

Highly parallelized laboratory evolution of wine yeasts for enhanced metabolic phenotypes

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Review Timeline:

Submission Date:	21st Mar 24
Editorial Decision:	22nd Mar 24
Revision Received:	3rd May 24
Editorial Decision:	16th May 24
Revision Received:	17th Jul 24
Accepted:	30th Jul 24

Editor: Maria Polychronidou / Poonam Bheda

Transaction Report: Please note that the manuscript was transferred from another journal where it was originally reviewed. Since the original reviews are not subject to EMBO's transparent review process policy, they cannot be published.

(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.)

25th Mar 2024

Manuscript Number: MSB-2024-12326

Title: Highly parallelized laboratory evolution of wine yeasts for enhanced metabolic phenotypes

Dear Kiran,

Thank you again for submitting your work to Molecular Systems Biology. I have now read your manuscript, the referee reports from the other journal and your responses to these comments and discussed them with the team. Overall, we think that the review process has been constructive and therefore we can consider the study for publication using these reports and without reviewing the study from scratch. We think that most technical and other core concerns seem to have been addressed in the previous rounds of revision at the other journal. Reviewer #1 is, already from the first revision round, satisfied with the performed revisions and supports publication. Reviewer #2 lists a few remaining concerns, which mostly pertain to the final follow up experiments on the three selected strains in a 80L setup and whether they convincingly demonstrate the relevance of the approach for industrial applications. We think that the performed additional analyses and text edits that clarify the proof-of-concept nature of the 80L setup for indicating industrial applicability seem to address these remaining concerns of reviewer #2. Moreover, we note that both reviewers acknowledge the relevance of the study both for evolutionary biologists and for industrial microbiology. Taken together, we have decided to proceed with publishing the study in Molecular Systems Biology, pending some minor revisions related to editorial issues listed below.

- Please provide 5 keywords.
- The "one sentence summary" should be removed.
- Please include a Disclosure and Competing Interests Statement in the main text.
- Please note that our editorial policy does not allow "Data not shown".
- Please provide a .doc version of the manuscript text (including legends for main figures and tables) and individual files for the main figures. The figure legends should be included at the end of the manuscript text, after the References.
- The Reference list should be formatted according to the Molecular Systems Biology style i.e. ordered alphabetically and listing the first 10 authors followed by et al.
- We have replaced Supplementary Information by the Expanded View (EV format). In this case, all additional figures can be included in a PDF called Appendix. Appendix figures and Tables should be labeled and called out as: "Appendix Figure S1, Appendix Figure S2... Appendix Table S1..." etc. Each legend should be below the corresponding Figure/Table in the Appendix. Please include a Table of Contents in the beginning of the Appendix. For detailed instructions regarding expanded view please refer to our Author Guidelines: .
- We would encourage you to provide the data that are currently deposited at Mendeley Data as EV Datasets. Please provide one file per EV Dataset. Please include the description of each EV Dataset in the dataset file itself, ie. in a separate tab for .xls files or as a README.txt file in .zip folders. The EV Datasets should be called out in the text (even if it is only in the Data Availability section).
- Please provide a "standfirst text" summarizing the study in one or two sentences (approximately 250 characters), three to four "bullet points" highlighting the main findings and a "synopsis image" (550px width and max 400px height, jpeg format) to highlight the paper on our homepage.
- All Materials and Methods need to be described in the main text. We would encourage you to use 'Structured Methods', our new Materials and Methods format. According to this format, the Material and Methods section should include a Reagents and Tools Table (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section in which we encourage the authors to describe their methods using a step-by-step protocol format with bullet points, to facilitate the adoption of the methodologies across labs. More information on how to adhere to this format as well as downloadable templates (.doc or .xls) for the Reagents and Tools Table can be found in our author guidelines: . An example of a Method paper with Structured Methods can be found here:
- Please include a Data availability section describing how the data, code etc. generated in this study have been made available. This section needs to be formatted according to the example below:
The datasets and computer code produced in this study are available in the following databases:
 - Chip-Seq data: Gene Expression Omnibus GSE46748 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46748>)

