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Lenacapavir-induced capsid damage uncovers HIV-1 genomes emanating from nuclear speckles

Thorsten Müller, Severina Klaus, Vojtech Zila, Bojana Lucic, Carlotta Penzo, Svenja Nopper, Gonen Golani, Maria Anders-Össwein, Vera Sonntag-Buck, Anke-Mareil Heuser, Ulrich Schwarz, Vibor Laketa, Marina Lusic, Barbara Müller, and Hans-Georg Kräusslich

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Review Timeline:

Submission Date:	8th Jul 25
Editorial Decision:	2nd Sep 25
Revision Received:	17th Oct 25
Editorial Decision:	7th Nov 25
Revision Received:	10th Nov 25
Accepted:	13th Nov 25

Editor: Hartmut Vodermaier

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.)

Dr Hans-Georg Krausslich
Heidelberg, Ruprecht-Karls-Universitt
Abteilung Virologie
Universitt Heidelberg
Im Neuenheimer Feld 324
Heidelberg D69120
Germany

2nd Sep 2025

Re: EMBOJ-2025-121832
Lenacapavir-induced capsid damage exposes HIV-1 genomes from nuclear speckles.

Dear Dr. Krausslich,

Thank you for submitting your manuscript on Lenacapavir effects on nuclear HIV-1 core uncoating. I apologize for the considerable delay in getting back to you with a decision - we sent your study to three expert referees, but have so far (despite multiple reminders sent from our office) still only received the reports of two of them. Since both of them are in fair agreement, and to prevent further loss of time, I have chosen to contact you now with an invitation to start revising the study based on the overall supportive comments copied below. As you will see, referee 2's points are mostly focused on presentational aspects, while referee 3 additionally raises important concerns about insufficient functional follow-up investigation. Should you be able to adequately improve this aspect, for example by heeding the well-taken experimental suggestions of the reviewer, we would be pleased to consider a revised manuscript further for publication.

Please note that this remains at present still a preliminary decision, and that any specific issues raised by the outstanding third report (should it arrive within the next two weeks) may still need to be incorporated before finalization and resubmission of the work. I should also point out that our policy to allow only a single round of (major) revision makes it important to carefully respond to all points raised at this point. As always, competing work appearing during the course of the revision period will not affect our final decision on your study. Should you have any further questions linked to this decision, the referee report or the revision guidelines, please do not hesitate to contact me.

Thank you again for the opportunity to consider this work for The EMBO Journal, and I shall be in contact shortly to either send you the outstanding third report, or to finalize the decision without it.

Yours sincerely,

Hartmut Vodermaier

Hartmut Vodermaier, PhD
Senior Editor, The EMBO Journal
h.vodermaier@embojournal.org

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- the number (n) and nature (biological or technical replicate) of independent experiments underlying each data point
- Figures may not include error bars for experiments with n<3; scatter plots showing individual data points should be used instead.

3) Revised manuscript text (including main tables, and figure legends for main and EV figures) has to be submitted as editable text file (e.g., .docx format). We encourage highlighting of changes (e.g., via text color) for the referees' reference.

4) Each main and each Expanded View (EV) figure should be uploaded as individual production-quality files (preferably in .eps, .tif, .jpg formats). For suggestions on figure preparation/layout, please refer to our Figure Preparation Guidelines: http://bit.ly/EMBOPressFigurePreparationGuideline

5) Point-by-point response letters should include the original referee comments in full together with your detailed responses to them (and to specific editor requests if applicable), and also be uploaded as editable (e.g., .docx) text files.

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In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (1st Dec 2025). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

Link Not Available

Referee #2:

This study uses cutting edge approaches including CLEM, ET, STED and ANCHOR to characterize how high concentrations of HIV-1 capsid inhibitors lenacapavir (LEN) and PF74 affect uncoating of viral cores in the nucleus. These findings are not surprising, but they are of sufficient general interest to be published in a high-impact journal. I recommend the following improvements.
1. The abstract states: "...speckle-associated factors appear to be required to promote genome uncoating." I strongly suggest rephrasing this sentence because there is no evidence presented in the present study to indicate that the speckle proteins "promote" uncoating. In fact, I like their discussion, where they state: "Drug induced displacement of the CPSF6 coat could make the capsid more sensible to breakage by dsDNA or could expose a previously broken capsid lattice that had been stabilized by the outer CPSF6 coat." In other words, CPSF6 could potentially be counteracting or shielding the uncoating process, whereas reverse transcribed dsDNA could be a primary force behind breaking HIV-1 cores. Accordingly, I suggest stating in the abstract that speckle-associated factors could regulate uncoating of HIV-1 cores in the nucleus.
2. The title of the sections of Results is: "Nuclear speckle localization of capsids can be pharmacologically perturbed using LEN". Remove "pharmacologically", which implies that this is a primary antiviral mechanism of action of LEN. In this section they nicely

