THE EMBO JOURNAL

The yeast mitochondrial pyruvate carrier is a hetero-dimer in its functional state

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Editor: Elisabetta Argenzio

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 7th November 2018

Thank you for submitting your manuscript on the characterization of mitochondrial pyruvate carrier in yeast to The EMBO Journal. Your study has been sent to three referees for evaluation, and we have now received reports from them, which are enclosed below for your information.

As you can see, the referees concur with us on the overall interest of your findings. However, they also raise some points that need to be addressed before they can support publication in The EMBO Journal. In particular, referees #1 and #2 request you to characterize pyruvate transport activity of the MPC1-MPC3 heterodimer. Also, referee #1 finds that differences between MCP protein oligomerization in yeast and mammals need to be discussed and points out that the specificity of the employed MPC inhibitor has to be assessed on the individual heterodimer subunits. Referee #3 asks you to test other MCP inhibitors by employing 14C-pyruvate exchange and thermostability assays.

Addressing these issues as suggested by the referees is required to warrant publication in The EMBO Journal. Given the overall interest of your study, I would like to invite you to revise the manuscript in response to the referee reports.

REFEREE REPORTS

Referee #1:

Tavoulari et al have provided data on the charaterisation of oligomers of the pyruvate carrier protein in the yeast S. cerevisiae. They have managed to purify hetero-oligomeric complexes form yeast and characterize them functionally in a reconstituted system. The question of the oligomeric state of the pyruvate carrier and how this is linked to its transport function is central to the understanding of the biological role of this protein. Since the discovery in 2012 of MPC proteins by two independent studies, several more studies appeared linkning the MPC to cell metabolism in major diseases

(cancer, diabetes etc). However, a thorough analysis of the oligomeric state is missing. In the absence of a detailed 3D structure, the determination of the active oligomer state will be important for future studies. The work presented is generally well executed. The authors argue that the functional entity for MPC in S cerevisae is a heterodimer between MPC1 and MPC3. The transport capacity of the heterodimer is sensitive to specific inhibitors, but the homodimer seems to be non-functional. The data are convincing, but there are a number of issues to be resolved. Also, the authors should tone down the statements that what they find in yeast is universally true for mammalian homologues.

1. Figure 2 shows that MPC1 and MPC3 form a hero-dimeric complex using size exclusion chromatography. It is not known what residues or surfaces may contribute to the formation of a heterodimer, and hetero-dimer is a prefered state as opposed to a homodimer when the partner subunit is co-epxressed. This should be addressed by some mutagenesis analysis.
This would be reinforced by mutagenesis in yeast to see (i) the effect of expressing one of the subunits in a mutated form incapable (or affected) in its hetero-dimer capacity and (ii) to see whether this translates also in affected transport of pyruvate in vivo.

2. It is an interesting result that the reconstituted heterodimer is sensitive to UK5099. However, the difference in the efficiency of inhibition between the mammalian and the yeast proteins reinforces the argument that the mammalian system may not necessarily oligomerize in the same manner as the one from yeast. The authors should discuss this possibility rather than dismissing the study of Nagampalli et al 2018 due to technical differences in the purification. To be fair, in the absence of an equivalent reconstituted system of the mammalian carriers one cannot draw safe conclusions about the properties of the mammalian carriers simply by analogy to the yeast system.

3. The use of the inhibitor Zaprinast is also interesting and adds to the functional analysis of the MPC. To see if the drug was specific for either the dimer interface or an individual subunit, a control using both monomeric proteins should be tested for thermal stability in the presence of the drug.

Minor point: In Figure 1B please indicate with arrows the MPC proteins and explain what the bands above the pure protein are.

Referee #2:

Mitochondrial pyruvate carrier (MPC) proteins facilitate the transport of pyruvate from the cytosol to the mitochondrial matrix. These are MPC1 and MPC2 in mammals and Mpc1, Mpc2 and Mpc3 in baker's yeast. The precise stoichiometry, oligomeric state and the functional unit of the MPC complexes have not been defined so far.

The work of Tavoulari et al. describes the successful co-expression, purification and characterization of the heterocomplexes Mpc1-Mpc2 and Mpc1-Mpc3 as well as the corresponding homodimers of Mpc1 and of Mpc3. The authors demonstrate that in principle MPC proteins from yeast are able to form both homo- and hetero-dimers. They also show that only the Mpc1-Mpc3 heterodimer (Mpc1-Mpc2 was not tested) is capable of transporting pyruvate and thus presents the functional unit of MPC.

Previous in vivo genetic study (e.g. Herzig et al., 2012) already indicated that MPCs function as hetero-multimeric complexes: Mpc1-Mpc2 under fermentative conditions and Mpc1-Mpc3 during respiration. Thus, the here presented in vitro biochemical approaches nicely confirm this hypothesis and extend our knowledge in the function of the MPC. However, I also think that this limits the novelty of the article in its present form. I however also think that these limitations could be overcome in a potential major revision.

