THE EMBO JOURNAL

mTORC1 senses glutamine and other amino acids through GCN2

Gianluca Figlia, Sandra Müller, Fabiola Garcia-Cortizo, Marilena Neff, Glynis Klinke, Gernot Poschet and Aurelio A. Teleman

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Transaction Report:

Please note that the manuscript was previously reviewed at another journal. As EMBO Press has a transfer agreement (including the identities of the referees) with that journal, revision was invited based on the reports from that previous external submission.
(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.)

Reviewer #1 (Remarks to the Author):

All my comments and concerns have been adequately addressed since the previous review and I would recommend this manuscript for publication.

Reviewer #2 (Remarks to the Author):

1) Fig. 1b. Because Gln starvation for 1 hour doesn't significantly decrease mTORC1 activity, it's hard to really see drastic changes here. It also appears that Glu, nicotinamide, and NH4+ rescues (at least to some extent) mTORC1 activity in Gln deprived conditions. In these conditions Asn is not in the media. Could just the addition of Asn activate mTORC1 in Gln starvation conditions? Asn has previously been shown by many groups to regulate mTORC1. It could be helpful to look at other mTORC1 substrates in Fig. 1b-c to see a more dramatic effect.

2) It's not clear how the authors can conclude that both Rag-dependent and Rag-independent mechanisms are involved in glutamine sensing from Fig. 2c, Sup. Fig. 3e-h.

3) There are other pathways that regulate mTORC1 besides GCN2 and AMPK. It's not clear why these were the only to pathways looked at.

4) Fig. 2a. doesn't seem surprising since amino acid starvation activates GCN2. Fig. 2e. Does GCN2 KO cells block the decrease in mTORC1 activity when starving cells of other single amino acids (Leu, Arg, Met, Asn)?

5) Sup. Fig. 5e. There doesn't appear to be a change in mTORC1 activity after Gln starvation when looking at the Western.

6) Sup. Fig. 6e. The authors state in the text (page 11): "Sestrin-triple knockout cells did show resistance to mTORC1 inhibition after overnight glutamine removal." From the figure it doesn't appear to show full resistance to mTORC1 inhibition.

7) Fig. 3d. The Western doesn't appear to match the quantifications looking at mTORC1 activity.

8) Reviewer 2 - Point 10: The most crucial question is how does asparagine specifically regulate (molecular mechanism) GCN2, compared to the removal of other amino acids?

Reviewer Response - As we show in Figure 5, our results indicate that the GCN2-dependent mechanism we discover here is not specific for asparagine (or glutamine). Instead, it relays the depletion of virtually any amino acid to mTORC1. The particular dependence of glutamine sensing on asparagine in the first hour after glutamine removal reflects the fact that asparagine is already basally very low in cells (Figure 1d) and that it is the only amino acid that is entirely dependent on glutamine for its synthesis. Thus, when glutamine is removed, cells experience limitation of asparagine well before the levels of other amino acids become limiting (Figure 1d-e). However, removal of almost any amino acid leads to GCN2 activation (Figure 5). To avoid this potential misunderstanding, we have now changed the title of the manuscript as well as multiple parts of the text.
This appears confusing because the title has glutamine in it, and the experiments throughout the manuscript focuses on glutamine and asparagine. It appears that amino acid starvation in general initiates these pathways through GCN2.

Reviewer #3 (Remarks to the Author):

In this resubmission, the authors provided additional evidence that the Asn sensing by mTORC1 occurs in several other cell lines. They propose that this is likely due to low levels of Asn, although it is not entirely clear if this is true for most cells or whether the expression levels of ASNS also play a role in most cases. This would be interesting to address given that asparaginase is effectively used in ALL but it is not clear whether this treatment may also be effective in particular cancer cells (where mTORC1 may be sensitive to Q or N limitation).

The studies propose that mTORC1 inhibition during Q starvation occurs via sequential GCN2-dependent mechanisms whereby acute Q limitation is mediated via a Rag-dependent and eIF2a/ISR-independent mechanism, whereas the prolonged response occurs via GCN2/ATF4/Ddit4/Sestrin2 mechanism. While they examined Sestrin2 as a Leu sensor, it is not clear if this is true for the other amino acid sensors in terms of addback responses. They can either try to address this further or limit their conclusion to Leu sensing.

