EMBO reports

ATR promotes mTORC1 activity via de novo cholesterol synthesis

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Thank you for the transfer of your manuscript to EMBO reports. We have now received the enclosed comments from 2 referees and given that both are in fair agreement I am making a decision on your ms now in order to save time.

As you will see, the referees acknowledge that the findings are interesting. However, they also have several suggestions for how your study could be strengthened and we think that all suggestions are good. While may be not all suggestions will need to be addressed, we do think that some further insight/data on how ATR impacts on mTORC1 activation will be required for the publication of your study here. I am happy to discuss a revision plan with you, either by email if you send me a proposed revision plan, or also a video chat would be fine. As you prefer. Please let me know how you would like to proceed.

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 (1st Mar 2025). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

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5) a complete author checklist, which you can download from our author guidelines . 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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Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

This study provides an intriguing look at how ATR regulates the mTORC1 pathway through cholesterol metabolism, bridging the DDR and cellular growth signaling. While the role of ATR in influencing mTORC1 under stress is known, this work highlights its significance in non-stressed conditions, particularly through cholesterol synthesis driven by LSS. The study reveals that ATR promotes mTORC1 activation independently of CHK1 and the TSC complex, with supplementation of cholesterol or lanosterol restoring mTORC1 activity after ATR inhibition. By facilitating mTOR localization to lysosomes, ATR underscores a novel metabolic mechanism with potential implications for cancer therapy and age-related processes like T-cell proliferation.
The manuscript is well-written, and its conclusions are generally well-supported by the data. However, a few points warrant further attention:
The connection between ATR and LSS is compelling but lacks mechanistic detail. It would be valuable to investigate whether ATR directly modulates LSS, perhaps via phosphorylation or transcriptional regulation.
The study heavily focuses on p16-deficient cells. Extending experiments to other cell types, such as immune or epithelial cells, or models with different genetic backgrounds, could establish whether the impact of ATR on cholesterol metabolism and mTORC1 is a generalizable phenomenon.
Given the emerging role of ATR in the cytoplasm, it would be worthwhile to test whether it interacts directly with lysosomal proteins such as LAMP2 or NPC1, which could add depth to the proposed mechanism.
Since DDR and mTORC1 are closely linked, exploring whether DNA damage influences the effect of ATR on cholesterol synthesis could provide deeper insights into the bidirectional relationship between these pathways.

Referee #2:

Tangudu and colleagues present an exciting connection between ATR and mTORC1 signaling, revealing cholesterol metabolism as a key intermediary. Their findings demonstrate that ATR promotes de novo cholesterol synthesis and mTORC1 activation by upregulating lanosterol synthase (LSS), independently of CHK1 and the TSC complex. Remarkably, the inhibition of ATR reduced mTORC1 activity, a defect rescued by lanosterol or cholesterol supplementation, emphasizing cholesterol's role in mTOR lysosomal localization and activation.
This study offers an exciting insight into how ATR and mTORC1 signaling are linked via cholesterol metabolism.

1) One fundamental point would be to address the molecular mechanisms underlying the regulation of LSS by ATR. Does ATR act through established DNA damage response (DDR) effectors, such as transcription factors like E2F1, or does it engage previously unrecognized regulatory pathways? Additionally, while ATR's potential cytoplasmic activity is noted, the possibility that ATR directly phosphorylates LSS to influence its stability or enzymatic function remains unexplored. Experimental validation of these mechanisms would significantly enhance our understanding of how ATR integrates DDR signaling with metabolic regulation.

2) While this study highlights cholesterol's role in mTORC1 activation, its broader implications in tumorigenesis warrant further discussion. Cholesterol also supports membrane synthesis in proliferating cells and contributes to steroid biosynthesis, both of which are crucial in cancer progression. Expanding the discussion to encompass these roles would highlight the ATR-LSS axis's multifaceted contributions to cancer biology.

