EMBO reports

Regulated microexon alternative splicing in single neurons tunes synaptic function

Bikash Choudhary, Rebekah Napier-Jameson, and Adam D Norris

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Editor: Esther Schnapp

Transaction Report:

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

Dear Dr. Norris,

Thank you for the submission of your manuscript to EMBO reports. We have now received the comments from 2 referees, which are pasted below. Given that both reports are in fair agreement that this ms is interesting and can be published after minor revisions, I am making a decision now in the interest of time.

As you will see, the referees acknowledge the quality of your work and that the findings are interesting. They only have rather minor comments that should all be addressed.

I would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (10th May 2025). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

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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). See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) 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.

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- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

4) 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.

5) a complete author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines
<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please remember to provide a reviewer password if the datasets are not yet public. The accession numbers and database should be listed in a formal "Data Availability" section placed after Materials & Method (see also https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

9) Our journal also encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

10) Regarding data quantification (see Figure Legends: https://www.embopress.org/page/journal/14693178/authorguide#figureformat)

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- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,

- the nature of the bars and error bars (s.d., s.e.m.),

- If the data are obtained from n Program fragment delivered error ``Can't locate object method "less" via package "than" (perhaps you forgot to load "than"?) at //ejpvfs23/sites23b/embor_www/letters/embor_decision_revise_and_review.txt line 56.' 2, use scatter blots showing the individual data points.

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- Please also include scale bars in all microscopy images.

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12) All Materials and Methods need to be described in the main text using our 'Structured Methods' format, which is required for all research articles. According to this format, the Methods section includes a separate Reagents and Tools Table file (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section describing the methods using a step-by-step protocol format. The aim is to facilitate adoption of the methodologies across labs. More information on how to adhere to this format as well as a downloadable template (.docx) for the Reagents and Tools Table can be found in our author guidelines:
https://www.embopress.org/page/journal/14693178/authorguide#structuredmethods.

An example of a Method paper with Structured Methods can be found here: https://www.embopress.org/doi/full/10.1038/s44320-024-00037-6#sec-4

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I look forward to seeing a revised form of your manuscript when it is ready.

Yours sincerely,

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

Choudhary et al. have generated a fluorescent two-color splicing reporter for the unc-13 microexon and uncovered neuron-type-specific microexon inclusion. In addition to PRP-40, a component of the U1 snRNP, they demonstrate that EXC-7 and MBL-1 cooperatively facilitate unc-13 microexon inclusion by binding to adjacent sites in the downstream intronic region. Furthermore, the authors provide evidence of genetic and physiological interactions between PRP-40 and the RNA-binding proteins (RBPs). By employing genome editing to enforce microexon inclusion (UNC-13included) or skipping (UNC-13skipped), they further demonstrate that (i) unc-13 microexon skipping is required for chemotaxis regulated by the AWA neuron, and (ii) unc-13 microexon inclusion is essential for locomotory behavior and neuromuscular synaptic transmission, particularly in inhibitory motor neurons. Notably, the presence and regulation of this microexon appear to be conserved across the unc-13 gene family in nematodes, as well as in one homolog in mice, suggesting a conserved role in nervous system function.

Overall, this study is well-structured, the data are clear, and the manuscript is well-written. The authors effectively demonstrate the biological significance of neuron-type-specific microexon inclusion and exclusion through genome editing in vivo. Particularly striking is their finding that the hypersensitivity of exc-7; mbl-1 double mutants to aldicarb can be rescued by forced inclusion of a single microexon in a specific neuron class. This highlights the functional significance of this microexon as a key target of EXC-7 and MBL-1 among other alternative exons.