Overall, the studies have some interesting findings and clarify some issues in amino acid sensing by mTORC1 via GCN2. The key mechanisms underlying Asn sensing via GCN2 or the acute GCN2 signals mediating Q starvation remain unclear.

Reviewer #2 (Remarks to the Author):

1) Fig. 1b. Because Gln starvation for 1 hour doesn't significantly decrease mTORC1 activity, it's hard to really see drastic changes here. It also appears that Glu, nicotinamide, and NH4+ rescues (at least to some extent) mTORC1 activity in Gln deprived conditions. In these conditions Asn is not in the media. Could just the addition of Asn activate mTORC1 in Gln starvation conditions? Asn has previously been shown by many groups to regulate mTORC1. It could be helpful to look at other mTORC1 substrates in Fig. 1b-c to see a more dramatic effect.

The main point of the paper is that glutamine regulates mTORC1 activity via multiple mechanisms that act sequentially and additively (Fig. 4h). Each of these mechanisms contributes partially to mTORC1 inhibition upon Gln starvation, and some of them take several hours to kick in because they require transcriptional regulation (e.g. activation of ATF4 and expression of Ddit4). We dissect these sequential mechanisms by looking at various timepoints of Gln removal, and Figure 1 focuses entirely on the 1-hour response, which, by the nature of the biological system as described above, is partial. That said, we disagree that the response is not significant – as we show in the new panel in Figure 1c, mTORC1 activity drops to 60%, this drop is statistically significant and can be rescued only with asparagine, among the metabolites tested. Quantifications of the other immunoblots in this figure have now also been added (Figure 1e and Figure 1i).

2) It's not clear how the authors can conclude that both Rag-dependent and Rag-independent mechanisms are involved in glutamine sensing from Fig. 2c, Sup. Fig. 3e-h.

In the figures the Reviewer is referring to, we did three different manipulations that either pin Rag activity to be maximally high (DEPDC5 KO cells in Fig. 2c-d or cells with a constitutively active Rag complex in Supp. Fig. 3e-f) or to be completely off (Rag knockout cells in Suppl. Fig. 3g-h). In all three cases one can see that the cells still respond partially to Gln removal, meaning that part of the response is Rag-independent, but part of the response is gone, meaning that part of the response is Rag-dependent. This can easily be seen by comparing the red curves (mutant genotypes) to the control black curves in the quantifications and, we believe, unequivocally shows that both Rag-dependent and Rag-independent mechanisms are at play.

3) There are other pathways that regulate mTORC1 besides GCN2 and AMPK. It's not clear why these were the only to pathways looked at.

We do not think that the reviewer's comment is relevant. Although many other pathways converge on mTORC1, the Rag GTPases, GCN2 and AMPK are the only ones we are aware of which could be directly modulated by glutamine abundance. Moreover, we clearly show in Fig. 2e-f that in GCN2 knockout cells, all the response of mTORC1 to glutamine removal is gone. This means no other pathway is contributing.

4) Fig. 2a. doesn't seem surprising since amino acid starvation activates GCN2. Fig. 2e. Does GCN2 KO cells block the decrease in mTORC1 activity when starving cells of other single amino acids (Leu, Arg, Met, Asn)?

Yes - this is clearly shown in Figure 5a-d and explicitly discussed at length in the Results text. GCN2 KO cells show only partial inhibition of mTORC1 activity when starved of Leu, Arg, Met (and also Ser), and no inhibition at all when starved of all the other amino acids individually.

5) Sup. Fig. 5e. There doesn't appear to be a change in mTORC1 activity after Gln starvation when looking at the Western.

The pS6K band in the 2nd lane is less than in the 1st lane, and this is quantified for 5 biological replicates in Suppl. Fig. 5f showing a statistically significant drop. In lanes 3-4, in the presence of GCN2 inhibitor, pS6K does not drop, which is precisely the point of the figure.