3) Given prior work showing LYCHOS as a cholesterol-sensing mediator for mTORC1, examining whether LYCHOS is involved downstream of ATR could clarify how cholesterol signals are transduced to mTORC1.

4) ATR inhibitors are under clinical development, yet this study suggests potential metabolic side effects related to mTORC1 signaling and cholesterol synthesis. Expanding on the implications for therapy design, including combinatorial strategies to mitigate side effects, would add translational value.

5) While ATR is highlighted, ATM inhibition also impacts mTORC1, albeit less strongly. A more detailed exploration of the differential and overlapping roles of ATR and ATM in cholesterol metabolism and mTORC1 regulation could provide a more comprehensive understanding of these pathways and their interplay.

We would like to express our sincere gratitude to the referees and the Editor for their constructive and thoughtful review of our review submitted to *EMBO Reports.* We are grateful for the acknowledgment from the Editor and all Reviewers regarding the significance and impact of our research, including ***"the findings are interesting"*** (Senior Editor); **"*This study provides an intriguing look at how ATR regulates the mTORC1 pathway through cholesterol metabolism*"** (Reviewer #1); **"*This study offers an exciting insight into how ATR and mTORC1 signaling are linked via cholesterol metabolism*"** (Reviewer #2). This positive evaluation validates the scientific merit of our work in investigating that ATR promotes mTORC1 activity via *de novo* cholesterol synthesis.

We truly appreciate the critical role and effort of this set of knowledgeable and helpful reviewers. Our commitment to addressing the Reviewers' comments is reflected in the comprehensive point-by-point response outlined below and a **significant amount of new data presented in the revised manuscript**. We believe that these revisions have resulted in a more compelling, robust, cohesive, and scientifically sound manuscript connecting ATR and mTORC1 signaling.

Reviewer #1

Comment 1: The connection between ATR and LSS is compelling but lacks mechanistic detail. It would be valuable to investigate whether ATR directly modulates LSS, perhaps via phosphorylation or transcriptional regulation.

Response: We thank the reviewer for this thoughtful suggestion to deepen the mechanistic insights of our work. We have provided new experimental evidence that ATR directly modulates LSS through phosphorylation but not transcription, which we will detail below.

Regulation of LSS by ATR-mediated phosphorylation: LSS has 5 potential ATR phospho sites (S*/T*Q) (**Fig. S3F**). Unfortunately, there are no commercially available LSS phospho-specific antibodies. Therefore, to investigate whether ATR directly modulates LSS through phosphorylation, we immunoprecipitated LSS in p16 knockdown cells with or without ATR knockdown and assessed potential phosphorylation sites via immunoblotting using a Phospho-ATM/ATR Substrate antibody. We found that ATR knockdown reduces phosphorylation of LSS (**new Fig. 3H**). These data point to LSS as a direct ATR substrate, which has been noted in the revised text (**Page 9-10, Lines 212-214**). Further mechanistic studies to determine which residue(s) ATR phosphorylates and whether/how they affect LSS levels, activity, or localization will be interesting for future studies. We have discussed this in the revised text (**Page 13, 272-278**).

Transcriptional Regulation by ATR: To evaluate whether ATR directly modulates *LSS* through transcriptional regulation, we performed RNA-Seq on p16 knockdown cells with or without ATR knockdown. We observed a modest (<1.5 fold) increase in *LSS* expression upon knockdown of p16; however, ATR knockdown did not rescue this effect (**new Fig. S3E**). These data demonstrate that ATR does not transcriptionally regulate *LSS*.

Comment 2: The study heavily focuses on p16-deficient cells. Extending experiments to other cell types, such as immune or epithelial cells, or models with different genetic backgrounds, could establish whether the impact of ATR on cholesterol metabolism and mTORC1 is a generalizable phenomenon.

