind.
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Statistical analysis was performed using Student's t test or one-way ANOVA. P<0.05 was considered statistically significant. *P<0.05, **P<0.01, and ***P<0.001 versus the corresponding controls are indicated. All statistical calculations were performed with GraphPad Prism software.
Is there an estimate of variation within each group of data?	We have presented ±SEM for each group of data as described in the figure legends.
Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

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http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jij.biochem.sun.ac.za
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6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Anti-Tim23 monoclonal antibody (BD Biosciences; cat. number 611223; 1:2,000 dilution),WB. Anti-PINK1 monoclonal antibody (Abgent, cat. number #AW5456-U100; 1:1000 dilution),WB Anti-Cytochrome c monoclonal antibody (BD Biosciences; cat. number 556433; 1:2000 dilution); WB. Anti-LC3B polyclonal antibody (MBL; cat. number PM036; 1:1000 dilution); WB. Anti-Actin monoclonal antibody(Sigma; cat. number, A5541; 1:10000 dilution); WB. Anti-UCP1 polyclonal antibody (Sigma; cat. number U6382; 1:2000 dilution);WB. Anti-Flag monoclonal antibody (Sigma; cat. number F3165; 1:1000 dilution); WB. Anti-VDAC1 monoclonal antibody (Abcam; cat. number ab14734; 1:3000 dilution); WB. Anti-NRF1 polyclonal antibody (Santa Cruz; cat. number sc33771;1:500 dilution); WB. Anti-PGC1α monoclonal antibody (Merck; cat. number ST1202;1:1000 dilution);WB. Anti-PGC1α Polyoclonal antibody (Merck; ST1204;1:1000 dilution);WB. Anti-PGC1β polyclonal antibody (Proteintech; cat. number 22378-1-AP;1:500 dilution); WB. Anti-PARKIN polyclonal antibody (Cell Signaling Technology; cat. number #2132; 1:1000 dilution);WB. Anti-TFAM polyclonal antibody (Cell Signaling Technology; cat. number #8076; 1:1000 dilution);WB. Anti-ATG5 polyclonal antibody (Proteintech; cat. number 10181-2-AP; 1:1000 dilution); WB. Anti-ULK1 polyclonal antibody (Proteintech; cat. number 20986-1-AP; 1:200 dilution); WB. Anti-HSP60 polyclonal antibody (Cell Signaling Technology; cat. number #12165; 1:2000 dilution);WB.
	Anti-Cytochrome c monoclonal antibody (BD Biosciences; cat. number 556432, 1:200 dilution), IHC Anti-LC3B polyclonal antibody (MBL; cat. number PM036; 1:200 dilution); IHC. Anti-UCP1 polyclonal antibody (Sigma; cat. number U6382; 1:200 dilution);IHC. Anti-FUNDC1 polyclonal antibody (anti serum was generated by immunizing rabbits with recombinant delta TM FUNDC1 protein; 1:500 dilution); IHC.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The following strains of mice were used: C57BL/6J mice ; Fundc1 fl/fl /mice; Fundc1 fl/fl /Ucp1 cre+ mice; Fundc1 fl/fl /Adiponectin cre+ mice; Fundc1 fl/fl /Adiponectin cre-/mito-Keima+ mice; Fundc1 fl/fl /Adiponectin cre+/mito-Keima+ mice. 8-12-week-old male mice were used in the experiments.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal experiments were performed according to procedures approved by the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No