6) Sup. Fig. 6e. The authors state in the text (page 11): "Sestrin-triple knockout cells did show resistance to mTORC1 inhibition after overnight glutamine removal." From the figure it doesn't appear to show full resistance to mTORC1 inhibition.

That is correct - the resistance is partial, in agreement with the main point of our paper which is that there are several mechanisms that contribute additively to mTORC1 regulation (Fig. 4h).

7) Fig. 3d. The Western doesn't appear to match the quantifications looking at mTORC1 activity.

The point of this figure is to show that loss of Ddit4 affects the response of mTORC1 to Gln removal during the later time-points of 2h, 4h and 8h. The quantification in Fig. 3e is from 6 biological replicates, one of which is shown in Fig. 3d. Nonetheless, also in the western blot in Fig. 3d one can clearly see that the pS6K band at 4h of Gln removal in the Ddit4 KO cells (lane 10) is higher than in the control cells (lane 4), as well as at 8 hours (lanes 11 versus 5).

8) Reviewer 2 - Point 10: The most crucial question is how does asparagine specifically regulate (molecular mechanism) GCN2, compared to the removal of other amino acids?

Reviewer Response - As we show in Figure 5, our results indicate that the GCN2-dependent mechanism we discover here is not specific for asparagine (or glutamine). Instead, it relays the depletion of virtually any amino acid to mTORC1. The particular dependence of glutamine sensing on asparagine in the first hour after glutamine removal reflects the fact that asparagine is already basally very low in cells (Figure 1d) and that it is the only amino acid that is entirely dependent on glutamine for its synthesis. Thus, when glutamine is removed, cells experience limitation of asparagine well before the levels of other amino acids become limiting (Figure 1d-e). However, removal of almost any amino acid leads to GCN2 activation (Figure 5). To avoid this potential misunderstanding, we have now changed the title of the manuscript as well as multiple parts of the text.
This appears confusing because the title has glutamine in it, and the experiments throughout the manuscript focuses on glutamine and asparagine. It appears that amino acid starvation in general initiates these pathways through GCN2.

Yes - indeed - our findings are more broadly applicable to how mTORC1 senses all amino acids, and not only glutamine. We have changed the title to make it more clear that our results are broadly applicable.

Reviewer #3 (Remarks to the Author):

In this resubmission, the authors provided additional evidence that the Asn sensing by mTORC1 occurs in several other cell lines. They propose that this is likely due to low levels of Asn, although it is not entirely clear if this is true for most cells or whether the expression levels of ASNS also play a role in most cases. This would be interesting to address given that asparaginase is effectively used in ALL but it is not clear whether this treatment may also be effective in particular cancer cells (where mTORC1 may be sensitive to Q or N limitation).

As we described in our previous response to the reviewer, we see that Gln is sensed via Asn in all the cell lines that we tested, both cancer cell lines and non-cancer cell lines. Hence this seems to be a general conclusion that applies to all cells. In ALL cells, which have low endogenous Asn synthesis, we would expect this to be the case even more-so.

The studies propose that mTORC1 inhibition during Q starvation occurs via sequential GCN2-dependent mechanisms whereby acute Q limitation is mediated via a Rag-dependent and eIF2a/ISR-independent mechanism, whereas the prolonged response occurs via GCN2/ATF4/Ddit4/Sestrin2 mechanism. While they examined Sestrin2 as a Leu sensor, it is not clear if this is true for

the other amino acid sensors in terms of addback responses. They can either try to address this further or limit their conclusion to Leu sensing.

It is unfortunately not clear what conclusion the reviewer is referring to by 'it is not clear if this is true'. Maybe the reviewer refers to the results shown in Figure 6, where we show that mTORC1 inhibition by GCN2 is released more slowly than mTORC1 inhibition through the dedicated leucine sensors Sestrin1/2/3. If this is the case, we have now adjusted the final section of the introduction and added “dedicated sensors Sestrin1/2/3” in the title of the paragraph corresponding to Figure 6 to make more clear that we tested this aspect only for leucine and its sensors. If the reviewer is referring to the contribution of GCN2 to sensing other amino acids, this is clearly shown in Fig. 5a-d and Suppl. Fig. 5i-j.