- [data type]: [full name of the resource] [accession number/identifier] ([doi or URL or identifiers.org/DATABASE:ACCESSION])
- Molecular Systems Biology supports formal data citations in the Reference list, to cite previously published datasets. In addition to citing the original papers that reported the data, we encourage you to also cite the relevant datasets directly in the Reference list. In the text, references to datasets are included as "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations are very similar to normal literature references but must be labeled with "[DATASET]" at the end of the reference. For detailed instructions please refer to our Author Guidelines .
- When you resubmit your manuscript, please download our CHECKLIST (<https://bit.ly/EMBOPressAuthorChecklist>) and include the completed form in your submission. *Please note* that the Author Checklist will be published alongside the paper as part of the transparent process (<https://www.embopress.org/page/journal/17444292/authorguide#transparentprocess>)

Please resubmit your revised manuscript online, with a covering letter listing amendments and responses to each point raised by the referees. Please resubmit the paper ****within one month**** and ideally as soon as possible. If we do not receive the revised manuscript within this time period, the file might be closed and any subsequent resubmission would be treated as a new manuscript. Please use the Manuscript Number (above) in all correspondence.

Thank you for submitting this paper to Molecular Systems Biology.

Kind regards,

Maria

Maria Polychronidou, PhD
Senior Editor
Molecular Systems Biology

If you do choose to resubmit, please click on the link below to submit the revision online before 21st Apr 2024.

IMPORTANT:

- When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
<https://bit.ly/EMBOPressFigurePreparationGuideline>
See also figure legend guidelines: <https://www.embopress.org/page/journal/17444292/authorguide#figureformat>

- Please note that corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (EMBO Press signed a joint statement to encourage ORCID adoption).
(<https://www.embopress.org/page/journal/17444292/authorguide#editorialprocess>)
Currently, our records indicate that the ORCID for your account is 0000-0002-6166-8640.

Please click the link below to modify this ORCID:
Link Not Available

As a matter of course, please make sure that you have correctly followed the instructions for authors as given on the submission website.

*** PLEASE NOTE *** As part of the EMBO Press transparent editorial process initiative (see our Editorial at <https://dx.doi.org/10.1038/msb.2010.72> , Molecular Systems Biology will publish online a Review Process File to accompany accepted manuscripts. When preparing your letter of response, please be aware that in the event of acceptance, your cover letter/point-by-point document will be included as part of this File, which will be available to the scientific community. More information about this initiative is available in our Instructions to Authors. If you have any questions about this initiative, please contact the editorial office (msb@embo.org).

Thank you for considering our submission MSB-2024-12326. Please find authors' (AU) responses to individual editorial comments (black) included below (red).

- Please provide 5 keywords.

AU: Five keywords are now included.

- The "one sentence summary" should be removed.

AU: The one sentence summary has been removed.

- Please include a Disclosure and Competing Interests Statement in the main text.

AU: We now declare no competing interests in the main text

- Please note that our editorial policy does not allow "Data not shown".

AU: All data referred to is shown.

- Please provide a .doc version of the manuscript text (including legends for main figures and tables) and individual files for the main figures. The figure legends should be included at the end of the manuscript text, after the References.

AU: The text is now included as .doc. Figure legends are included at the end of the text.

- The Reference list should be formatted according to the Molecular Systems Biology style i.e. ordered alphabetically and listing the first 10 authors followed by et al.

AU: The reference list is now formatted according to MSB style.

- We have replaced Supplementary Information by the Expanded View (EV format). In this case, all additional figures can be included in a PDF called Appendix. Appendix figures and Tables should be labeled and called out as: "Appendix Figure S1, Appendix Figure S2... Appendix Table S1..." etc. Each legend should be below the corresponding Figure/Table in the Appendix. Please include a Table of Contents in the beginning of the Appendix. For detailed instructions regarding expanded view please refer to our Author

Guidelines: <http://msb.embopress.org/authorguide#expandedview>.

AU: We have designated supplementary figures 1, 3, 4, 6 and 8 as EV figures 1-5, while supplementary figures 2, 5, 7, 9, 10 and 11 and all tables are now appendix.

- We would encourage you to provide the data that are currently deposited at Mendeley Data as EV Datasets. Please provide one file per EV Dataset. Please include the description of each EV Dataset in the dataset file itself, ie. in a separate tab for .xls files or as a README.txt file in .zip folders. The EV Datasets should be called out in the text (even if it is only in the Data Availability section).

AU: We have now included the Mendeley data as source data files in the submission, as instructed.