Response: We appreciate the reviewer's thoughtful suggestion. In addition to the p16-deficient melanoma cells, we also found that ATR regulates mTORC1 activation in HeLa cells (a human epithelial adenocarcinoma cell line), HEK293 cells (Human Embryonic Kidney cells, an epithelial cell line), and MEFs (Mouse Embryonic Fibroblasts) (**Fig. 1A-F, EV1,** and **EV2C**), suggesting that the mechanism of ATR mediating mTORC1 activity is a generalizable phenomenon. To better understand whether this is mediated via cholesterol in all cell lines, we inhibited ATR in these cell lines and supplemented them with cholesterol and assessed mTORC1 activation via S6K phosphorylation. Unexpectedly, we found that cholesterol rescues pS6K in both of our cancer cell models (**new Fig. 4A-B, 4E, 4G and Fig. EV4A-B**) while cholesterol did not rescue pS6K in normal cells (**new Fig. EV4C-D**). Together, these data have uncovered a surprising finding that ATR can regulate mTORC1 activity via cholesterol in cancer cells, whereas normal cells may have an alternative pathway. Although outside the scope of the current study, it is intriguing to speculate that this is due to lipid metabolic derangement or high basal replication stress of cancer cells. It is also possible that these cells take up less cholesterol. We have noted that this phenomenon seems to be generalizable to cancer but not normal cells (**Page 10, Lines 225-229**) and expanded on this interesting observation in the revised Discussion (**Page 13, Lines 282-286**).

Comment 3: Given the emerging role of ATR in the cytoplasm, it would be worthwhile to test whether it interacts directly with lysosomal proteins such as LAMP2 or NPC1, which could add depth to the proposed mechanism.

Response: We appreciate the reviewer for this intriguing comment regarding ATR in the cytoplasm. We found that ATR is upstream of LSS, which is not known to be at the lysosome. Interestingly, prior high throughput proteomics studies found LSS in or near the nucleus (MacDonald et al., 2024; Wang et al., 2017). Together, these data indicate it is likely that the ATR and LSS interaction does not occur at the lysosome. Moreover, we agree with the reviewer that further assessment of ATR and LSS localization would provide valuable information regarding the mechanism and may also point to why this mechanism is cancer cell specific. We did find that LSS is phosphorylated by ATR (**new Fig. 3H**; an experiment in response to Comment #1), and future studies will be aimed at probing whether this occurs in the nucleus. We have expanded on this idea in the revised Discussion (**Page 13, Lines 272-276**).

Comment 4: Since DDR and mTORC1 are closely linked, exploring whether DNA damage influences the effect of ATR on cholesterol synthesis could provide deeper insights into the bidirectional relationship between these pathways.

Response: We thank the reviewer for this suggestion. To explore whether DNA damage influences the effect of ATR on cholesterol synthesis, we induced replication stress in cancer cells by treating with hydroxyurea to activate ATR and quantified cholesterol using filipin staining. Indeed, inducing replication stress in cancer cells increased cholesterol levels, which was abrogated by ATR inhibition (**new Fig. EV3D-E**). These data provide important insights into the bidirectional relationship between the DNA damage and mTORC1 pathways. We also believe these data may provide additional evidence to explain why cancer cells, which have high basal levels of replication stress, have a more active ATR-cholesterol-mTORC1 signaling pathway than normal cells. This point has been added to the revised text (**Page 13, Lines 282-286**).

Reviewer #2

Comment 1: One fundamental point would be to address the molecular mechanisms underlying the regulation of LSS by ATR. **A)** Does ATR act through established DNA damage response (DDR) effectors, such as transcription factors like E2F1, or does it engage previously unrecognized regulatory pathways? **B)** Additionally, while ATR's potential cytoplasmic activity is noted, the possibility that ATR directly phosphorylates LSS to influence its stability or enzymatic function remains unexplored. Experimental validation of these mechanisms would significantly enhance our understanding of how ATR integrates DDR signaling with metabolic regulation.

Response: We thank the reviewer for this insightful suggestion. **Reviewer #1** had a similar question regarding the transcriptional regulation of *LSS* by ATR (Comment #1 above). Using RNA-Seq, we found that ATR does not affect *LSS* mRNA expression (**new Fig. EV4F**), demonstrating that ATR does not upregulate LSS through a transcriptional factor like E2F1.