Overall, the studies have some interesting findings and clarify some issues in amino acid sensing by mTORC1 via GCN2. The key mechanisms underlying Asn sensing via GCN2 or the acute GCN2 signals mediating Q starvation remain unclear.

The mechanisms how GCN2 senses amino acid deficiency, including Asn deficiency, have been broadly studied - this is known to occur via sensing of uncharged tRNAs and by sensing ribosome collisions. While the exact mechanism how GCN2 acutely inhibits mTORC1 still needs to be elucidated, we provide nonetheless an important mechanistic insight by showing that this is mediated by the Rag GTPases downstream of the GATOR complex.

Dear Aurelio,

Thank you again for the submission of your amended manuscript (EMBOJ-2025-121219-T) to The EMBO Journal. Please accept my apologies for the protraction due to delayed expert input. We have carefully assessed your manuscript and the point-by-point response provided to the referee concerns that were raised during peer-review at a different journal. In addition, and as mentioned before, we decided to involve the previous referee #1 as an arbitrating expert to evaluate the revised version of your work, with respect to technical robustness, conceptual advance and overall suitability for publication in The EMBO Journal.

As you will see from his/her comment enclosed below, the arbitrating advisor is broadly in favour of the work stating the interest and value of your results and s/he is supportive of publication at The EMBO Journal.

We are thus pleased to inform you that we can offer to swiftly move forward towards acceptance of this work at The EMBO Journal.

We now need you to take care of a number of minor issues related to formatting and data annotation, which I will share shortly in a separate message, together with additional changes and requests by our production team for Source Data provision.

Please submit a revised version of the manuscript using the link enclosed below, addressing the advisor's comments.

As you might have seen on our web page, every paper at the EMBO Journal now includes a 'Synopsis', displayed on the html and freely accessible to all readers. The synopsis includes a 'model' figure as well as 2-5 one-short-sentence bullet points that summarize the article. I would appreciate if you could provide this figure and the bullet points.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal, I look forward to hearing from you and receiving your final revised version of the manuscript.

Kind regards,

Daniel

Daniel Klimmeck PhD
Senior Editor
The EMBO Journal

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EMBOJ-2025-121219-T

Referee #1, additional arbitrating comment:

'I am of the opinion that the results are novel, and the conclusions described within the manuscript are wholly justified by the data.

The mechanism underlying some of those results are unexplained, but the experiments conducted are to a high quality and the resulting observations are likely valid. All possible current understanding of how these pathways operate are unable to fully explain the carefully produced results seen within the manuscript.

In terms as to whether it is suitable for EMBO J. I would say the quality of the data and the novelty of the findings are of sufficient quality for publication in EMBO J. The mechanistic detail is lacking, but, as the manuscript does not present a model of how these observations may be explained (as there is no existing framework, given that experiments have been conducted which exclude facile models based on prior data), I feel this is fully justified. There may be another two papers' worth of systems/molecular cell biology/metabolic data necessary to fully elucidate the underlying cause of their results.

I, personally, would rather read a high-quality paper that ends with "this is new biology that requires further investigation" than a paper which fails to report novelty or include currently inexplicable results.'

Additional Correspondence **22nd May 2025**

Dear Aurelio,

Further to below message, please find the mentioned additional formatting requests on your study enclosed below.

Please let us know any time should there be questions related.

Best regards,
Daniel

Daniel Klimmeck, PhD
d.klimmeck@embojournal.org

Formatting changes required for the revised version of the manuscript:

>> Please provide the main manuscript text as .docx file.

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>> Adjust the title of the 'Competing Interests' section to 'Disclosure and Competing Interests Statement'.

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Methods should be after Discussion; References should be placed before the figure legends

>> Figures in separate files: figures should be uploaded as individual, high resolution figure files. The supplementary figures should be renamed "Figure EV1" - EV11 and also uploaded as separate files. Their legends should be placed after the main figure legends and under the heading "Expanded View Figure Legends".

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>> Figure callouts: please recheck callouts for Fig 3H and Suppl Table 2 in the main text.

>> Please add a Reagents and Tools table to the Methods section, as a separate file using the existing template in the Guide For Authors, listing key reagents, experimental models, software and relevant equipment.

