

Determinants of epigenetic resistance to HDAC inhibitors in dystrophic fibro-adipogenic progenitors

Silvia Consalvi, Luca Tucciarone, Elisa Macrì, Marco De Bardi, Mario Picozza, Illari Salvatori, Alessandra Renzini, Sergio Valente, Antonello Mai, Viviana Moresi, and Pier Lorenzo Puri

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Submission Date:	24th Jan 22
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Accepted:	23rd Mar 22

Editor: Achim Breiling

Transaction Report: This manuscript was transferred to EMBO reports following peer review at The EMBO Journal.

(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 Dr. Puri,

Thank you for the transfer of your revised manuscript to our editorial offices. I now went through the referee reports from The EMBO Journal, your point-by-point-response, the comments of referee #2 regarding the revised manuscript (I attached again below) and your response to the comments of referee #2. Original referee #1 apparently refused to re-assess the study but going through your p-b-p-response, I consider his/her points as adequately addressed. Referee #3 now fully supports the publication of your study.

Given the overall positive evaluation, I would like to invite a final revision of the manuscript. Please address the remaining points of referee #2 that can be addressed by text changes or different data presentation, and also provide a detailed p-b-p-response to his/her report (as in your response letter). If you have further data to strengthen the paper in response to the referee points at hand, I would welcome their addition. Please address the first major point of referee #2 (regarding statistics) and the minor points as requested by the referee.

Moreover, I have these editorial points I also ask you to address:

- Please provide the abstract written in present tense.
- Please add subheadings and paragraphs to the results section to render it more structured and comprehensible for the reader.
- Please gather all the primer information presently in the methods section into a table, that I suggest adding to the Appendix. Please name this Appendix Table S1 and add callouts using this name to the manuscript. Please add the table with a legend at the end of the Appendix file.
- Please add a title, page numbers and a table of contents (TOC) including page numbers to the Appendix file. Please also move the legends for each figure below the figures. I think this is more comprehensible.
- Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also those in the Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. See also the minor comments of referee #2.
- For microscopic images, please add scale bars of similar style and thickness to all the microscopic images (e.g. images in Fig. 4 lack scale bars), using clearly visible black or white bars (depending on the background). Please do not write on or near the bars in the image but define the size in the respective figure legend.
- Please format the references according to our journal style. See:
<http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>
- Please make sure that all the funding information is also entered into the online submission system and that it is complete and similar to the one in the acknowledgement section of the manuscript text file.
- We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy <https://www.embopress.org/competing-interests> and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement'.
- Please remove the sentence 'The data will be private until publication' and the referee tokens from the 'Data availability' section and make sure that the data is public upon publication of the study.
- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address (mainly in the figure legends). Please provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:

- a short, two-sentence summary of the manuscript (not more than 35 words).
- three to four short (2 lines) bullet points highlighting the key findings of your study.
- a schematic summary figure (synopsis image) in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels that can be used as a visual synopsis on our website.

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

Best,

Achim Breiling
Senior Editor
EMBO Reports

Referee #2:

As the authors have nicely articulated in the cover letter, this manuscript addresses a significant topic and has a number of strengths. It was therefore surprising that the response letter and revised manuscript addressed only a subset of the "addressable" concerns raised in the initial review.

Most notably:

