EMBO Molecular Medicine

Dominant negative ATP5F1A variants disrupt oxidative phosphorylation causing neurological disorders

Sara Fielder, Marisa Friederich, Daniella Hock, Jessie Zhang, Liana Valin, Jill Rosenfeld, Kevin Booth, Natasha Brown, Rocio Rius, Tanavi Sharma, Liana Semcesen, Kim Worley, Lindsay Burrage, Kayla Treat, Tara Samson, Sarah Govert, Sara DaCunha, Weimin Yuan, Jian Chen, Jacob Lesinski, Hieu Hoang, Stephanie Morrison, Farah Ladha, Roxanne Van Hove, Cole Michel, Richard Reisdorph, Eric Tycksen, Dustin Baldridge, Gary Silverman, Claudia Soler-Alfonso, Erin Conboy, Francesco Vetrini, Lisa Emrick, William Craigen, Undiagnosed Diseases Network, Stephen Sykes, David Stroud, Johan Van Hove, Tim Schedl, and Stephen Pak

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Editor: Zeljko Durdevic

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

28th Apr 2025

Dear Dr. Pak,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the two reviewers who agreed to evaluate your manuscript. Both referees recognize interest of the study but also raise important concerns that should be addressed in a major revision. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

Further consideration of a revision that addresses reviewers' concerns in full will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

We would welcome the submission of a revised version within three months for further consideration. Please let us know if you require longer to complete the revision.

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Editor
EMBO Molecular Medicine

*****

When submitting your revised manuscript, please carefully review the instructions that follow below. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

1) A .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': (https://www.embopress.org/page/journal/17574684/authorguide#figureformat).

3) A .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) A complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/17574684/authorguide#submissionofrevisions). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

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6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and

database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/17574684/authorguide#dataavailability).

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.). See also 'Figure Legend' guidelines: https://www.embopress.org/page/journal/17574684/authorguide#figureformat

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10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc.

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- the results obtained and

- their clinical impact.

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13) A Conflict of Interest statement should be provided in the main text.

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15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines (https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods)

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***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

The authors have extensively tested their hyopthesis in a C. elegans model and in cultured patient fibroblasts. The choice of model organism is convincing.

Referee #1 (Remarks for Author):

Fielder et al present a compelling study in which they describe a novel mechanism by which heterozygous de novo dominant mutations in the ATP5F1A gene cause a mitochondrial disease in six patients carrying four different ATP5F1A variants. Most of the probands manifested with dystonia and/or spasticity and developmental delay.

This is a very carefully executed study that uses structural argument and a Caenorhabditis elegans animal model to demonstrate that mutations at the interacting surfaces / contact points of the alpha-subunit with neighboring structures of the F1F0-ATPase cause dysfunction and destabilization of complex V thereby reducing ATP synthesis, increasing uncoupling and reducing the mitochondrial membrane potential in patient fibroblasts. This stands in stark contrast to previously described recessive disease mutations in the same gene.

The authors show in the worm model that the dominant negative effect can be overcome by increasing the number of wildtype atp-1 mRNA copies in the cells. They also show that haploinsufficiency by removing one entire allele of the atp-1 gene (homolog of ATP5F1A) did not show any phenotype. Further they investigated in their worm model the presence of mitochondrial stress by visualizing the unfolded protein response with a hsp-6p::gfp reporter and the mitochondrial morphology.

I just have some minor suggestions:

[1] It would be of interest to know, whether the p.Leu335Pro (Ganetzky et al. 2022) and p.Thr334Pro, p.Val482Ala (Nasca et al. 2023) in ATP5F1B would also be located at contact point between alpha- und beta-subunits. The position of this mutation could be highlighted in Figure 2, giving more arguments in favor of the authors hypothesis on pathophysiology.

Please also locate the de novo mutations described by Zech et al. 2022 and Lines et al. 2021 on the cryo-structure of Figure 2.

[2] The fact that the here described dominant mutations are found at the contact points between the subunits should be mentioned in the abstract.

[3] As the majority of the here described patients presented with dystonia I would like to see their cranial MRI scans in the supplementary section of the manuscript, preferably the T2-weighted images of the basal ganglia/striatum and the brain stem.