Major comments:
1- Although the authors report on the successful Mpc1-Mpc2 heterodimer purification, they did not characterize its pyruvate transport activity. Especially in the light of the potential differential use of the Mpc1-Mpc2 and Mpc1-Mpc3 heterodimers during different metabolic conditions, an

experimental comparison of the transport properties of both complexes appears critical.

2- Homodimers do not exhibit pyruvate transport. How can this be explained? Is it possible to turn such a homodimer by directed mutagenesis into a transporter of pyruvate?

Referee #3:

In this Ms, the authors present a strong set of evidence supporting the heterodimeric nature of functional MPC. The experiments with an inducible bidirectional vector transduced in mpc deletion strain led to the purification of a stable Mpc1/Mpc3 1 :1 heterocomplex. 14C-pyruvate measurements into proteoliposomes are convincing, and particularly relevant with differential pHi=8/pHe=7.2.
With their setup evaluating 14C-pyruvate exchange (figs 3E-F) and possibly thermostability (fig 4C) (if compound characterisctics allow it), the authors should provide the scientific community working in the field with informations on the handful MPC inhibitors reported so far. Besides UK5099 and Zaprinast, thiazolidinediones (Proc Natl Acad Sci U S A. 2013 Apr 2; 110(14): 5422-5427.), lonidamine (http://www.biochemj.org/content/473/7/929.long) and aminocarboxycoumarins 7ACC2 (https://www.nature.com/articles/s41467-018-03525-0) have been reported. This information would considerably broaden the interest of the manuscript for the EMBO J readership.

1st Revision - authors' response 4th Februray 2019

Thank you for the opportunity to revise our manuscript in light of the reviewers' comments. We appreciate the positive evaluation and the helpful comments by all the reviewers. Following is a point-by-point response to each of the comments:

Referee #1
Tavoulari et al have provided data on the characterisation of oligomers of the pyruvate carrier protein in the yeast *S. cerevisiae*. They have managed to purify hetero-oligomeric complexes form yeast and characterize them functionally in a reconstituted system. The question of the oligomeric state of the pyruvate carrier and how this is linked to its transport function is central to the understanding of the biological role of this protein. Since the discovery in 2012 of MPC proteins by two independent studies, several more studies appeared linking the MPC to cell metabolism in major diseases (cancer, diabetes etc). However, a thorough analysis of the oligomeric state is missing. In the absence of a detailed 3D structure, the determination of the active oligomer state will be important for future studies. The work presented is generally well executed. The authors argue that the functional entity for MPC in *S. cerevisae* is a heterodimer between MPC1 and MPC3. The transport capacity of the heterodimer is sensitive to specific inhibitors, but the homodimer seems to be non-functional. The data are convincing, but there are a number of issues to be resolved. Also, the authors should tone down the statements that what they find in yeast is universally true for mammalian homologues.

1) Figure 2 shows that MPC1 and MPC3 form a hetero-dimeric complex using size exclusion chromatography. It is not known what residues or surfaces may contribute to the formation of a heterodimer, and hetero-dimer is a preferred state as opposed to a homodimer when the partner subunit is co-expressed. This should be addressed by some mutagenesis analysis. This would be reinforced by mutagenesis in yeast to see (i) the effect of expressing one of the subunits in a mutated form incapable (or affected) in its hetero-dimer capacity and (ii) to see whether this translates also in affected transport of pyruvate in vivo.

Response: We agree with the reviewer that it would be interesting to elucidate the dimer interface. However, in the absence of structures to guide the mutagenesis analysis we would have to investigate a large number of mutant complexes (>100 to mutate one of two protomers), each requiring a technically challenging purification and functional analysis. The other problem is that this approach might lead to false positives and false negatives. Mutation of a single interface residue

might not be sufficient for disrupting the interface (a false negative) and thus a combination of different mutants might be required. If a mutation affects the structure, it will prevent dimerisation even when the residue is not in the interface (a false positive). Functional assays would not be conclusive, as the activity can be affected by other reasons, such as an impaired substrate binding and transport mechanism or by protein misfolding, none of which have anything to do with the dimerization interface. We hope that the reviewer will agree that obtaining structures of the hetero-dimers would be the preferred way to address this question, which is beyond the scope of this manuscript.

2) It is an interesting result that the reconstituted heterodimer is sensitive to UK5099. However, the difference in the efficiency of inhibition between the mammalian and the yeast proteins reinforces the argument that the mammalian system may not necessarily oligomerize in the same manner as the one from yeast. The authors should discuss this possibility rather than dismissing the study of Nagampalli et al 2018 due to technical differences in the purification. To be fair, in the absence of an equivalent reconstituted system of the mammalian carriers one cannot draw safe conclusions about the properties of the mammalian carriers simply by analogy to the yeast system.