1. The authors state "both FAPs and MuSCs (but not macrophages) of late stages mdx mice exhibited activation of common secretory patterns implicated in Senescence Associated Secretory Phenotype (SASP) and related multiple inflammatory responses". But they only provided the p value for Fig 3B and C, while no normalized enrichment score was provided, nor the FDR q value. It's very hard to read the NES from figure 2A especially for SASP and Cellular Senescence. From Figure 2B and C, it looks like a lot of genes of SASP and cellular senescence are negatively "enriched". Please add the NES and FDR q value in Figure 2B and C.
2. The authors are well-versed in aging, yet, in Figure 3, refer to 12-month-old mice as old. This is not an appropriate comparison, and use of tissue from 24+-month-old mice is needed.
3. "It will be especially interesting to the senescence field if the mechanisms apply to other senescent cell types; HDACi..senomorphic to inhibit SASP." This comment was not addressed. A relatively straightforward experiment in IMR90s or the like would show relevance and increase potential impact of study.
4. Related to #3, "Is this mechanism specific to mdx FAPs or is it a more general mechanism in other cell types? Since the authors have senescent IMR90 experiments (Figure 2J), can they confirm in this model?" Again, for example, do senescent IMR90 cells have higher HDAC activity? Is this a hallmark that may provide utility to the senescence field?
5. The inquiry ""What is the HDAC activity in skeletal muscle from wild-type young and old mice?", also was not addressed. At 12 months, mice are mid-adult and senescence is a later life event (e.g., to our knowledge, not evident in most tissues until at least 18 months of age). Thus, the statement "deregulation of HDAC activity does not occur as a consequence of chronological aging" is not supported by the data.
6. For the question "The loss of activation of cell cycle genes in older mdx muscle by HDACi (Sup Fig 6) couldn't be explained by senescence. Senescent cells are always a very small fraction of all, which suggests there may be other mechanisms leading to the resistance to HDACi." The authors responded "Again, this concern refers to the issue related to the use of the term "senescence features" - see response to point 1 (as well as to point 1 of referee 2)". This concern is not just about labels. In older MDX samples, HDACi treatment did not activate gene expression compared to the young samples. Do the authors conclude ALL or most FAPs gain the "disease-associated features of senescence"? This would be contrary to what has been described in other tissues at advanced ages or in the context of disease. Could this instead reflect an altered biological state unrelated to senescence?

Minor concerns:

Presumably, "LamininB1" is meant to be LaminB1. Likely a typo, but please confirm appropriate antibodies were used for desired stains.

1. Inquiry: "The p21 expression level wasn't consistent between Figure 1K and Sup Figure 3. It's lower in old samples in Fig 1K but higher in Sup Figure 3." The authors responded: "see response to point 7 of reviewer 1", which was about a new figure 2, and it was not related to the wrong labels in Figure 1K. Please address.
2. Statistical tests are provided for data represented in Figure 2B, E, J, K, and O, however, no error bars are provided for the YC samples. It is up to the journal to require plotting of individual data points, but error bars at least provide a sense of distribution.

Dear editor

Thank you for inviting us to transfer our revised manuscript to the editorial office of EMBO Reports. Below is the point-to-point rebuttal letter, in which we have addressed the remaining points of referee #2. We have also addressed the following additional editorial requests:

Please provide the abstract written in present tense

RE: Done

Please add subheadings and paragraphs to the results section to render it more structured and comprehensible for the reader.

RE: Done

Please gather all the primer information presently in the methods section into a table, that I suggest adding to the Appendix. Please name this Appendix Table S1 and add callouts using this name to the manuscript. Please add the table with a legend at the end of the Appendix file.

RE: Done

Please add a title, page numbers and a table of contents (TOC) including page numbers to the Appendix file. Please also move the legends for each figure below the figures. I think this is more comprehensible.

RE: Done

Regarding data quantification and statistics, please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (also those in the Appendix). Please also check that all the p-values are explained in the legend, and that these fit to those shown in the figure. Please provide statistical testing where applicable. Please avoid the phrase 'independent experiment', but clearly state if these were biological or technical replicates. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. See also the minor comments of referee #2.

RE: Done

For microscopic images, please add scale bars of similar style and thickness to all the microscopic images (e.g. images in Fig. 4 lack scale bars), using clearly visible black or white bars (depending on the background). Please do not write on or near the bars in the image but define the size in the respective figure

RE: Done

Please format the references according to our journal style.
See: <http://www.embopress.org/page/journal/14693178/authorguide#referencesformat>
RE: Done

Please make sure that all the funding information is also entered into the online submission system and that it is complete and similar to the one in the acknowledgement section of the manuscript text file.
RE: Done

We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy <https://www.embopress.org/competing-interests> and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement'.
RE: Done

Please remove the sentence 'The data will be private until publication' and the referee tokens from the 'Data availability' section and make sure that the data is public upon publication of the study.
RE: Done

Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address (mainly in the figure legends). Please provide your final manuscript file with track changes, in order that we can see any modifications done.
RE: Done

In addition, I would need from you:

- a short, two-sentence summary of the manuscript (not more than 35 words).