- Please provide a "standfirst text" summarizing the study in one or two sentences (approximately 250 characters), three to four "bullet points" highlighting the main findings and a "synopsis image" (550px width and max 400px height, jpeg format) to highlight the paper on our homepage.

AU: A standfirst text and a synopsis image is now included.

- All Materials and Methods need to be described in the main text. We would encourage you to use 'Structured Methods', our new Materials and Methods format. According to this format, the Material and Methods section should include a Reagents and Tools Table (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section in which we encourage the authors to describe their methods using a step-by-step protocol format with bullet points, to facilitate the adoption of the methodologies across labs. More information on how to adhere to this format as well as downloadable templates (.doc or .xls) for the Reagents and Tools Table can be found in our author guidelines: <https://www.embopress.org/page/journal/17444292/authorguide#textformat>. An example of a Method paper with Structured Methods can be found here: <https://www.embopress.org/doi/10.15252/msb.20178071>.

AU: We have now added a Reagents and tools tables. However, we feel that a step-by-step protocol format with bullet points does not fit our Materials and Methods section well.

- Please include a Data availability section describing how the data, code etc. generated in this study have been made available. This section needs to be formatted according to the example below:

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

- Chip-Seq data: Gene Expression Omnibus GSE46748

(<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46748>)

- [data type]: [full name of the resource] [accession number/identifier] ([doi or URL or identifiers.org/DATABASE:ACCESSION])

AU: The data availability section is designed as instructed.

- Molecular Systems Biology supports formal data citations in the Reference list, to cite previously published datasets. In addition to citing the original papers that reported the data, we encourage you to also cite the relevant datasets directly in the Reference list. In the text, references to datasets are included as "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations are very similar to normal literature references but must be labeled with "[DATASET]" at the end of the reference. For detailed instructions please refer to our Author Guidelines <http://msb.embopress.org/authorguide#datacitation>.

AU: We do not use or refer to any previously published data sets in this paper.

- When you resubmit your manuscript, please download our CHECKLIST (<https://bit.ly/EMBOPressAuthorChecklist>) and include the completed form in your submission. *Please note* that the Author Checklist will be published alongside the paper as part of the transparent process

<https://www.embopress.org/page/journal/17444292/authorguide#transparentprocess>)

AU: A check list has been included in the submission.

Please resubmit your revised manuscript online, with a covering letter listing amendments and responses to each point raised by the referees. Please resubmit the paper ****within one month**** and ideally as soon as possible. If we do not receive the revised manuscript within this time period, the file might be closed and any subsequent resubmission would be treated as a new manuscript. Please use the Manuscript Number (above) in all correspondence.

16th May 2024

Manuscript Number: MSB-2024-12326R

Title: Highly parallelized laboratory evolution of wine yeasts for enhanced metabolic phenotypes

Dear Kiran,

Thank you for sending us your revised manuscript. We have gone through the performed revisions and I am glad to inform you that we can soon accept the manuscript for publication, pending some final editorial requests listed below.

- Our data editors have noted the following issues that need to be fixed:

-- Please note that the figure 1 is missing in the manuscript, although the legend is provided. Figures 2-7 are mislabeled in the submissions system as figures 1-6 This needs to be corrected.

-- Please note that the legend for figure panels 2a-b is not labelled in the manuscript. Further, the legends 2c-d are mislabeled as 2a-b. This needs to be rectified.

-- Please indicate the exact p values in the legends of figures 7c; EV 5a, c, d-e.

-- Please include the information related to n in the legend of figure EV 3a.

-- Please note that n=2 in figure EV 5a. For n <5 we request that the actual individual data from each experiment should be plotted alongside an error bar.

-- Please describe the nature of entity for 'n' (biological? technical?) in the legends of figures 2c; 3c; 7a-b; EV 5a-f.

- The funding information provided in the manuscript text (Acknowledgements) should match the information entered in the online submission system. Currently the Research Council of Sweden grant no. 325-2014-6547 is missing from the submission system.

- The References should be formatted according to the Molecular Systems Biology reference style (i.e., ordered alphabetically and listing the first 10 authors followed by et al).

- The first reference in the References list seems to be misplaced, there is no mention of it in the text.