Referee #2 (Comments on Novelty/Model System for Author):

This is an interesting well written paper on the discovery of several de novo heterozygous missense mutations in the gene encoding ATP5F1A subunit of mitochondrial atp-synthase. The alpha subunit encoded by ATP51A works in combination. with the beta subunit forming dynamically open-close structures moved by the central stalk of ATPsynthse during its rotation caused by the movement of the c-subunit cylinder actioned by the passage of protons through the oblique channel largely formed by the mtDNA subunit 6. The mutations found in this paper are in close proximity if not in junction with residues of the beta subunit. This suggests that the effects of the missense mutation in the alpha subunit can be expanded to malfunctioning of the beta subunit as well, therefore amplifying the effect of the mutation and explaining the dominant effect demonstrated also by different

and convincing approaches, including biochemistry, animal C. elegans) models, respirometry etc. The paper is multidisciplinary, experiment look impeccable, conclusions are convincing. I have a couple of questions: what was the main symptomatology prompting the Authors to suspect a mitochondrial disease? Was there a free window or the symptoms were congenital? Although this is not the case in the series presented here, other subjects with the mutation in the same gene showed improved age-related clinical conditions. Any hypothesis regarding this interesting phenomenon? Any hint that one of the patients of the present series may undergo the same clinical mitigation/improvement. Concerning the uncoupling phenomenon observed in the present series, shouldn't this determine any dysfunction in heat production, dysregulation of the body temperature, or any other phenomenon related to the discrepancy between increased oxygen consumption and lower than normal ATP production?

Referee #2 (Remarks for Author):

This is an impeccable pieace of clinical and experimental work with several new findings and interestn g conclusion about the damage of mitochondrial ATPsynthase through a convincingly demonstrated dominant negative effect.
I think the paperris worth to be published by EMBO Mol. Medicine.

Response to Referee's Comments

The authors would like to thank the reviewers for their thoughtful review of our manuscript. Below is a point-by-point response to each of the two reviewers' comments (in blue), with reference to specific revisions in the current version.

Referee #1

Fielder et al present a compelling study in which they describe a novel mechanism by which heterozygous de novo dominant mutations in the ATP5F1A gene cause a mitochondrial disease in six patients carrying four different ATP5F1A variants. Most of the probands manifested with dystonia and/or spasticity and developmental delay.

This is a very carefully executed study that uses structural argument and a Caenorhabditis elegans animal model to demonstrate that mutations at the interacting surfaces / contact points of the alpha-subunit with neighboring structures of the F1F0-ATPase cause dysfunction and destabilization of complex V thereby reducing ATP synthesis, increasing uncoupling and reducing the mitochondrial membrane potential in patient fibroblasts. This stands in stark contrast to previously described recessive disease mutations in the same gene.

The authors show in the worm model that the dominant negative effect can be overcome by increasing the number of wildtype atp-1 mRNA copies in the cells. They also show that haploinsufficiency by removing one entire allele of the atp-1 gene (homolog of ATP5F1A) did not show any phenotype. Further they investigated in their worm model the presence of mitochondrial stress by visualizing the unfolded protein response with a hsp-6p::gfp reporter and the mitochondrial morphology.

I just have some minor suggestions:

[1] It would be of interest to know, whether the p.Leu335Pro (Ganetzky et al. 2022) and p.Thr334Pro, p.Val482Ala (Nasca et al. 2023) in ATP5F1B would also be located at contact point between alpha- und beta-subunits. The position of this mutation could be highlighted in Figure 2, giving more arguments in favor of the authors hypothesis on pathophysiology. Please also locate the de novo mutations described by Zech et al. 2022 and Lines et al. 2021 on the cryo-structure of Figure 2.

As suggested by the reviewer, we have included the locations of the previously reported p.Arg207His variant in *ATP5F1A* (Lines et al. 2021 and Zech et al. 2022), and the p.Leu335Pro (Ganetzky et al. 2022) p.Thr334Pro, and p.Val482Ala (Nasca et al. 2023) variants in *ATP5F1B* on the cryo-structure. This information is presented in a new **Expanded View Figure (Fig. EV1)**. Six out of seven dominant variants reported so far are located at or near the contact points between ATP5F1A and ATP5F1B subunits highlighting the importance of protein conformation on coordinated interaction between the α- and β-subunits for ATP synthase function. Further

studies are required to better understand the structural and functional consequences of these variants and how they contribute to different clinical presentations.

[2] The fact that the here described dominant mutations are found at the contact points between the subunits should be mentioned in the abstract.

We have revised the **Abstract** to include that the variants are located at the contact points between the α- and β-subunits.

[3] As the majority of the here described patients presented with dystonia I would like to see their cranial MRI scans in the supplementary section of the manuscript, preferably the T2-weighted images of the basal ganglia/striatum and the brain stem.

MRIs for probands #1, #3, #5 and #6 were performed externally so images were not available. However, according to the clinical notes, MRIs of proband #1 and proband #3 were unremarkable (no comments on basal ganglia or brainstem). Proband #5, had stable right sided ventriculomegaly associated with posterior predominate white matter volume loss. Volume loss of the right hippocampus and suspected of the right mesial temporal lobe indicate concurrent right mesial temporal lobe sclerosis. Decreased perinasal sinus inflammatory changes now with opacification only in the right maxillary sinus. Proband #6 (latest MRI at age 5) was negative for acute intracranial, orbital or sinonasal abnormalities. Right-sided serous mastoid air cell effusion. MRIs for probands #2 and #4 were performed internally and are included in the revised manuscript as new **Appendix Fig. S1**. No abnormalities were noted.