The second question regarding whether ATR directly phosphorylates LSS is an intriguing one. LSS has 5 potential ATR phospho sites (S*/T*Q) (**Fig. EV4G**). Unfortunately, there are no commercially available LSS phospho-specific antibodies. Therefore, to investigate whether ATR directly modulates LSS through phosphorylation, we immunoprecipitated LSS in p16 knockdown cells with or without ATR knockdown and assessed potential phosphorylation sites via immunoblotting using a Phospho-ATM/ATR Substrate antibody. We found that ATR knockdown reduces phosphorylation of LSS (**new Fig. 3H**). These data point to LSS as a direct ATR substrate, which has been noted in the revised text (**Pages 9-10, Lines 212-215**). How this phosphorylation affects LSS remains to be explored. Further mechanistic studies to determine the residue(s) ATR phosphorylates and how that event direct affects LSS stability, activity, or localization will be interesting for future studies. We have discussed this in the revised text (**Page 13, Lines 272-276**).

Comment 2: While this study highlights cholesterol's role in mTORC1 activation, its broader implications in tumorigenesis warrant further discussion. Cholesterol also supports membrane synthesis in proliferating cells and contributes to steroid biosynthesis, both of which are crucial in cancer progression. Expanding the discussion to encompass these roles would highlight the ATR-LSS axis's multifaceted contributions to cancer biology.

Response: We thank reviewer for this comment. We have discussed these points in the manuscript and expanded the revised text by adding more discussion points to emphasize the ATR-LSS axis's multifaceted contributions to cancer biology, including tumor progression, tumor microenvironment reprogramming, and metastasis (**Page 14, Lines 296-306**).

Comment 3: Given prior work showing LYCHOS as a cholesterol-sensing mediator for mTORC1, examining whether LYCHOS is involved downstream of ATR could clarify how cholesterol signals are transduced to mTORC1.

Response: We thank reviewer for this suggestion. Unfortunately, due to unanticipated technical challenges, we have been unable to knockdown LYCHOS in our cell lines. In fact, we attempted this knock down experiment four independent times with 5 independent shRNAs. To our knowledge, LYCHOS is the only cholesterol sensor at the lysosome (Shin et al., 2022). Therefore, given that we observed increased mTOR at the lysosome in the context of high cholesterol (**Fig. 5**), it is probable that the cholesterol generated downstream of ATR is sensed by LYCHOS to activate mTORC1. We have clarified this point in the revised text (**Page 13-14, Lines 291-296**).

Comment 4: ATR inhibitors are under clinical development, yet this study suggests potential metabolic side effects related to mTORC1 signaling and cholesterol synthesis. Expanding on the implications for therapy design, including combinatorial strategies to mitigate side effects, would add translational value.

Response: We thank reviewer for this helpful suggestion. We expanded our discussion to address this thoughtful comment from the reviewer on implications of reduced mTORC1 activity downstream of ATR inhibitors, effects of cholesterol reduction on tumor growth and the tumor microenvironment, effects on the immune system and response to immunotherapies, and implications for treatment regimens and mitigating potential toxicities. We further discussed these points in the revised discussion section (**Pages 14-15, Lines 308-323**).

Comment 5: While ATR is highlighted, ATM inhibition also impacts mTORC1, albeit less strongly. A more detailed exploration of the differential and overlapping roles of ATR and ATM in cholesterol metabolism and mTORC1 regulation could provide a more comprehensive understanding of these pathways and their interplay.

