EMBO reports

Scaf1 promotes respiratory supercomplexes and metabolic efficiency in zebrafish

Carolina García-Poyatos, Sara Cogliati, Enrique Calvo, Pablo Hernansanz-Agustín, Sylviane Lagarrigue, Ricardo Magni, Marius Botos, Xavier Langa, Francesca Amati, Jesús Vázquez, Jose Enriquez, and Nadia Mercader
DOI: 10.15252/embr.202050287

Corresponding author(s): Nadia Mercader (nadia.mercader@ana.unibe.ch) , Jose Enriquez (jaenriquez@cnic.es)

Review Timeline:

Submission Date:	24th Feb 20
Editorial Decision:	17th Mar 20
Revision Received:	13th Apr 20
Accepted:	28th Apr 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.)

Dear Nadia,

Thank you for submitting your manuscript to EMBO Reports. Your manuscript has been reviewed at another journal. I have heard back from our arbitrating advisor, who looked at the revised manuscript and your response to the original referee concerns, whose comments are below. Having looked at everything carefully, I would like to invite a minor revision, before I can accept the manuscript.

• Please address the minor concerns of the arbitrating advisor.
• 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 fill out and include an author checklist as listed in out online guidelines (https://www.embopress.org/page/journal/14693178/authorguide)
• The reference format currently does not follow EMBO Reports guidelines. Please see https://www.embopress.org/page/journal/14693178/authorguide#referencesformat for further information.
• We noted that Fig 6L is called out, but the figure has 2 panel E's so only goes to K.
• There is a callout to Table 2, but there is no such table.
• We noted the following regarding Dataset EV Legends: The Tables have an incorrect nonenclature. Table S2 is small and could be included in an Appendix file, other it should be Table EV2. The others should be Dataset EV# files.
• We noticed that there are currently 7 supplemental figure files. Some could be (for technical reasons max 5) Expanded View figures, the rest should make up an Appendix with a table of contents.
• We realized that Figure S4 is in landscape format, which should be converted to portrait.
• Data and Code Availability section - RNAseq data (GSE133487) need to be made publicly available.
• Data and Code Availability section - please provide an URL for zebrafish line information at ZFIN.
• Fig 7D-F is missing labelling in the figure itself.
• Methods section needs to be renamed as Materials and Methods.
• Papers published in EMBO Reports include a 'Synopsis' to further enhance discoverability. Synopses 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 are provided by the authors and streamlined by the handling editor. I would therefore ask you to include your synopsis blurb - as well as 2-5 one-sentence bullet points that summarize the key findings of the paper.
• 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.

Referee #1:

This report investigates the role of SCAf1 in the assembly of respiratory supercomplexes in Zebrafish. Scaf1 is known to coassemble with respiratory complexes and that scaf1 mutant mice display altered distribution of complexes into supercomplexes. However, these mice do not show overt physiological phenotype, raising the question of the functional importance of these supercomplexes.

Here, the authors generate scaf1-null zebrafishes and observe that, in addition to a supercomplex assembly defect, they also have a metabolic phenotype, being smaller and accumulating fat, altogether indicating a respiratory deficiency. Interestingly, most phenotypes can be suppressed by doubling food doses. This prompted them to reevaluate the scaf1 mutant and knock-out mice in condition where food is limiting, and here again they evidence a physiological phenotype. Altogether they find that scaf1 plays an important physiological role, most likely through promoting the assembly of supercomplexes.

The authors responded adequately to the reviewers concerns. Many of the points of reviewer 1 were not addressed, but their bearing on the story is unclear (for many experiments proposed, whatever outcome would not affect the main cnclusions of the paper). Reviewer 2 raises a tricky but important point. While the data hereing definitely indicate a physiological role for scaf1, they do not ensure that this effect is related to the supercomplex assembly deffect observed in the same mutants. The way to definitly test this causality inference would be to somehow rescue supercomplex assembly in the absence of Scaf1. Since there is no easy way to do it and since the causality, while unproven, is still very likely for reasons the authors explain clearly in the manuscript and their rebuttal, this should ne be a prerequisite for publication. A sentence in the discussion stating that while it is most likely that scaf1 affects physiology via the assembly of supercomplexes, one cannot, at this stage, exclude the unlikely possibility that it does so through other mechanisms.

Minor points
p8 lines 165-169. Could be rephrased. The appearance of greater number of band is not due to the discontinuation of the antibody, but to the use of a substitute. An d this has nothing to do with mammal vs zebrafish, as the first sentence implies.

Figure 7, there is a wildtype control for both delta1 and delta2 alleles. these are labelled delta1 scaf1+ and the mutants delta1 scaf- which is very misleading. Why not WT1 and WT2 versus delta1 and delta2?

Referee #1:

This report investigates the role of SCAf1 in the assembly of respiratory supercomplexes in Zebrafish. Scaf1 is known to coassemble with respiratory complexes and that scaf1 mutant mice display altered distribution of complexes into supercomplexes. However, these mice do not show overt physiological phenotype, raising the question of the functional importance of these supercomplexes.

Here, the authors generate scaf1-null zebrafishes and observe that, in addition to a supercomplex assembly defect, they also have a metabolic phenotype, being smaller and accumulating fat, altogether indicating a respiratory deficiency. Interestingly, most phenotypes can be suppressed by doubling food doses. This prompted them to reevaluate the scaf1 mutant and knock-out mice in condition where food is limiting, and here again they evidence a physiological phenotype. Altogether they find that scaf1 plays an important physiological role, most likely through promoting the assembly of supercomplexes.