Referee #2 (Comments on Novelty/Model System for Author):

This is an interesting well written paper on the discovery of several de novo heterozygous missense mutations in the gene encoding ATP5F1A subunit of mitochondrial atp-synthase. The alpha subunit encoded by ATP51A works in combination with the beta subunit forming dynamically open-close structures moved by the central stalk of ATPsynthse during its rotation caused by the movement of the c-subunit cylinder actioned by the passage of protons through the oblique channel largely formed by the mtDNA subunit 6. The mutations found in this paper are in close proximity if not in junction with residues of the beta subunit. This suggests that the effects of the missense mutation in the alpha subunit can be expanded to malfunctioning of the beta subunit as well, therefore amplifying the effect of the mutation and explaining the dominant effect demonstrated also by different and convincing approaches, including biochemistry, animal C. elegans) models, respirometry etc. The paper is multidisciplinary, experiment look impeccable, conclusions are convincing.

I have a couple of questions:

1) what was the main symptomatology prompting the Authors to suspect a mitochondrial disease?

Mitochondrial disease was not suspected in the probands as their presentations were relatively non-specific. Metabolic workup showed some elevated lactate and pyruvate, but overall profiles were not consistent with a known metabolic disorder. Mitochondrial disease was only suspected after the identification of candidate *ATP5F1A* variants following trio exome/whole genome sequencing and confirmed by functional studies.

2) Was there a free window or the symptoms were congenital?

For proband 4, the first noted symptom was developmental delays at age 1 year. For all other probands, symptom onset was congenital.

3) Although this is not the case in the series presented here, other subjects with the mutation in the same gene showed improved age-related clinical conditions. Any hypothesis regarding this interesting phenomenon?

This is certainly intriguing and not easily explained. Insufficient data are available to even generate a hypothesis. Prior to the report by Lines et al. 2021, transient symptoms in primary mitochondrial disorders were only noted for Reversible mitochondrial myopathy with COX deficiency and reversible hepatopathy in TRMU disorder. All other primary mitochondrial disorders are progressive. It will be interesting to hear from the authors of those cases what the long term follow up will be. There have been cases where symptoms have appeared many years after a transient period of apparent clinical resolution.

Any hint that one of the patients of the present series may undergo the same clinical mitigation/improvement.

Most of our probands are currently teenagers. Their conditions have either remained steady or worsened over time. None have shown signs of clinical improvement.

4) Concerning the uncoupling phenomenon observed in the present series, shouldn't this determine any dysfunction in heat production, dysregulation of the body temperature, or any other phenomenon related to the discrepancy between increased oxygen consumption and lower than normal ATP production?

Although hyperthermia was reported for the individuals with the uncoupling variant, *ATP5F1B* p.Leu335Pro (Ganetzky et al. 2022), dysregulation of body temperature was not reported for the probands in the current study. These observations suggest that uncoupling variants in core subunits of ATP synthase can present with different clinical phenotypes. Whether this is due to differences in the severity of the uncoupling or due to another mechanism remains to be determined. Of note, at the most recent United Mitochondrial Disease Foundation (UMDF) conference (June, 2025), other cases with some evidence of uncoupling were presented, none of them with hyperthermia.

Referee #2 (Remarks for Author):

This is an impeccable pieace of clinical and experimental work with several new findings and interestn g conclusion about the damage of mitochondrial ATPsynthase through a convincingly demonstrated dominant negative effect. I think the paperris worth to be published by EMBO Mol. Medicine.

We thank the reviewer for their positive comments.

18th Jul 2025

Dear Dr. Pak,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Authors: Please define the consortium "Undiagnosed Diseases Network" using the attached guidelines. Please note, that the consortium should also be entered as an author in our submission system, if it is included in the main author list, together with the contact details of a nominated consortium representative.
2) Please address all comments suggested by our data editors listed below:
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- Limit keywords to max. 5.
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- Add "Disclosure and competing interests statement". 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.
- In Methods, provide the statement that informed consent was obtained from all human subjects and confirm 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.
- In Methods, provide antibody dilutions that were used for each antibody.
- In Methods, a statistical paragraph should reflect all information that you have filled in the Authors Checklist, especially regarding randomization, blinding, replication.
- Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.
- Raw data from large-scale datasets e.g. WES, RNA sequencing or Mass Spectrometry should be deposited in one of the relevant databases and made freely available prior the publication of the manuscript. The journal encourages authors to provide access to genotype and clinical data with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. A signed statement of informed consent to publish any human clinical, large-scale, and genomic datasets must be obtained from each person (parents or legal guardians for minors) who appears in a study. Please check "Author Guidelines" for more information https://www.embopress.org/page/journal/17574684/authorguide#datadeposition Use the following format to report the accession number of your data:

The datasets produced in this study are available in the following databases:
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Please check "Author Guidelines" for more information.
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- Please correct the reference citation in the reference list. Where there are more than 10 authors on a paper, 10 will be listed, followed by "et al.". Also, please remove DOIs. DOIs should only be used for preprints and datasets that have not been published. Please check "Author Guidelines" for more information.
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3) Figures: Please mark all splices in Figure 6B. Use a visible line or space (e.g., a thin white or black line) to indicate where the blot was spliced.
4) Reagent Table: Please correct callout "Table S3" to "Appendix Table S3".
5) Appendix: Please place the figure legends underneath the corresponding figure, add page numbers to the table of contents, and correct the nomenclature to "Appendix Table S1" etc.
6) Funding: Please ensure that all funding sources listed in the Acknowledgements are entered into our system - currently the National Institute of Neurological Disorders and Stroke of the National Institutes of Health U01HG007709 and U01HG007942, Children's Discovery Institute, St Louis Children's Hospital Foundation etc. are only mentioned in the manuscript text.
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you agree with the publication of the RPF and as here, if you want to remove or not any figures from it prior to publication. Please note that the Authors checklist will be published at the end of the RPF.
10) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file).

I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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*** PLEASE NOTE *** As part of the EMBO Publications transparent editorial process initiative (see our Editorial at https://www.embopress.org/doi/pdf/10.1002/emmm.201000094), EMBO Molecular Medicine will publish online a Review Process File to accompany accepted manuscripts.

In the event of acceptance, this file will be published in conjunction with your paper and will include the anonymous 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 at contact@embomolmed.org.

When submitting your revised manuscript, please include:

1) a .docx formatted version of the manuscript text (including Figure legends and tables)

2) Separate figure files*

3) supplemental information as Expanded View and/or Appendix. Please carefully check the authors guidelines for formatting Expanded view and Appendix figures and tables at
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4) a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word file).

5) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting
- the medical issue you are addressing,
- the results obtained and
- their clinical impact.
This may be edited to ensure that readers understand the significance and context of the research.
Please refer to any of our published articles for an example.

6) Author contributions: the contribution of every author must be detailed in a separate section.

7) EMBO Molecular Medicine now requires a complete author checklist (https://www.embopress.org/page/journal/17574684/authorguide) to be submitted with all revised manuscripts. Please use the checklist as guideline for the sort of information we need WITHIN the manuscript. The checklist should only be filled with page numbers were the information can be found. This is particularly important for animal reporting, antibody dilutions (missing) and exact values and n that should be indicted instead of a range.

8) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one sentence bullet points that summarise the paper. Please write the bullet points to summarise the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

You are also welcome to suggest a striking image or visual abstract to illustrate your article. If you do please provide a jpeg file 550 px-wide x 300-600px high.

9) A Conflict of Interest statement should be provided in the main text

10) Please note that we now mandate that all corresponding authors list an ORCID digital identifier. This takes <90 seconds to complete. We encourage all authors to supply an ORCID identifier, which will be linked to their name for unambiguous name identification.

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*Additional important information regarding Figures

Each figure should be given in a separate file and should have the following resolution:
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Figures are not edited by the production team. All lettering should be the same size and style; figure panels should be indicated by capital letters (A, B, C etc). Gridlines are not allowed except for log plots. Figures should be numbered in the order of their appearance in the text with Arabic numerals. Each Figure must have a separate legend and a caption is needed for each panel.

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The authors addressed the remaining editorial issues.

25th Jul 2025

Dear Dr. Pak,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine. Please be aware that datasets deposited in public repositories should be freely available upon publication.

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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 EMBO Molecular Medicine.

Yours sincerely,
Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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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). 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, Material and Methods, Table S3 & S4

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	Yes	Reagents and Tools Table, Material and Methods

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, Material and Methods, Table S3 & S4

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.	Yes	Reagents and Tools Table, Material and Methods
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Material and Methods, Full clinical report (Appendix)
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.	Yes	Reagents and Tools Table, Material and Methods, Table S3
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Material and Methods

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	Material and Methods

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.	Yes	Full clinical report (Appendix)

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	Acknowledgments

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	Figure legends
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.	Yes	Material and Methods
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	
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	Material and Methods, Figure legends

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	Material and Methods, Figure legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure legends

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 number for approval.	Yes	Material and Methods
Studies involving **human participants**: 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.	Yes	Material and Methods
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 approva**l (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	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

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.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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 these guidelines.	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 Section
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	