RE: Please, see below

Progressive increase of HDAC activity in Fibro-adipogenic progenitors of muscles from a mouse model of Duchenne Muscular Dystrophy leads to genome-wide alterations of histone acetylation profiles associated with development of features of senescence at late stages of disease progression that are not fully recovered by HDAC inhibitors.

three to four short (2 lines) bullet points highlighting the key findings of your study.

RE: Please, see below

HDAC activity increases in FAPs of muscles from mdx mice at late stages of disease progression
FAPs of late stage mdx muscles exhibit altered profile of histone acetylation
FAPs of late stage mdx muscles develop features of senescence
HDAC inhibitors fail to reverse cell cycle arrest and glycolysis in FAPs of late stage mdx muscles
HDAC inhibitors inhibit activation of SASP genes in FAPs of late stage mdx muscles

a schematic summary figure (synopsis image) in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels that can be used as a visual synopsis on our website. Coherent with the short summary above

RE: Done (Figure uploaded, as visualabstract)

Specific point-to-point rebuttal to Referee #2:

As the authors have nicely articulated in the cover letter, this manuscript addresses a significant topic and has a number of strengths. It was therefore surprising that the response letter and revised manuscript addressed only a subset of the "addressable" concerns raised in the initial review.

Most

notably:

1. The authors state "both FAPs and MuSCs (but not macrophages) of late stages mdx mice exhibited activation of common secretory patterns implicated in Senescence Associated Secretory Phenotype (SASP) and related multiple inflammatory responses". But they only provided the p value for Fig 3B and C, while no normalized enrichment score was provided, nor the FDR q value. It's very hard to read the NES from figure 2A especially for SASP and Cellular Senescence. From Figure 2B and C, it looks like a lot of genes of SASP and cellular senescence are negatively "enriched". Please add the NES and FDR q value in Figure 2B and C.

RE: We have included in the revised manuscript the requested statistical points as well as improved the data visibility in Fig. 2A

2. The authors are well-versed in aging, yet, in Figure 3, refer to 12-month-old mice as old. This is not an appropriate comparison, and use of tissue from 24+-month-old mice is needed.

RE: The reviewer did not seem to correctly understand the rationale of this experiment, as we meant to compare the phenotype of FAPs from 12 month old mdx mice with that of the corresponding chronological age (12 month old wild type counterpart) to show that the features of senescence observed in mdx FAPs are indeed a consequence of disease progression (but not chronological aging), as they are not observed in wild type counterpart at the same age. That 24 month old "geriatric" mice develop classical features of senescence in few cells of various tissues has been already published by others and it is not relevant to our manuscript. This conclusion also applies to the issue that classical cellular senescence develops in only few cells within a tissue, which is clearly not the case of disease-associated features of senescence that instead we show uniformly develops in muscle-derived FAPs as bulk. We have explained this concept in the text of the revised manuscript.

3. "It will be especially interesting to the senescence field if the mechanisms apply to other senescent cell types; HDACi..senomorphic to inhibit SASP." This comment was not addressed. A relatively straightforward experiment in IMR90s or the like would show relevance and increase potential impact of study.

RE: This reviewer continues to propose performing experiment in primary human IMR90 cultured fibroblasts induced to undergo conventional senescence in culture. However, we have explained that this is "an in culture model of senescence" that substantially differs from the disease-associated features of senescence by dystrophic FAPs. Although these experiments are definitely feasible (evaluation of HDAC activity and effect of HDACi in cultured IMR90 cells could be easily performed), we decided not to perform them, as we stand on our point that results from these experiments do not provide any meaningful data with respect to our story and would just reiterate conclusions already published that HDACi have a senolytic effects in condtions of model conventional cellular senescence (Vizioli et al. 2020).

4. Related to #3, "Is this mechanism specific to mdx FAPs or is it a more general mechanism in other cell

types? Since the authors have senescent IMR90 experiments (Figure 2J), can they confirm in this model?" Again, for example, do senescent IMR90 cells have higher HDAC activity? Is this a hallmark that may provide utility to the senescence field?