Response: We thank reviewer for this intriguing comment. As the reviewer pointed out, in **Fig. 1 and 2D** we have shown that ATM knockdown and inhibition impacts mTORC1, albeit less strongly. To explore the role of ATM in cholesterol metabolism and mTORC1 regulation, we inhibited ATM in both control and TSC2 deficient cells and supplemented with cholesterol to assess mTORC1 activation via pS6K expression. We found that unlike ATR, which mediates mTORC1 via cholesterol in cancer cell lines, and independent of the TSC complex (**Fig. 1E, 3-4 and EV4A-B**), ATM mediates mTORC1 via the TSC complex (**Fig. 1G**), and ATM inhibition is not rescued by cholesterol even in cancer cells (**Fig. EV4B**). Together, our data demonstrate that ATR and ATMs have differential roles in cholesterol metabolism and mTORC1 regulation.

References

MacDonald, K.M., Khan, S., Lin, B., Hurren, R., Schimmer, A.D., Kislinger, T., and Harding, S.M. (2024). The proteomic landscape of genotoxic stress-induced micronuclei. Molecular cell *84*, 1377-1391.e1376.

Shin, H.R., Citron, Y.R., Wang, L., Tribouillard, L., Goul, C.S., Stipp, R., Sugasawa, Y., Jain, A., Samson, N., Lim, C.Y.*, et al.* (2022). Lysosomal GPCR-like protein LYCHOS signals cholesterol sufficiency to mTORC1. Science (New York, NY) *377*, 1290-1298.
Wang, J., Mauvoisin, D., Martin, E., Atger, F., Galindo, A.N., Dayon, L., Sizzano, F., Palini, A., Kussmann, M., Waridel, P.*, et al.* (2017). Nuclear Proteomics Uncovers Diurnal Regulatory Landscapes in Mouse Liver. Cell metabolism *25*, 102-117.

Dear Dr. Aird,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees and I am happy to say that both support its publication now. Only a few editorial requests will need to be addressed before we can proceed with the official acceptance of your manuscript:

- Please reduce the number of keywords to 5.

- Please correct the conflict of interest subheading to "Disclosure and Competing Interests Statement"

- There are 2 author name discrepancies: Naveen Kumar Tangudu in the ms vs. Naveen Tangudu in our online system; Katarzyna M. Kedziora in the ms vs. Kasia Kedziora in the online system. Please correct.

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I would like to suggest some minor changes to the abstract that needs to be written in present tense:

DNA damage and cellular metabolism exhibit a complex interplay characterized by bidirectional feedback. Key mediators of these pathways include Ataxia Telangiectasia and Rad3-related protein (ATR) and the mechanistic Target of Rapamycin Complex 1 (mTORC1), respectively. Previous studies established ATR as a regulatory upstream factor of mTORC1 during replication stress; however, the precise mechanisms remain poorly defined. Additionally, the activity of this signaling axis in unperturbed cells has not been extensively investigated. We demonstrate that ATR promotes mTORC1 activity across various cellular models under basal conditions. This effect is enhanced in cells following the loss of p16. Mechanistically, ATR promotes de novo cholesterol synthesis and mTORC1 activation through the phosphorylation and upregulation of lanosterol synthase (LSS), independently of both CHK1 and the TSC complex. Interestingly, this pathway is distinct from the regulation of mTORC1 by ATM and may be specific to cancer cells. Finally, ATR-mediated cholesterol increase correlates with enhanced localization of mTOR to lysosomes. Collectively, our findings demonstrate a novel connection linking ATR and mTORC1 signaling through the modulation of cholesterol metabolism.

I think it would be good to specify the cells used in your study a little more in the abstract. And to specify what p16 is.

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

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor

Referee #1:

The authors have adequately addressed the Reviewers' concerns/remarks.

Referee #2:

The authors have addressed my comments. I congratulate the authors on this excellent research article!

All editorial and formatting issues were resolved by the authors.

Katherine Aird
University of Pittsburgh School of Medicine
United States

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Esther Schnapp, PhD
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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.
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- 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?	Not Applicable	

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 section

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; Materials and Methods section

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; Materials and Methods section
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.	Yes	Materials and Methods section

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

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

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If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Materials and Methods section

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	

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
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 section
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	Figure and Materials and Methods section

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

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)
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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	Reagents and tools table; Materials and Methods 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**.	Yes	References