Response: We agree with the reviewer that there might be differences between the yeast and the mammalian MPC complexes and that the same analysis needs to be performed on the mammalian complexes. However, we would like to note that previous studies on mammalian MPCs attributed functionality to the hetero-complexes and not to single proteins (Bricker *et al.*, 2012, Compan *et al.*, 2015, Herzig *et al.*, 2012, Vanderperre *et al.*, 2016). To comply with the comments of the reviewer, we have emphasised in the discussion that there are possible differences between the yeast and mammalian MPCs, including the oligomeric state, and we have removed our criticism on the human MPC purification.

3) The use of the inhibitor Zaprinast is also interesting and adds to the functional analysis of the MPC. To see if the drug was specific for either the dimer interface or an individual subunit, a control using both monomeric proteins should be tested for thermal stability in the presence of the drug.

Response: We thank the reviewer for bringing up this control. We had partially addressed it by testing the effect of Zaprinast on the thermostability of Mpc3 alone (Figure 4, panel C) and found that Mpc3 is not stabilized as the hetero-complex. In the CPM assay, the purified sample of Mpc1 does not show a denaturation profile (Figure 1), so cannot be used. However, we have now tested the effect of Zaprinast on Mpc1 with the nanoDSF (Figure 4, panel D) and found that there was no stabilizing shift on Mpc1 alone, further supporting that only the hetero-dimer can bind Zaprinast.

Minor point: In Figure 1B please indicate with arrows the MPC proteins and explain what the bands above the pure protein are.

Response: We apologise for not making clear that the samples in Figure 1B are not purified protein but crude mitochondrial preparations. We have now indicated that in the main text and the figure legend. We have also indicated the bands that are most likely Mpc proteins, based on molecular weight, with arrows. The smears detected above the Mpc bands are possibly due to non-specific binding of the polyclonal hen antibodies.

Referee #2:

Mitochondrial pyruvate carrier (MPC) proteins facilitate the transport of pyruvate from the cytosol to the mitochondrial matrix. These are MPC1 and MPC2 in mammals and Mpc1, Mpc2 and Mpc3 in baker's yeast. The precise stoichiometry, oligomeric state and the functional unit of the MPC complexes have not been defined so far.

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Previous in vivo genetic study (e.g. Herzig et al., 2012) already indicated that MPCs function as hetero-multimeric complexes: Mpc1-Mpc2 under fermentative conditions and Mpc1-Mpc3 during respiration. Thus, the here presented in vitro biochemical approaches nicely confirm this hypothesis and extend our knowledge in the function of the MPC. However, I also think that this limits the novelty of the article in its present form. I however also think that these limitations could be overcome in a potential major revision.

Response: We would like to thank the reviewer for the positive comments and for noting the misconception about MPCs being multimeric complexes. We think that our work is novel in terms of providing the precise stoichiometry, oligomeric state and functional unit of MPC.

Major comments:

1) Although the authors report on the successful Mpc1-Mpc2 heterodimer purification, they did not characterize its pyruvate transport activity. Especially in the light of the potential differential use of the Mpc1-Mpc2 and Mpc1-Mpc3 heterodimers during different metabolic conditions, an experimental comparison of the transport properties of both complexes appears critical.

Response: We agree that a comparison between the two alternative MPC complexes is important. In our original manuscript we have mentioned the problems with the stability of the Mpc1/Mpc2 purified protein. As we had anticipated, the reconstitution of Mpc1/Mpc2 into liposomes was challenging mainly due to the low yield of the purified protein and its stability. However, by introducing modifications in the purification and reconstitution protocols we managed to measure pyruvate transport by the Mpc1/Mpc2 hetero-complex, shown now in Figure EV4, panel D. The low yield, concentration and stability of the purified Mpc1/Mpc2 (one order of magnitude less than Mpc1/Mpc3) was a limiting factor for the reconstitution procedures. Given the incredible difficulty in handling of this complex, it is not possible to carry out an extensive comparison.

2) Homodimers do not exhibit pyruvate transport. How can this be explained? Is it possible to turn such a homodimer by directed mutagenesis into a transporter of pyruvate?

Response: The reviewer raises an interesting question. Since pyruvate is an asymmetric substrate it is expected to bind to an asymmetric binding site, which can be provided by a hetero-dimer but not by a homo-dimer. Other transporters (i.e. some ABC transporters), are also known to function as obligate hetero-dimers. Since the binding site, dimerization interface, and conformational changes have not been defined for MPC to guide mutagenesis, it would be nearly impossible to experimentally convert the homo-dimers into active pyruvate transporters at this point.