Specific Comments

1. Page 5, third paragraph, last two lines: The statement, "EXC-7 and MBL-1 cooperate in non-additive ways to facilitate unc-13 microexon inclusion," as well as similar descriptions in the subsequent text, may not be appropriate for non-quantitative results. Even though the ΔPSI values (Figure 7G) suggest a measurable effect, they may not be linearly correlated with RBP function or the effects of RBP loss. I suggest rephrasing to more precisely describe the nature of the observed interaction.
2. Page 9, first paragraph, lines 3-4: The statement, "the prp-40 mutant phenotype (complete microexon skipping) is dominant over either the exc-7 or mbl-1 mutant phenotypes," could be refined for clarity. Since the prp-40 mutant already exhibits complete microexon skipping (GFP expression), no further reduction is possible. As a result, it is expected that the exc-7; prp-40 and prp-40; mbl-1 double mutants display the same phenotype as the prp-40 single mutant. Typically, epistasis analysis is more informative when comparing mutants with opposing phenotypes.
3. Page 9, first section: The manuscript first describes genetic interactions, followed by physiological interactions. It may improve readability to reverse this order by presenting the physiological interaction of MBL-1 and PRP-40 in vivo (third paragraph) before discussing genetic interactions (first and second paragraphs). Since EXC-7 has already been shown to interact with PRP-40, this revised structure would provide a more logical flow of evidence supporting the proposed model.

Minor Points
1. Page 5, third paragraph, lines 3-4: In the sentence, "mbl-1 mutation results in an decrease in microexon inclusion," the article "an" should be corrected to "a."
2. Page 8, Figure 3A: The orange line above the downstream intron appears to be separated into two segments. If this represents a bipartite deletion, please clarify this in the Figure legend.

Referee #2:

In this very impressive paper, Choudhary et al carefully study the regulation of microexon inclusion in an important synaptic protein, unc-13. Using elegant and rigorous analysis of not just RNAseq data, but also in vivo splicing reporters, the authors discover cell-type specific splicing patterns, identify regulators for these patterns and demonstrate the functional relevance of these splicing pattern.

Both breadth and depth of this analysis are impressive and I'm hesitant, but nevertheless insistent in pointing out one glaring issue that the authors should consider addressing - and that's the expression pattern of exc-7 and mbl-1. This is a relevant request because one big picture questions is whether exc-7 and mbl-1 are together sufficient to explain microexon splicing patterns and/or whether other factors remaining to be identified and/or whether things don't presently make sense. Notably, the authors of this paper had described expression patterns of exc-7 and mbl-1 in previous papers and there are good reporters out there (fosmid-based and reporter alleles). It appears that exc-7 and mbl-1 are co-expressed in cholinergic MNs but NOT in GABAergic neurons - and that's based simply on published data. Yet the authors are remarkably vague in describing the changes of the microexon splicing reporter in VNC MNs in exc-7/mbl-1 double mutants - are effects restricted to cholinergic neurons? I'm suspicious that this may not be the case because of the authors reluctance to raise this issue - but I do think this deserves further discussion. I find the discussion of the authors in the Discussion section not helpful at all -> they only refer to scRNA data ("This model is supported by single-cell expression data [50] indicating that olfactory neurons (e.g. AWA, AWB) exhibit low or undetectable levels of both RBPs, while motor neurons (e.g. DA, DB) exhibit high expression levels"). Why not referring to the reporter data? It would also be nice to explicitly confirm with the reporter the absence in sensory neurons (just pick the dye-fillable ones).

Two minor issues:

Page 2 & Fig.1 legend: Ref 24 -> change to Ref 22; make sure to reference updated bioRxiv and update further once in proofs (the paper is currently at eLife).

Page 5: please state if molecular nulls

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Overall, this study is well-structured, the data are clear, and the manuscript is well-written. The authors effectively demonstrate the biological significance of neuron-type-specific microexon inclusion and exclusion through genome editing in vivo. Particularly striking is their finding that the hypersensitivity of exc-7; mbl-1 double mutants to aldicarb can be rescued by forced inclusion of a single microexon in a specific neuron class. This highlights the functional significance of this microexon as a key target of EXC-7 and MBL-1 among other alternative exons.

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We have now adjusted the text (lines 143-146) to match the qualitative nature of the data, removing the quantitative-sounding terminology of “additive” and “synergistic.” We have also tried to make the language more precise for Figure 7, where actual quantitative data is presented (lines 399-405).

2. Page 9, first paragraph, lines 3-4: The statement, "the prp-40 mutant phenotype (complete microexon skipping) is dominant over either the exc-7 or mbl-1 mutant

phenotypes," could be refined for clarity. Since the prp-40 mutant already exhibits complete microexon skipping (GFP expression), no further reduction is possible. As a result, it is expected that the exc-7; prp-40 and prp-40; mbl-1 double mutants display the same phenotype as the prp-40 single mutant. Typically, epistasis analysis is more informative when comparing mutants with opposing phenotypes.