- Please correct the callouts for Appendix tables: i.e. for Appendix Tables S2 and S3 ("S" is missing from the callouts), Appendix Table S5 ("Appendix" is missing), Appendix Tables S6-S8 ("S" is missing and there isn't any Appendix Table S8), Appendix Figs. S1-S3 ("S" is missing). Moreover, the following callouts need to be corrected: Supplementary Fig. 1 and Supplementary Table 1 should be corrected to the respective EV or Appendix Figure/Table. Table EV8 is called out but doesn't exist.

- Table EV1A-D should be combined in a single Dataset EV1 file, with separate sheets and a description of the data in each sheet.

- Table EV2 should be provided as a single Excel file, with the description of the dataset in a separate sheet (instead of a .txt file in a .zip folder).

- Please include page numbers in the Appendix Table of Contents. Within the Appendix, Appendix Figure 1 needs "S" in the name.

Please resubmit your revised manuscript online, with a covering letter listing amendments and responses to each point raised by the referees. Please resubmit the paper ****within one month**** and ideally as soon as possible. If we do not receive the revised manuscript within this time period, the file might be closed and any subsequent resubmission would be treated as a new manuscript. Please use the Manuscript Number (above) in all correspondence.

As a matter of course, please make sure that you have correctly followed the instructions for authors as given on the submission website.

Kind regards,

Maria

Maria Polychronidou, PhD

Senior Editor
Molecular Systems Biology

If you do choose to resubmit, please click on the link below to submit the revision online before 15th Jun 2024.

*** PLEASE NOTE *** As part of the EMBO Press transparent editorial process initiative (see our Editorial at <https://dx.doi.org/10.1038/msb.2010.72> , Molecular Systems Biology will publish online a Review Process File to accompany accepted manuscripts. When preparing your letter of response, please be aware that in the event of acceptance, your cover letter/point-by-point document will be included as part of this File, which will be available to the scientific community. More information about this initiative is available in our Instructions to Authors. If you have any questions about this initiative, please contact the editorial office (msb@embo.org).

Dear Kiran,

Thank you for sending us your revised manuscript. We have gone through the performed revisions and I am glad to inform you that we can soon accept the manuscript for publication, pending some final editorial requests listed below.

- Our data editors have noted the following issues that need to be fixed:

AU: Comments are included in red below

-- Please note that the figure 1 is missing in the manuscript, although the legend is provided. Figures 2-7 are mislabeled in the submissions system as figures 1-6 This needs to be corrected.

AU: We apologize. Figure 2 had failed to export correctly. The figure is now included and subsequent figures re-labelled. Figure 1 is identical to the synopsis figure. We have now included both in the submission.

-- Please note that the legend for figure panels 2a-b is not labelled in the manuscript. Further, the legends 2c-d are mislabeled as 2a-b. This needs to be rectified.

AU: The legend for figure 2 is now correct. The mismatch of legend to figure followed from Figure 2 not exporting correctly.

-- Please indicate the exact p values in the legends of figures 7c; EV 5a, c, d-e.

AU: We have now added the exact p-values to the figures 7c, EV 5a, b, d and e.

-- Please include the information related to n in the legend of figure EV 3a.

AU: This is now reported as: "mean of (\log_2 , normalized) cell doubling time for each wine yeast ($n=24$ replicate ALE populations, extinct populations excluded, in each environment) across all environments ($n=8$ environments). Error bars: SEM ($n=8$)."

-- Please note that $n=2$ in figure EV 5a. For $n < 5$ we request that the actual individual data from each experiment should be plotted alongside an error bar.

AU: We have now added individual data points for figure EV5 b, d, and e. For Figure EV5A, measurement errors are too small for individual data points to be distinguishable. We note that individual data points for all figures are reported in the supplementary data.

-- Please describe the nature of entity for 'n' (biological? technical?) in the legends of figures 2c; 3c; 7a-b; EV 5a-f.

AU: We have multiple layers of biological replication. We now distinguish between these in all legends as "strains", "replicate ALE populations" and "biological replicates" respectively, in all instances where "n" is mentioned. There are no technical replicates in the study.

- The funding information provided in the manuscript text (Acknowledgements) should match the information entered in the online submission system. Currently the Research Council of Sweden grant no. 325-2014-6547 is missing from the submission system.

AU: We have now added the grant to the online submission system.

- The References should be formatted according to the Molecular Systems Biology reference style (i.e., ordered alphabetically and listing the first 10 authors followed by et al).

AU: The references have now been formatted correctly.

- The first reference in the References list seems to be misplaced, there is no mention of it in the text.