cite the previous work to state that LEN EC50 values are ~50 pM in several cell lines. Yet, ~10,000-fold higher concentration (500 nM) of LEN was needed to displace CPSF6 from nuclear subviral complexes in MDMs (Figure 2). Similarly, for the CLEM-ET experiments in Figure 5 they used 500 nM LEN. There is nothing wrong with using such high concentrations of LEN as an investigational probe to study HIV-1 biology but avoid using terms such as "pharmacological". Furthermore, I suggest clarifying once again in Discussion that observed capsid alterations were detected at LEN concentrations that substantially exceed antiviral EC50 values of the inhibitor.
3. A previous study (PMID: 36202818) examined the CPSF6 displacement from HIV-1 cores by LEN in TZM-bl cells and the reasons for the need of high LEN concentrations have been discussed in the indicated report. The present studies corroborate with the previous findings. However, PMID: 36202818, which is highly relevant to the present study, is not referenced or discussed.

Referee #3:

The manuscript by Mueller et al. investigates the pre-integration steps in the HIV-1 life cycle and specifically ask the question if reverse transcription of the viral genome, which is completed in the nucleus, is the key driver of uncoating of the viral core. The authors use elegant imaging techniques, combining super-resolution microscopy with correlative light and electron microscopy, and label different components of the virus to study a narrow window of time during the late steps of reverse transcription in the nucleus of primary macrophages. In agreement with previous reports, the authors localized HIV-1 capsid core clusters in nuclear speckels and showed that treatment with drugs lenacapavir (LEN) or PF74, which bind and alter the viral core structure, displaced host-cofactor CPSF6 from the capsid core. In addition, these drugs removed the viral cores from speckels simultaneously breaking them. Such damaged cores appeared to release nucleic acids from the narrow end, becoming more permeable to fluorescent molecules that label the viral genome once reverse transcription is nearly completed. The authors conclude that the late steps of reverse transcription take place in an intact core coated by CPSF6 in nuclear speckels and that LEN perturbs these normal events by prematurely breaking the cores.

The manuscript addresses an interesting topic, which is poorly understood. There is some controversy on the role of reverse transcription in uncoating, with some studies suggesting that the conversion of RNA into bulkier DNA is sufficient for uncoating and others suggesting that there are host-cofactors that help. The results shown here are of good quality and well-presented and the manuscript is clearly written. However, although some of the results are novel, the manuscript does not provide much information on the putative uncoating factor and lacks functional follow up.

1. One question that remains unaddressed is if the breaking of the cores in the nucleus by LEN affects function. The authors have a nice experimental set-up that is timed to detect the late steps of reverse transcription in the nucleus. They know that in these conditions nuclear import and reverse transcription are unaffected by the drugs. But is integration impaired? Can the breaking of cores in the nucleus be correlated with efficiency of integration? Perhaps this can be tested by Alu-LTR qPCR, or perhaps even by imaging.
2. A recent study (Ay et a. EMBO J. 2025 PMID: 39623137), which is cited by the authors, also shows that CPSF6 condensates protect and stabilize viral cores but Ay et al. found that reverse transcription reduces the number of apparently intact cores and, notably, observe similar filamentous-like structures protruding from their narrow end. Their interpretation is that reverse transcription per se induces uncoating. This discrepancy should be discussed.
3. Can the results (more cores with positive labelling for late DNA transcripts) also be interpreted to suggest that breaking of the cores promotes more efficient reverse transcription?
4. Figure 2e. It would be helpful to show the quantification of these images showing displacement of cores from speckels.

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We changed the wording as suggested to "*speckle-associated factors could regulate genome uncoating*".

