EMBO reports

Succinate induces skeletal muscle fiber remodeling via SUNCR1 Signaling Pathway

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Review timeline:

Submission date:	7 February 2019
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Revision received:	10 May 2019
Editorial Decision:	7 June 2019
Revision received:	13 June 2019
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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 15 March 2019

Thank you for submitting your manuscript for consideration by EMBO Reports. It has now been seen by two referees whose comments are shown below.

As you can see, both referees express interest in your study demonstrating that succinate activates muscle remodeling. However, they also raise concerns that need to be addressed in full before we can consider publication of the manuscript here.

Given these constructive comments, I would like to invite you to revise your manuscript with the understanding that the referee must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Of note, please address the point 4 of referee #1 by measuring mitochondrial membrane potential with another method such as TMRE staining rather than omitting the data. 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.

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.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
http://emboj.embopress.org/about#Transparent_Process

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 also include scale bars in all microscopy images.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
(In order to avoid delays later in the publication process please check our figure guidelines before preparing the figures for your manuscript:
http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf)
- a separate PDF file of any Supplementary information (in its final format)
- all corresponding authors are required to provide an ORCID ID for their name. 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).

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

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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."

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.

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REFEREE REPORTS

Referee #1:

Overview

This is an interesting paper that provides strong evidence for the role of circulating succinate in activating muscle fiber remodelling through the SUCNR1 receptor. In general the work is of high quality and the data support the interpretation. Overall, I think this is a solid paper that adds considerably to an emerging field. However, I do have some comments and technical points that I feel will require more experiments.

Major points

1 I think that since GPR91 was de-orphaned that it is now caller SUNCR1.

2 I don't believe the data in Fig 1A - assuming SUA means succinic acid? The control concentration of succinic acid is about 85 mg/l. As the Mw of succinic acid is 118, this is a concentration of 720 µM!! The normal serum concentration of succinate is about 1 - 2 µM (eg see Journal of the American Heart Association (2018) 7 e007546). The use of the Sigma kit for plasma and tissue is not acceptable and this needs to be redone properly by LC-MS.

3 The food is labelled as 0.5% or 1% succinate but assuming this is a salt then the counterion isn't defined.

4 The use of JC1 to assess changes in mitochondrial membrane potential is not a good method as it is artifact prone and not quantitative . I suggest repeating with a more reliable method (eg TMRM) or omitting the data.

5 The analysis of mtDNA does not seem to have been normalised to a nuclear gene? Without that the data are hard to interpret and should be redone.

6 A major source of succinate seems to be ischemic tissue which accumulates succinate (see Nature 2014 515 431-435) and releases it into the circulation Journal of the American Heart Association (2018) 7 e007546: Cell Reports 2018 23 2617). This should be discussed and its possible link to SUNCR1 activation.

7 In the recent Nature paper in 2018 it was shown that succinate activated BAT and led to weight loss. The reasons why you don't see weight loss here should be discussed.

Minor points

1 SUA (succinate acid?) wasn't defined in the abbreviations.

2 The paper was well written and logically constructed, but in a few places there were typos and nonidiomatic phrases - a quick edit by a native speaker would be helpful.

Referee #2:

In the manuscript Titled "Succinate Induces skeletal muscle fiber remodeling via GPR91 Signaling Pathway" the authors analyzed the effect of succinate increases in endurance exercise ability, slow vs. fast -twitch fiber types markers, aerobic enzyme activity, oxygen consumption and mitochondrial biogenesis in skeletal muscle.
In addition, the authors show evidence for a GPR91 role in mediating succinate effect on skeletal muscle fiber type remodeling suggesting a potential use of succinate-based compounds in both athletic and sedentary populations.

Overall, the work is might provide valuable information regarding the role of succinate in fast/slow

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muscle fiber twitch and their metabolism.

However, in order to be suitable for publication, the manuscript deserves further experiments.
Major points:
1. In 2017 the same group published the following paper: Succinate promotes skeletal muscle protein synthesis via Erk1/2 signaling pathway from the same group Yexian Yuan, et al. published in Mol Med Rep. 2017 Nov; 16(5): 7361-7366. In this paper the authors provide evidence that succinate stimulates protein synthesis along with the increase of the Akt/mTOR/FoxO pathway. How the authors explain the discrepancy between the observed succinate-mediated protein synthesis (Yexian Yuan, et al.) and the data presented in the present paper regarding the lack of increased muscles weight?
In the present work, the authors should include a biochemical analysis of the Akt/mTOR/FoxO pathway on WT not treated and in vivo fed with succinate-supplemented chow and in GPR91 KO mice.