We agree that the 'epistasis' experiments mentioned (Fig 3F) are fairly unsurprising. We have now modified the language, removing the word epistasis entirely, and simply concluding that the results are consistent with the already-discussed notion that PRP-40 recruitment is the necessary downstream molecular event required for microexon inclusion (lines 205-211), as supported by the more-informative biochemical and trans-heterozygote experiments.

3. Page 9, first section: The manuscript first describes genetic interactions, followed by physiological interactions. It may improve readability to reverse this order by presenting the physiological interaction of MBL-1 and PRP-40 in vivo (third paragraph) before discussing genetic interactions (first and second paragraphs). Since EXC-7 has already been shown to interact with PRP-40, this revised structure would provide a more logical flow of evidence supporting the proposed model.

We have rearranged the ordering of this section according to the reviewer's suggestions (Lines 196-220, Figure 3F-H, and Figure 3 legend)

Minor Points

1. Page 5, third paragraph, lines 3-4: In the sentence, "mbl-1 mutation results in an decrease in microexon inclusion," the article "an" should be corrected to "a."

Fixed!

2. Page 8, Figure 3A: The orange line above the downstream intron appears to be separated into two segments. If this represents a bipartite deletion, please clarify this in the Figure legend.

Yes, this is indeed a bipartite deletion, and we now clarify this in the figure legend (Lines 184-187).

Referee #2:

In this very impressive paper, Choudhary et al carefully study the regulation of microexon inclusion in an important synaptic protein, unc-13. Using elegant and rigorous analysis of not just RNAseq data, but also in vivo splicing reporters, the authors discover cell-type specific splicing patterns, identify regulators for these patterns and demonstrate the functional relevance of these splicing pattern.

Both breadth and depth of this analysis are impressive and I'm hesitant, but nevertheless insistent in pointing out one glaring issue that the authors should consider addressing - and that's the expression pattern of exc-7 and mbl-1. This is a relevant request because one big picture questions is whether exc-7 and mbl-1 are together sufficient to explain microexon splicing patterns and/or whether other factors remaining to be identified and/or whether things don't presently make sense. Notably, the authors of this paper had described expression patterns of exc-7 and mbl-1 in previous papers and there are good reporters out there (fosmid-based and reporter alleles). It appears that exc-7 and mbl-1 are co-expressed in cholinergic MNs but NOT in GABAergic neurons - and that's based simply on published data. Yet the authors are remarkably vague in describing the changes of the microexon splicing reporter in VNC MNs in exc-7/mbl-1 double mutants - are effects restricted to cholinergic neurons? I'm suspicious that this may not be the case because of the authors reluctance to raise this issue - but I do think this deserves further discussion. I find the discussion of the authors in the Discussion section not helpful at all -> they only refer to scRNA data ("This model is supported by single-cell expression data [50] indicating that olfactory neurons (e.g. AWA, AWB) exhibit low or undetectable levels of both RBPs, while motor neurons (e.g. DA, DB) exhibit high expression levels"). Why not referring to the reporter data? It would also be nice to explicitly confirm with the reporter the absence in sensory neurons (just pick the dye-fillable ones).

Thank you to the reviewer for pointing out this important matter that we did not do justice to in the manuscript. We have now analyzed both transcriptome data and fosmid reporters, and have added the following observations to the manuscript:

1. Indeed, ALL ventral cord motor neurons (both cholinergic and GABAergic) express only the skipped isoform in *exc-7; mbl-1* double mutants (added in line 142-143).
2. Revisiting our fosmid reporters generated for Thompson *et al.*, 2019, we find that indeed, *exc-7* expression is highly restricted to cholinergic, but not GABAergic, ventral cord motor neurons (now added to fig S6, and lines 462-465). *mbl-1*, however, while its signal is very bright in cholinergic neurons, is also expressed at low

but detectable levels in GABAergic neurons as well (added to fig S6). This is in contrast to *exc-7*, where no signal at all is detected in GABA motor neurons. Transcriptomic data from CeNGEN and other single-cell sequencing experiments also support this finding, with *exc-7* levels being high in cholinergic and absent in GABAergic, while *mbl-1* levels are high in both cholinergic and GABAergic motor neurons (Fig S6, and lines 462-465). Finally, we note in passing that the *mbl-1* fosmid used is a C-terminal protein-RFP fusion, and there is evidence for complex alternative C-terminal *mbl-1* isoforms (both exon skipping and intron retention) that could further contribute to the complex relationship between the fosmid expression levels, the transcriptomic data, and the functional cell-specific splicing regulation controlled by MBL-1.