The authors responded adequately to the reviewers concerns. Many of the points of reviewer 1 were not addressed, but their bearing on the story is unclear (for many experiments proposed, whatever outcome would not affect the main cnclusions of the paper). Reviewer 2 raises a tricky but important point. While the data hereing definitely indicate a physiological role for scaf1, they do not ensure that this effect is related to the supercomplex assembly deffect observed in the same mutants. The way to definitly test this causality inference would be to somehow rescue supercomplex assembly in the absence of Scaf1. Since there is no easy way to do it and since the causality, while unproven, is still very likely for reasons the authors explain clearly in the manuscript and their rebuttal, this should ne be a prerequisite for publication. A sentence in the discussion stating that while it is most likely that scaf1 affects physiology via the assembly of supercomplexes, one cannot, at this stage, exclude the unlikely possibility that it does so through other mechanisms.

We thank the reviewer very much for this evaluation. We have included a sentence in the discussion pointing out the unlikely possibility of SC assembly independent functions of SCAF1:

"However, we cannot exclude an unlikely possibility that the physiological consequences of Scaf1 loss of function are due to unknown functions independent on its role as a SC assembly factor."

Minor points
p8 lines 165-169. Could be rephrased. The appearance of greater number of band is not due to the discontinuation of the antibody, but to the use of a substitute. An d this has nothing to do with mammal vs zebrafish, as the first sentence implies.

We changed the sentence:" We found that this is due to the use of new Scaf1 antibody generated against the entire protein, as the previously well-established antibody generated against a Scaf1-specific peptide was discontinued.

Figure 7, there is a wildtype control for both delta1 and delta2 alleles. these are labelled delta1 scaf1+ and the mutants delta1 scaf- which is very misleading. Why not WT1 and WT2 versus delta1 and delta2?

We changed the labelling according to the request.

1st Revision - Editorial Decision 28th Apr 2020

Dear Nadia,

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

Congratulations on a nice study!

Before we can transfer your manuscript to our production team, we need to sort out a couple of minor points.

1. Our data editors have asked you to clarify a couple of points in the legend of the newly added data (please see attached). Please address those with 'track changes on' in the attached word file. Your can return the file to me per email.

2. I have taken the liberty of performing some minor changes in the items below to increase clarity/accessibility. Please take a look and confirm, or feel free to suggest further changes.

Synopsis: This study reveals a physiological function of mitochondrial respiratory supercomplex assembly in metabolism by analyzing scaf1 mutant zebrafish that are unable to form supercomplexes.

Bullet points:
- Comprehensive characterization of zebrafish respiratory supercomplexes reveals presence of supercomplexes similar to those of mice, as well as zebrafish specific ones.
- Null mutants for the super-assembly factor scaf1 lack CIII+CIV supercomplex
- scaf1 null mutant zebrafish exhibit impaired mitochondrial bioenergetics
- scaf1 null mutants are smaller, weigh less and display reduced fertility
- Phenotypic consequences of scaf1 mutation can be rescued through by doubling food supply.

Abstract:
The oxidative phosphorylation (OXPHOS) system is a dynamic system in which the respiratory complexes coexist with super-assembled quaternary structures called supercomplexes (SCs). The physiological role of SCs is still disputed. Here we use zebrafish to study the relevance of respiratory SCs. We combined immunodetection analysis and deep data-independent proteomics to characterize these structures and found similar SCs to those described in mice, as well as previously unidentified SCs including III2+IV2, I+IV and I+III2+IV2. To study the physiological role of SCs, we generated two null allele zebrafish lines for supercomplex assembly factor 1 (scaf1). scaf1-/- fish display altered OXPHOS activity due to the disrupted interaction of complex III and IV. scaf1-/- fish are smaller in size and show abnormal fat deposition and decreased female fertility. Doubling the food supply rescues these physiological phenotypes, which in parallel improves bioenergetics and alters the metabolic gene expression program without affecting SC assembly. These results reveal that SC assembly by Scaf1 modulates OXPHOS efficiency and ensures the optimization of metabolic resources.

Kind regards,

Deniz

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Nadia Mercader & Jose Antonio Enriquez
Journal Submitted to: EMBO REPORTS
Manuscript Number: EMBOR-2020-50287V1

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?	no specific program for prediction was used. The sample size was chosen empirically by comparing all animals from one clutch
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample size depends on the amount of females/males in each fish clutch.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals were excluded. Samples were excluded only in case of technical error during the processing of the sample. Data outlyers were identified using ROUT method and, when found, they were still represented in the figure, indicated and not taken into account for the statistic.
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.	No
For animal studies, include a statement about randomization even if no randomization was used.	Fish were randomly selected for all experiments. For diet experiments, fish were randomly selected and distributed in equal groups according to their length and weight.
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.	Image analysis was performed in a blind manner.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Experiments with risk of bias were performed in a blind manner. They are indicated in Material and Methods.
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.	no, a Fisher exact test was used to assess if distribution was normal or no to then apply either parametric or non-parametric tests.

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
http://www.consort-statement.org
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://jij.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	yes
Is the variance similar between the groups that are being statistically compared?	yes

C- Reagents

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).	done
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	does not apply

* 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.	done
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	done
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.	done

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	does not apply
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.	does not apply
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	does not apply
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	does not apply
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	does not apply
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.	does not apply
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.	does not apply

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	done
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).	done
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).	does not apply
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.	done

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