2. Since the Gastrocnemius is a mixed muscle, the authors should include evidences in EDL (fast/fast) and soleus (slow/slow) muscles.

3. To assess the distribution of the slow vs. fast fibers, the authors should perform a metachromatic staining (NADH or SDH staining).

1st Revision - authors' response 10 May 2019

Referee #1:

Overview
This is an interesting paper that provides strong evidence for the role of circulating succinate in activating muscle fiber remodelling through the SUCNR1 receptor. In general the work is of high quality and the data support the interpretation. Overall, I think this is a solid paper that adds considerably to an emerging field. However, I do have some comments and technical points that I feel will require more experiments.

Major points

1. I think that since GPR91 was de-orphaned that it is now caller SUNCR1.

This point is well taken. GPR91 has been replaced by SUNCR1 in this manuscript revision.

2 I don't believe the data in Fig 1A - assuming SUA means succinic acid? The control concentration of succinic acid is about 85 mg/l. As the Mw of succinic acid is 118, this is a concentration of 720 µM!! The normal serum concentration of succinate is about 1-2 µM (eg see Journal of the American Heart Association (2018) 7 e007546). The use of the Sigma kit for plasma and tissue is not acceptable and this needs to be redone properly by LC-MS.

We highly appreciate this point. The mouse serum succinic acid concentration was re-measured by LC-MS and Fig 1A was replaced by new results. The control serum succinic acid level is around 0.4 ng/µL (mg/L), which is equal to 3.4 µM and comparable to normal serum succinate in human patients (1-2 µM, Journal of the American Heart Association (2018) 7 e007546).

3 The food is labelled as 0.5% or 1% succinate but assuming this is a salt then the counterion isn't defined.

We appreciate the point. Succinic acid sodium salt was used for dietary supplementation. The following description has been added in line 589-590:

"Three groups of mice were fed with normal standard diets containing 0, 0.5% or 1% Succinic acid sodium salt, respectively".

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4 The use of JC1 to assess changes in mitochondrial membrane potential is not a good method as it is artifact prone and not quantitative. I suggest repeating with a more reliable method (eg TMRM) or omitting the data.

This is an excellent point. The mitochondrial membrane potential was re-tested by TMRM and supplementary Fig EV2B-C was replaced by new results.

5 The analysis of mtDNA does not seem to have been normalized to a nuclear gene? Without that the data are hard to interpret and should be redone.

Sorry for any confusion it may have caused. For mtDNA assay, we used β-globin as a nuclear reference gene for calibration. The detailed method description has been updated in the present version as following:

"Total cellular DNA was extracted from C2C12 cells with DNAzol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Mitochondrial DNA copy number was determined by quantification of four mitochondrial marker genes, including mitochondrially encoded ATP synthase membrane subunit 6 (ATPase6), cytochrome c oxidase subunit 2 (COX2), Mit-1000 and mitochondrial encoded cytochrome b (mt-Cytb). The expression level of ATPase6, COX2, Mit-1000 and mt-Cytb was tested by quantitative real-time PCR and normalized to an intro of the nuclear-encoded β-globin gene as descripted before [67,68]. The primer sequences can be found in the Supplementary Table 1".

6 A major source of succinate seems to be ischemic tissue which accumulates succinate (see Nature 2014 515 431-435) and releases it into the circulation Journal of the American Heart Association (2018) 7 e007546: Cell Reports 2018 23 2617). This should be discussed and its possible link to SUNCR1 activation.

This is an excellent point. The following discussion has been added.

"Regular exercise and chronic hypoxia are natural stimuli that produce sustainable carioprotection against ischemia-reperfusion [53]. Consistent with the important role of succinate in muscle metabolism and fiber re-modeling we showed, succinate is elevated in the blood in response to exercise [32] and accumulated rapidly in hypoxic/ischemic tissues [33, 54, 55], suggesting a potential role of succinate in exercise/hypoxia-mediated carioprotection. Succinate may acts as a paracrine or endocrine signaling molecules via SUCNR1 to regulate local cellular metabolism [56], or increase tissue blood supply through the renin-angiotensin system, thereby alleviating tissue hypoxia and hypoxia adaptation of metabolism in the environment [57-59]. Consistently, augmentation of succinate has been shown to improve cardiac ischemic energetics, a source of damage at reperfusion [60]. Therefore, succinic acid may not only play an important role in autocrine regulation of skeletal muscle metabolism and fiber type conversion, but also improve the adaptability of cardiovascular and brain tissues to the ischemic environment."