3. Focusing on olfactory neurons, we provide evidence both from the fosmid reporters and the transcriptomic data that olfactory neurons in the head exhibit no/low levels of *exc-7* and *mbl-1* expression, while motor neurons exhibit high levels of both (Fig S6, and lines 462-465).

Two minor issues:

Page 2 & Fig.1 legend: Ref 24 -> change to Ref 22; make sure to reference updated bioRxiv and update further once in proofs (the paper is currently at eLife).

We have now changed the references as requested, and since the preprint is now reviewed and publicly available at eLife, we have updated to the current eLife version.

Page 5: please state if molecular nulls

This information has now been added (Lines 154-155).

Dear Dr. Norris,

Thank you for the submission of your revised manuscript. We have now received the enclosed comment from referee 2, who was asked to assess it, and I am happy to say that we can in principle accept your ms.

Only a few editorial requests will need to be addressed:

- Please remove the figures from the ms file. The figure legends should be kept at the end of the ms - first the main figure legends should be listed and then EV figure legends.

- The Disclosure and competing interest statement is provided twice: the one before the Acknowledgments is the correct one, but needs to be placed after the Acknowledgments.

- The REFERENCE format needs to be correct. It needs to be alphabetical, not numerical; et al needs to be used after 10 author names. Please use the EMBO reports style.

- The author CHECKLIST is missing responses to the pull down menus in cells D106-D108, please complete and send us the completed checklist.

- The individual EV Figures have incorrect titles - Figure S1, etc. They need to be corrected to Figure EV1, etc.

- The Reagents & Tools TABLE needs to be removed from the ms and uploaded as a separate file.

- Our routine figure check of revised ms detected some potential image duplications that are not explained in the figure legends:
Cell reuse between Figure 1F and Figure 2D.
Cell reuse between Figure 1F and Appendix Fig S4A.
Cell reuse between Figure 2D and Appendix Fig S4A.
Can you please clarify what happened? If these are indeed the same images, this needs to be justified and explained in the figure legends.

Figure Legends - Comments

- Please note that the exact p values are not provided in the legends of figures 4C, F, H; 5B, C, G, H; 6B, D, F, G, I, J; 7H. Please provide exact p-values are reasonable.
- Please indicate the statistical test used for data analysis in the legends of figures 4C, E, F, H; 5B, C, G, H; 6B, D, F, G, I, J; 7H
- Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers, and percentile in the legend of figure 7D
- Please note that information related to n is missing in the legends of figures 1C, 2A
- Please note that the error bars are not defined in the legends of figures 1C, 2A
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- Please mention in the title or abstract that this study is done on C. elegans.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). The synopsis image should provide a sketch of the major findings, like a graphical abstract. Please note that text needs to be readable at the final size. Please send us this information along with the final manuscript.

I look forward to seeing a final version of your manuscript as soon as possible.

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #2:

The authors have provided satisfying responses to my comments and I find the paper ready for publication, as is.

Greetings Esther,

Thank you for the comments. We believe we have now addressed all of the items you mentioned. New files are attached, and new text in the manuscript file is in red (text in response to the reviewers' previous comments remains in blue).

Sincerely,

Adam Norris

Adam D Norris
University of California, Riverside
United States

Dear Dr. Norris,

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.

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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.

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Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: Adam Norris
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Manuscript Number: EMBOR-2025-61184-T

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

Abridged guidelines for figures
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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:
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- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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	Materials and Methods

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	Materials 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	Materials and Methods

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.	Yes	Materials and Methods
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Materials 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.	Not Applicable	

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?	Not Applicable	

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, 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	Materials 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.	Yes	Results, Materials and Methods
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	Materials 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	Figure Legends, Materials and Methods
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.	Not Applicable	
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.	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 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?	Not Applicable	
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	