AU: The first reference has been removed.

- Please correct the callouts for Appendix tables: i.e. for Appendix Tables S2 and S3 ("S" is missing from the callouts), Appendix Table S5 ("Appendix" is missing), Appendix Tables S6-S8 ("S" is missing and there isn't any Appendix Table S8), Appendix Figs. S1-S3 ("S" is missing). Moreover, the following callouts need to be corrected: Supplementary Fig. 1 and Supplementary Table 1 should be corrected to the respective EV or Appendix Figure/Table. Table EV8 is called out but doesn't exist.

AU: Appendix Tables are now correctly called out. Supplementary Fig. 1 and Fig S1 is now correctly called out as Fig EV1. Supplementary Table 1 is now correctly referred to as Appendix Table S1. Appendix Table S7 is now correctly called out as Table EV1. Tables EV8 and Appendix Table S8 are now correctly referred to as EV2. We have also changed one instance of Fig S8C to Fig EV5C.

- Table EV1A-D should be combined in a single Dataset EV1 file, with separate sheets and a description of the data in each sheet.

AU: Done. Correspondingly, we now refer to table EV1A-D as table EV1

- Table EV2 should be provided as a single Excel file, with the description of the dataset in a separate sheet (instead of a .txt file in a .zip folder).

AU: Done.

- Please include page numbers in the Appendix Table of Contents. Within the Appendix, Appendix Figure 1 needs "S" in the name.

AU: Page numbers are included in the Appendix and referred to in the appendix table of contents. Appendix Figure S1 is now correctly labelled.

30th Jul 2024

Manuscript number: MSB-2024-12326RR

Title: Highly parallelized laboratory evolution of wine yeasts for enhanced metabolic phenotypes

Dear Prof Patil,

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

Your manuscript will be processed for publication by EMBO Press. It will be copy edited and you will receive page proofs prior to publication. Please note that you will be contacted by Springer Nature Author Services to complete licensing and payment information.

You may qualify for financial assistance for your publication charges - either via a Springer Nature fully open access agreement or an EMBO initiative. Check your eligibility: <https://www.embopress.org/page/journal/17444292/authorguide#chargesguide>

Should you be planning a Press Release on your article, please get in contact with embo_production@springernature.com as early as possible in order to coordinate publication and release dates.

If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to Molecular Systems Biology.

Yours sincerely,

Poonam Bheda, PhD
Scientific Editor
Molecular Systems Biology

>>> Please note that it is Molecular Systems Biology policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://www.embopress.org/transparent-process#Review_Process

EMBO Press Author Checklist

Corresponding Author Name: Kiran Patil
Journal Submitted to: Molecular Systems Biology
Manuscript Number: MSB-2024-12326

USEFUL LINKS FOR COMPLETING THIS FORM

- [The EMBO Journal - Author Guidelines](#)
- [EMBO Reports - Author Guidelines](#)
- [Molecular Systems Biology - Author Guidelines](#)
- [EMBO Molecular Medicine - Author Guidelines](#)

Reporting Checklist for Life Science Articles (updated January)

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if $n < 5$, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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 χ^2 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.

**Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.**

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Reagents and Tools Table. Commercially obtained strains and evolved derivatives of these are subject to patent law restrictions as per the suppliers instructions
Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	
DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Reagents and Tools Table
Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, and/ OR RRID.	Not Applicable	
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	
Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail housing and husbandry conditions .	Not Applicable	

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Reagents and Tools Table

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been pre-registered , provide DOI in the manuscript . For clinical trials, provide the trial registration number OR cite DOI.	Not Applicable	
Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if external detailed step-by-step protocols are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Yes	Materials and Methods and Figures
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, have they been described?	Not Applicable	
Include a statement about blinding even if no blinding was done.	Not Applicable	
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not Applicable	
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Data analyzed by parametric tests are approximately normally distributed.

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was replicated in laboratory.	Yes	Figures and Material and Methods
In the figure legends: define whether data describe technical or biological replicates .	Yes	Figures and Material and Methods

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving human participants : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference	Not Applicable	
Studies involving human participants : Include a statement confirming that informed consent was obtained from all subjects and that the experiments	Not Applicable	
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	

Studies involving experimental animals : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving specimen and field samples : State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
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Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

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State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Not Applicable	
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	Not Applicable	
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have primary datasets been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data availability
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	