7 In the recent Nature paper in 2018 it was shown that succinate activated BAT and led to weight loss. The reasons why you don't see weight loss here should be discussed.

This is an excellent point. We appreciate the suggestion and agree the necessity to discuss the discrepancy between the recent Nature paper (PMID: 30022159) and our results. The following discussion has been added.

"Our results demonstrated that dietary succinate supplementation led to remodeling of muscle fiber without changing body weight or fat distribution, suggesting that the primary function of succinate is to regulate muscle type transition but not body weight. However, our study was carried out under normal chow diet (low fat diet), which may have concealed a phenotype relevant for human obesity normally induced by high-energy/fat diet. Indeed, a recent study has shown that water supplementation of 1.5% but not 1% succinate stimulates uncoupling protein 1 (UCP1)-dependent thermogenesis from BAT, which induces robust protection against HFD-induced obesity [22]. This

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discrepancy suggests a diet-dependent anti-obesity effect of succinate, which may be attribute to different baseline UCP1 activation in chow and HFD condition. It has been shown that HFD significantly inhibits the expression and metabolic activity of UCP-1 in BAT [61]. The inconsistency may also be due to different supplementary method and dose (1.5% in water vs 1% diet). The effective dose of succinate to remodel skeletal muscle fiber type may be lower than that to reduce body weight and fat mass."

Minor points
1 SUA (succinate acid?) wasn't defined in the abbreviations.

SUA has been defined in FOOTNOTES.

2 The paper was well written and logically constructed, but in a few places there were typos and nonidiomatic phrases - a quick edit by a native speaker would be helpful.

Proofreading has been done by a native speaker.

Referee #2:

In the manuscript Titled "Succinate Induces skeletal muscle fiber remodeling via GPR91 Signaling Pathway" the authors analyzed the effect of succinate increases in endurance exercise ability, slow vs. fast -twitch fiber types markers, aerobic enzyme activity, oxygen consumption and mitochondrial biogenesis in skeletal muscle. In addition, the authors show evidence for a GPR91 role in mediating succinate effect on skeletal muscle fiber type remodeling suggesting a potential use of succinate-based compounds in both athletic and sedentary populations. Overall, the work is might provide valuable information regarding the role of succinate in fast/slow muscle fiber twitch and their metabolism. However, in order to be suitable for publication, the manuscript deserves further experiments.

Major points:

1. In 2017 the same group published the following paper: Succinate promotes skeletal muscle protein synthesis via Erk1/2 signaling pathway from the same group Yexian Yuan, et al. published in Mol Med Rep. 2017 Nov; 16(5): 7361-7366. In this paper the authors provide evidence that succinate stimulates protein synthesis along with the increase of the Akt/mTOR/FoxO pathway. How the authors explain the discrepancy between the observed succinate-mediated protein synthesis (Yexian Yuan, et al.) and the data presented in the present paper regarding the lack of increased muscles weight? In the present work, the authors should include a biochemical analysis of the Akt/mTOR/FoxO pathway on WT not treated and in vivo fed with succinate-supplemented chow and in GPR91 KO mice.

This is an excellent point. A biochemical analysis of AKT/mTOR/FOXo3a signaling in the gastrocnemius has been added in both WT and GPR91 KO in the current revision. We found a similar stimulatory effect of succinate on AKT/mTOR/FOXo3a pathway in WT (Figs. EV1C-D), which is blocked in GPR91 KO mice (Figs. EV4H-I), suggesting a GPR91-mediated activation on protein synthesis. We speculate that the discrepancy between increased protein synthesis and unchanged muscle mass may be due to succinate-induced muscle type remodeling. The following discussion has been added in the manuscript revision.