2. The title of the sections of Results is: "Nuclear speckle localization of capsids can be pharmacologically perturbed using LEN". Remove "pharmacologically", which implies that this is a primary antiviral mechanism of action of LEN. In this section they nicely cite the previous work to state that LEN EC50 values are ~50 pM in several cell lines. Yet, ~10,000-fold higher concentration (500 nM) of LEN was needed to displace CPSF6 from nuclear subviral complexes in MDMs (Figure 2). Similarly, for the CLEM-ET experiments in Figure 5 they used 500 nM LEN. There is nothing wrong with using such high concentrations of LEN as an investigational probe to study HIV-1 biology but avoid using terms such as "pharmacological". Furthermore, I suggest clarifying once again in Discussion that observed capsid alterations were detected at LEN concentrations that substantially exceed antiviral EC50 values of the inhibitor.

We agree with the reviewer and have removed the word "pharmacologically" from the section heading. In the Discussion we added the following sentence to clarify the effect of different Lenacapavir concentrations: *"These alterations were detected at LEN concentrations that remove the CPSF6-layer from the hydrophobic binding pocket, and which substantially exceed antiviral EC50 values of the inhibitor."*

3. A previous study (PMID: 36202818) examined the CPSF6 displacement from HIV-1 cores by LEN in TZM-bl cells and the reasons for the need of high LEN concentrations have been discussed in the indicated report. The present studies corroborate with the previous findings. However, PMID: 36202818, which is highly relevant to the present study, is not referenced or discussed.

We thank the reviewer for pointing out this relevant oversight. In the revised manuscript, we added the citation to the following sentence: *"We hypothesize that not only the immersion of capsids into the phase-separated speckle environment (Xu et al, 2022), but also this specific*

subdomain localization is driven by the biophysical properties of the multimeric CPSF6 coat (Wei et al, 2022) encasing nuclear capsids."

We now also discuss this report in the Discussion section as follows: *"Anti-viral LEN activity at lower concentrations was previously attributed to binding of LEN to unoccupied hydrophobic pockets without displacing cellular cofactors (Wei et al, 2022)."*

Referee #3:

The manuscript by Mueller et al. investigates the pre-integration steps in the HIV-1 life cycle and specifically ask the question if reverse transcription of the viral genome, which is completed in the nucleus, is the key driver of uncoating of the viral core. The authors use elegant imaging techniques, combining super-resolution microscopy with correlative light and electron microscopy, and label different components of the virus to study a narrow window of time during the late steps of reverse transcription in the nucleus of primary macrophages. In agreement with previous reports, the authors localized HIV-1 capsid core clusters in nuclear speckels and showed that treatment with drugs lenacapavir (LEN) or PF74, which bind and alter the viral core structure, displaced host-cofactor CPSF6 from the capsid core. In addition, these drugs removed the viral cores from speckels simultaneously breaking them. Such damaged cores appeared to release nucleic acids from the narrow end, becoming more permeable to fluorescent molecules that label the viral genome once reverse transcription is nearly completed. The authors conclude that the late steps of reverse transcription take place in an intact core coated by CPSF6 in nuclear speckels and that LEN perturbs these normal events by prematurely breaking the cores.

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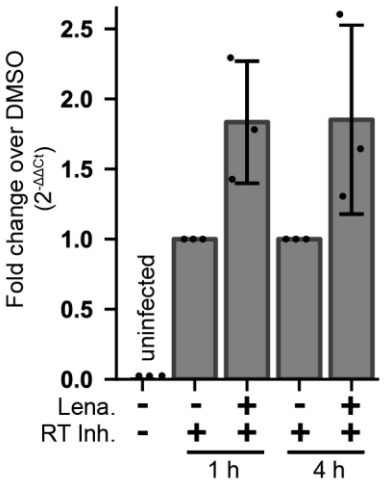
We thank the reviewer for suggesting this important experiment. Following the reviewer's suggestion, we have performed HIV-1 infection experiments in primary MDM from three independent donors and quantified the amount of integrated HIV-1 genomes following LEN treatment using Alu-LTR qPCR. MDM infected with wild-type HIV-1 were treated with LEN or DMSO for 1h or 4h at 70h post infection, similar to the previously shown experiments where the integration-deficient variant NNHIV had been used. To avoid further reverse transcription during the period of drug addition, the RT inhibitor Efavirenz was added to all samples together with LEN or DMSO.

This experiment showed a twofold increase of integrated genomes both after 1h and 4h incubation with LEN compared to the control. These results are presented in the revised manuscript as new panel G in Fig. 4, and we have pasted this panel below.