>> Dataset EV legends: the supplementary tables should be renamed "Dataset EV1" and "Dataset EV2", and both files should

have legends added in a separate tab/worksheet, with the titles and short descriptions.

>> Please provide source data for the study as to the separate request e-mail. Source data should be uploaded as one (zipped) file per figure.

>> Funding: please enter all funding information into the list of funders in our online system and detail them in addition in the Acknowledgments section of the manuscript.

>> Data availability section: Please provide a URL for the PRIDE dataset and ensure privacy is released and the data is public.

>> Methods: Please specify if reblotting of Western nitrocellulose membranes was applied.

>> Data integrity checks: Please recheck annotation and display for Western Blot loading controls and samples in the following Figure panels:

Figure 2A, 2G
Figure 3D, 3F
Figure 4A, 4C, 4E
Figure 5G, 5I
Figure 6A, 6E
Supp. Figure 2F
Supp. Figure 3E, 3G
Supp. Figure 4A
Supp. Figure 5I
Supp. Figure 6G
Supp. Figure 7B, 7D, 7G
Supp. Figure 8A
Supp. Figure 9E
Supp. Figure 10A, 10C
Supp. Figure 11A, 11C

Comparisons between and stacked display of samples on/from different gels/blots are discouraged. If a 'representative' loading control is shown for multiple gels/blots, the intra-gel controls should be shown in the source data files and the figure legends should describe the data displayed accurately.

Please also consider below respective text from author guidelines regarding this issue.

Quantitative comparisons between samples on different gels/blots are discouraged, even if the samples derive from one experiment, as confounding factors reduce comparability. If unavoidable, the figure legend must state that the samples derive from the same experiment and that gels/blots were processed in parallel. Loading controls must be run on the same gel.

If a 'representative' loading control is shown for multiple gels/blots, the intra-gel controls should be shown in the source data files and the figure legends should describe the data displayed accurately. Quantitative comparisons require that the experimental setup produces data within the linear signal range, unless a titration is added for standardisation. Where experimental limitations prevent reliable quantitative comparisons, we encourage qualitative interpretation instead.

>> Consider additional changes and comments from our production team as indicated below:

- DAS:
1. Please note that the specific URL for PXD055740 dataset is not provided in the data availability statement.

- Figure legends:
Please note that the exact p values are not provided in the legends of figures 1F, G, E, I; 4G

Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

The authors addressed the editorial issues.

Dear Dr Teleman,

As discussed I hereby invite you for an additional minor revision of your manuscript files.

Please

** explicitly state in the Methods that '... The same samples were loaded on different gels and blotted in parallel with the indicated antibodies. Protein concentration was controlled by blotting a-tubulin on a separate gel. Sample volumes were controlled by subsequent Ponceau stain.'

** state in the Figure legends 'Protein concentration was controlled by blotting a-tubulin on a separate gel.'

** group/indicate blots deriving from the same gel in the SD with dashed lines.

Please let me us know if there are any questions related.

Best regards,

Daniel Klimmeck

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The authors addressed the remaining editorial issues.

2nd Revision - Editorial Decision **30th Jun 2025**

Dear Aurelio,

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript and concluded that the remaining minor concerns have been sufficiently addressed.

I am thus pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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

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On a different note, I would like to alert you that EMBO Press offers a format for a video-synopsis of work published with us, which essentially is a short, author-generated film explaining the core findings in hand drawings, and, as we believe, can be very useful to increase visibility of the work. Please see the following link for representative examples and their integration into the article web page:
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Please let me know, should you be interested to engage in commissioning a similar video synopsis for your work. According operation instructions are available and intuitive.

If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to The EMBO Journal.

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

Best regards,

Daniel

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EMBO Press Author Checklist

Corresponding Author Name: Gianluca Figlia & Aurelio A. Teleman
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2025-121219-T

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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

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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

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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?
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  - definition of 'center values' as median or average;
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Please complete ALL of the questions below.
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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	Data Availability

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, Materials and Methods

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Dataset EV2

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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)
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Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

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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.	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	Materials and Methods

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
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	
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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
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**.	Yes	Reference list