"Consistent with our previous report on the stimulatory effects of succinate on protein synthesis in skeletal muscle [53], we found dietary supplementation of succinate activated Akt/mTOR cascade and inhibited FoxO3a in WT mice. These regulatory effects of succinate were diminished in SUNCR1 KO mice, suggesting a SUNCR1-mediated activation on protein synthesis. In this context, a seemingly paradoxical finding is that dietary supplementation of succinate failed to increase muscle mass. How can succinate increases skeletal muscle protein synthesis without changing muscle weight? We speculate that this inconsistency may be due to succinate-induced muscle type remodeling from fast-twitch fibers to slow-twitch fibers. It is known that slow-twitch fibers have lower fiber size and higher oxidative proteins and capacity for protein synthesis compared to fast-

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twitch fibers [54]. Succinate-induced hypertrophy of skeletal muscle may be neutralized by the discrepancy in fiber size of slow- and fast-twitch or mass of large myofibrillar proteins and much smaller oxidative proteins. Alternatively, it is also possible that the protein synthesis is balanced by a high rate of protein degradation resulting in a higher turnover rate in the high oxidative fibers."

2. Since the Gastrocnemius is a mixed muscle, the authors should include evidences in EDL (fast/fast) and soleus (slow/slow) muscles.

This is an excellent point. We examined MyHC I and IIb protein expression in both soleus (SOL) and extensor digitorum longus (EDL) muscle by immunofluorescence after dietary supplementation of 0.5% or 1% succinate in WT mice (Figs. EV1K-N). We found that succinate dose-dependently increased MyHC I but not MyHC IIb protein expression in SOL, suggesting an increased proportion of slow-twitch fiber. On the other hand, succinate failed to affect the muscle fiber composition of EDL muscles.

3. To assess the distribution of the slow vs. fast fibers, the authors should perform a metachromatic staining (NADH or SDH staining).

We appreciate this constructive suggestion. Oxidative capacity of SOL, EDL and gastrocnemius muscles has been analyzed by succinate dehydrogenase (SDH) staining after dietary supplementation of 0.5% or 1% succinate in WT mice (Figs. EV1E-J). We found that succinate dose-dependently increased the percentage of SDH-positive fibers in SOL, EDL and gastrocnemius muscles, suggesting succinate is sufficient to improve mitochondrial content and oxidative capacity of mixed (gastrocnemius), slow/slow (SOL) or fast/fast (EDL) muscles.

2nd Editorial Decision 7 June 2019

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

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

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

REFEREE REPORTS

Referee #1:

The authors have addressed all the points I raised to my satisfaction.

Referee #2:

In the revised manuscript titled "Succinate induces skeletal muscle fiber remodeling via SUNCR1 Signaling Pathway" the authors address all the suggested comments made by the reviewers, making the work suitable for publication.
Minor points:
- This reviewer suggests re-ordering the figures in progressive order based on the mention in the text (i.e. Fig2D become Fig2A and Fig 2A become Fig 2D; FigEV1K-L and EV1M-N should be numbered before Figs. EV1E-J; Fig 5C is mentioned after fig 5F; Fig EV3B is mentioned after Fig. EV3C-E and so on.....). This will facilitate the reading.
- In Fig. EV1H the graph relative to the % of SDH positive fibers in Soleus do not correspond.

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2nd Revision - authors' response 13 June 2019

Referee #2:

In Fig. EV1H the graph relative to the % of SDH positive fibers in Soleus do not correspond.
The graph relative to the % of SDH positive fibers in Soleus has been reanalyzed. Only darkly stained SDH fibers are treated as SDH positive fibers.

3rd Editorial Decision 26 June 2019

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 the very nice work!

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name:Gang Shu
Journal Submitted to: EMBOR
Manuscript Number:EMBOR-2019-47892