RE: See reply to point #3

5. The inquiry ""What is the HDAC activity in skeletal muscle from wild-type young and old mice?", also was not addressed. At 12 months, mice are mid-adult and senescence is a later life event (e.g., to our knowledge, not evident in most tissues until at least 18 months of age). Thus, the statement "deregulation of HDAC activity does not occur as a consequence of chronological aging" is not supported by the data.

RE: See reply to point # 2

6. For the question "The loss of activation of cell cycle genes in older mdx muscle by HDACi (Sup Fig 6) couldn't be explained by senescence. Senescent cells are always a very small fraction of all, which suggests there may be other mechanisms leading to the resistance to HDACi." The authors responded "Again, this concern refers to the issue related to the use of the term "senescence features" - see response to point 1 (as well as to point 1 of referee 2)". This concern is not just about labels. In older MDX samples, HDACi treatment did not activate gene expression compared to the young samples. Do the authors conclude ALL or most FAPs gain the "disease-associated features of senescence"? This would be contrary to what has been described in other tissues at advanced ages or in the context of disease. Could this instead reflect an altered biological state unrelated to senescence?

RE: See reply to point 2

Minor concerns: Presumably, "LamininB1" is meant to be LaminB1. Likely a typo, but please confirm appropriate antibodies were used for desired stains.

RE: Done

Inquiry: "The p21 expression level wasn't consistent between Figure 1K and Sup Figure 3. It's lower in old samples in Fig 1K but higher in Sup Figure 3." The authors responded: "see response to point 7 of reviewer 1", which was about a new figure 2, and it was not related to the wrong labels in Figure 1K. Please address.

RE: We corrected the figure labels

2. Statistical tests are provided for data represented in Figure 2B, E, J, K, and O, however, no error bars are provided for the YC samples. It is up to the journal to require plotting of individual data points, but error bars at least provide a sense of distribution.

RE: We have included error bars to YC samples

Dr. Pier Lorenzo Puri
Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute
Muscle Development & Regeneration
10901 North Torrey Pines Road
La Jolla, La Jolla, CA 92037
United States

Dear Dr. Puri,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

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

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Pier Lorenzo Puri

Journal Submitted to: EMBO J

Manuscript Number: EMBOJ-2021-108699

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 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.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size were not predetermined for a specific effect size. Experiments were performed with at least three biological replicates for each experimental point.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For all the experiments, at least three mice for each experimental point were used.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded from our analyses
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 randomization was used in this study.
For animal studies, include a statement about randomization even if no randomization was used.	No randomization was used in this study.
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.	No blinding was used in this study.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was used in this study.
5. For every figure, are statistical tests justified as appropriate?	Yes, all statistical tests used are described in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. We have used multiple statistical tools to test the significance, as described in the manuscript.
Is there an estimate of variation within each group of data?	Yes. Error bar was used to show variation. Variation within groups is presented as +/-S.E.M.

USEFUL LINKS FOR COMPLETING THIS FORM

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Is the variance similar between the groups that are being statistically compared?	Yes.
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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).	Citation and catalog number have been provided for all antibodies used in this study in methods and materials.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	IMR90 fibroblasts (from the Coriell Institute for Medical Research) are routinely tested for mycoplasma contamination.

* 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.	Normal wild-type C57/BL6 and C57BL6 mdx mice (DMD murine model) were purchased from Jackson Laboratories. Wild-type C57/BL6 were sacrificed at 1.5, 12 and 30 months of age. C57BL6 mdx mice were sacrificed at 1.5 and 12 months of age. Experiments involved a mix of males and females mice.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Mice were bred, handled and maintained according to the standard animal facility procedures and the internal Animal Research Ethical Committee in agreement to the Italian Ministry of Health approved experimental protocols and the ethiccommittee of the Fondazione Santa Lucia (FSL) approved protocols.
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.	The study is in compliance with the guideline.

E- Human Subjects

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

F- Data Accessibility

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

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA
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