Referee #3:

In this Ms, the authors present a strong set of evidence supporting the heterodimeric nature of functional MPC. The experiments with an inducible bidirectional vector transduced in mpc deletion strain led to the purification of a stable Mpc1/Mpc3 1 :1 heterocomplex. 14C-pyruvate measurements into proteoliposomes are convincing, and particularly relevant with differential pHi=8/pHe=7.2.
With their setup evaluating 14C-pyruvate exchange (figs 3E-F) and possibly thermostability (fig 4C) (if compound characteristics allow it), the authors should provide the scientific community working in the field with informations on the handful MPC inhibitors reported so far. Besides UK5099 and Zaprinast, thiazolidinediones (Proc Natl Acad Sci U S A. 2013 Apr 2; 110(14): 5422-5427), lonidamine (http://www.biochemj.org/content/473/7/929.long) and aminocarboxycoumarins 7ACC2 (https://www.nature.com/articles/s41467-018-03525-0) have been reported. This information would considerably broaden the interest of the manuscript for the EMBO J readership.

Response: We would like to thank the reviewer for the positive comments on our work. We have tested the suggested compounds for their ability to inhibit pyruvate transport by the yeast Mpc1/Mpc3 reconstituted in proteoliposomes. In addition to UK5099 and Zaprinast, we have now measured IC_{50} values for lonidamine and 7ACC2 and we have incorporated IC_{50} data for all compounds in Figure 3, panel E. We have also tested two different thiazolidinediones, pioglitazone and rosiglitazone, for their ability to inhibit pyruvate exchange. Even in high concentrations of 500

μM pioglitazone had no effect on transport activity. At the same high concentration, however, rosiglitazone inhibited pyruvate transport to 52%. We could not test higher concentrations because beyond the 500 μM these compounds were not soluble in our buffer system. As we have emphasized in the discussion section, the inhibitor co-ordination appears different between the yeast and mammalian MPC complexes and we cannot draw any conclusions for the ability of the TZDs to inhibit the human MPC.

References

Bricker DK, Taylor EB, Schell JC, Orsak T, Boutron A, Chen YC, Cox JE, Cardon CM, Van Vranken JG, Dephoure N, Redin C, Boudina S, Gygi SP, Brivet M, Thummel CS, Rutter J (2012) A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. *Science* 337: 96-100

Herzig S, Raemy E, Montessuit S, Veuthey JL, Zamboni N, Westermann B, Kunji ER, Martinou JC (2012) Identification and functional expression of the mitochondrial pyruvate carrier. *Science* 337: 93-96

Compan V, Pierredon S, Vanderperre B, Krznar P, Marchiq I, Zamboni N, Pouyssegur J, Martinou JC (2015) Monitoring Mitochondrial Pyruvate Carrier Activity in Real Time Using a BRET-Based Biosensor: Investigation of the Warburg Effect. *Mol Cell* 59: 491-501

Vanderperre B, Cermakova K, Escoffier J, Kaba M, Bender T, Nef S, Martinou JC (2016) MPC1-like Is a Placental Mammal-specific Mitochondrial Pyruvate Carrier Subunit Expressed in Postmeiotic Male Germ Cells. *J Biol Chem* 291: 16448-16461

2nd Editorial Decision 7th March 2019

Thank you for submitting a revised version of your manuscript. It has now been seen by one of the original referees, whose comments are appended below.

As you will see, s/he finds that all criticisms have been sufficiently addressed and recommends the study for publication. However, before we can officially accept the manuscript, I kindly ask you to review and approve the text edits to the legends made by our production/data editors (in attachment to this e-mail).

REFEREE REPORTS

Referee #2:

The authors have addressed my comments in writing and experimentally. I appreciate the difficulties of the purification of membrane proteins and that the authors undertook the effort to nevertheless try to characterize the Mpc1-Mpc2 heterodimer.

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: Sotiria Tavoulari
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2018-100785

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 oligomeric state analysis has been performed with five biological repeats for the Mpc1/Mpc3 hetero-complex and three biological repeats for Mpc3, which is more than the typical for similar published analyses. Thermostability analysis has been performed in three independent experiments in triplicates. To evaluate IC50 of inhibition and kinetic parameters we have performed three independent experiments in triplicates. Time course experiments have been performed 2-4 independent times in duplicates, as the observed differences were at least 50%.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N//A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	In rare cases, transport data points need to get excluded if filtrartion of the proteoliposomes gets disrupted due to dicontinuous vacuum and yields unreasonably high numbers.
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.	N/A
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	N/A
5. For every figure, are statistical tests justified as appropriate?	N/A
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A
Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

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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).	We have designed our own antibodies against Mpc1 and Mpc3, which were raised in hen by Agrisera (www.agrisera.com). The synthetic peptides used to raise the antibodies correspond to residues 111-126 of Mpc1 and residues 40–54 of Mpc3.
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.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
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.	N/A

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
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.	N/A

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).	All Data will be uploaded to Dryad Digital Repository upon acceptance.
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.	N/A