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?	The number of my experimental samples is statistically significant. The number of samples in animal experiments is greater than 6, the number of samples tested by the enzyme-linked immunosorbent assay kit is greater than 6, and the number of WB protein samples is greater than 3.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Our animal experiment is divided into three parts, the succinate test for C57BL/6J drinking water, the GPR91KO and the gastrocnemius specific knockout GPR91 experiment. The repeat number of the animal model is greater than 6, which is statistically significant.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Yes, we have checked if the value is a variation value by Dixon's Q test(Dean R B , Dixon W J . Simplified Statistics for Small Numbers of Observations[J]. Analytical Chemistry, 1951, 23(4):636-638).
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.	We will ensure the randomness of sample detection during the experiment, we will ensure the randomness of position, light and so on in cell experiments.
For animal studies, include a statement about randomization even if no randomization was used.	Operation step (1) No.: N test units are numbered from 1 to N. Animals are numbered by weight (2) to obtain random numbers: starting from any number in the random number table, sequentially obtain a random number of experimental units in the same direction. (3) Find the remainder of the random number divided by the number of groups to find the remainder. If divisible, the number of groups is divided into groups (4), and the packets are grouped by remainder. (5) If there are a total of n cases to be adjusted, one case needs to be extracted from it, then a random number is copied, and the remainder obtained by dividing n is used as the The serial number of the experimental unit (if the division is divisible, the remainder is n).
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.	In the process of our cell and animal experiments, we will try our best to ensure that many people assist in the operation and prevent experimental errors caused by subjective consciousness.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Our animal experiments ensure that multiple people operate simultaneously in the experimental grouping and data collection process to avoid experimental errors caused by subjective consciousness.
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.	We use Spss software for reasonable data analysis.
Is there an estimate of variation within each group of data?	Yes, we will estimate the variance value.
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
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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-tun

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://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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-β-Tubulin (bs-1482M,1:5000, Bioss), rabbit anti-GPR91 (NBP1-00861,1:1000, Novus), mouse anti-MyHC I (ab11083,1:1000, ABCAM), rabbit anti-MyHC IIa (ab124937,1:1000, ABCAM), goat anti-MyHC IIb (sc-168672,1:500, Santa Cruz), mouse anti-PGC-1α (ST1202,1:1000, Millipore), rabbit anti-Histone (4499S,1:2000, CST), mouse anti-NFAT (sc-7294,1:500, Santa Cruz), rabbit anti-NRF-1 (#12381s,1:2000, CST), rabbit anti-calcineurin (#2614s,1:2000, CST), rabbit anti-Myoglobin (ab77232,1:1000, ABCAM), rabbit anti-MEF2A (#97365,1:2000, CST).mouse anti-MyHC I(BA-D5-S 1:100, DSHB), mouse anti-MyHC IIb (BF-F3 1:100, DSHB), rabbit anti-Laminin (PA1-16730 1:1000, Thermo Fisher).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The cell originates from the Cell Center , Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Numbering:3111C0001CCC000099.We are authorized and they are doing relevant tests in the near future.

* 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.	Animal experiments—C57BL/6J about 3 weeks old mice were purchased from the Medical Experimental Animal Center of Guangdong Province (Guangzhou, Guangdong, China) ' Sucnr1 knockout mouse model used in this study was designed and developed by Shanghai Model Organisms Center, Inc (Shanghai, China). Four independent sgRNAs designed to delete exon 2 of Sucnr1 were 5'- TGGACCTTCAATACGAGGGC -3', 5'- GGCATGGACCTTCAATACGA -3', 5'-CGCCCATGATTAAATTCCAC -3', and 5'- GATCCTGTGGAATTTAATCA-3'. The sgRNAs were in vitro transcribed using the MEGAshortscript Kit (ThermoFisher, USA). In vitro-transcribed Cas9 mRNA and sgRNAs were injected into zygotes of C57BL/6J mouse, and transferred to pseudopregnant recipients. Obtained F0 mice were screened by PCR and sequencing using primer pairs: F1-5'-GGCTGGCCATGAAGATACA-3'; R1-5'- TTGGAATGCCGAGAACTGAG-3'. The positive F0 mice were chosen and crossed with C57BL/6J mice to obtain F1 heterozygous Sucnr1 knockout mice. The genotype of F1 mice was identified by PCR and confirmed by sequencing. Male and female F1 heterozygous mice were intercrossed to produce the homozygous Sucnr1 knockout mice..All mice were housed in an individual cage under the controlled room temperature (23 °C ± 3 °C) and relative humidity (70 ± 10%) conditions with a 12h-12h light-dark cycle. C57BL/6J mice were left to acclimate 1 week, then randomly divided into three groups (n=11) based on their body weight.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal raised and experiments were permitted by the College of Animal Science, South China Agricultural University, and in line with "the instructive notions with respect to caring for laboratory animals" issued by the Ministry of Science and Technology of the People's Republic of China.
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.	Yes.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

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	NA
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).	NA
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).	NA
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.	NA

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.	NA