We have also added the respective methodology to the Methods section. Furthermore, to perform these challenging experiments within the short revision period, we turned to our colleagues in the institute with long experience in Alu-LTR qPCR. Because of their important contributions, we have expanded the list of authors accordingly.

Importantly, the results of this additional experiment confirmed that functionally complete and integration-competent HIV-1 cDNA genomes can be released from capsids not accessible to the fusion protein prior to LEN treatment. This conclusion could not be drawn from the ANCHOR detection using integration-defective viruses (as used throughout the manuscript) since the recognition sequence does not require full-length cDNA synthesis. Accordingly, we had previously written that complete or almost complete genomes could be released by LEN from closed capsid structures. This has now been revised based on the new data, and we are very grateful to the reviewer for leading us into this direction.

The results of this experiment are now included in the Results section of the manuscript and put into context in the Discussion:

Results: "*To expand this analysis to MDM and test whether HIV-1 cDNA revealed by LEN from CPSF6-coated nuclear capsids is competent for integration into host cell chromatin, we performed quantitation of integrated HIV-1 proviral sequences in LEN- or control-treated MDM using Alu-LTR qPCR. Since NNHIV is not competent for integration, we infected primary MDM from three different donors with VSV-G pseudotyped NL4-3 wild-type virus for 70 h before treating the cells with LEN or DMSO for 1h or 4h, respectively. To exclude the possibility that a change in the number of integration events after drug treatment could be due to continued nuclear import and reverse transcription, thereby confounding the result, we added EFV to all samples at the time of DMSO or LEN treatment, effectively blocking further reverse transcription. LEN treatment for 1h or 4h of LEN treatment led to a ca. twofold increase of Alu-LTR qPCR products (Fig. 4G), indicating that integration-competent, functionally complete proviral HIV-1 cDNA could be revealed from CPSF6-coated nuclear capsids by this treatment.*"

Discussion: "*Furthermore, LEN treatment also induced a twofold increase of integrated HIV-1 genomes when primary MDM were infected by wild-type virus. This effect did not require ongoing reverse transcription indicating that functionally complete and integration-competent genomic*

HIV-1 DNA had been synthesized inside nuclear capsids and could be revealed by LEN treatment. These observations are consistent with the ability of HIV-1 to evade activation of innate immune responses (Cingöz & Goff, 2019) unless cGAS-mediated sensing is induced by PF74 (Sumner et al, 2020; Ay et al, 2025) or LEN (Scott et al, 2025).

Our findings are consistent with reports suggesting that synthesis of long dsDNA inside the HIV-1 capsid is important for capsid breakage and genome uncoating, but suggest that reverse transcription may not be sufficient. ANCHOR detection in our system requires the dsDNA to be completed to at least 7.3 kbp. This DNA length is well above the critical limit of ~ 6 kbp dsDNA for efficient HIV-1 genome uncoating reported in a previous study (Burdick et al, 2024). Furthermore, the observed increase of integration events as shown by Alu-LTR qPCR and independent of ongoing reverse transcription shows that at least some of the nuclear capsids contain functionally complete viral genomes that were not accessible prior to LEN treatment."

2. A recent study (Ay et a. EMBO J. 2025 PMID: 39623137), which is cited by the authors, also shows that CPSF6 condensates protect and stabilize viral cores but Ay et al. found that reverse transcription reduces the number of apparently intact cores and, notably, observe similar filamentous-like structures protruding from their narrow end. Their interpretation is that reverse transcription per se induces uncoating. This discrepancy should be discussed.

Ay et al. used Immuno-Gold EM analysis of the infected THP-1 cell line in the presence or absence of the RT inhibitor NEV. They did not, however, analyse the effect of Lenacapavir or PF74 on the viral ultrastructure. Note that Immuno Gold-EM uses an extraction procedure that may impede the structural integrity of the sample. The authors showed an example of an empty core (they termed it "ghost") with electron density in close vicinity to the core. This diffuse density seems to be at the wide end of the core (see panel C below). In comparison, we observed, upon treatment with LEN, clear protrusions from the narrow ends of cones. We discussed what these could represent in the discussion section of the manuscript.

LEN treatment in our experiments consistently revealed elongated protrusions at the narrow end (Figure 5C right panel). Importantly, our observations are not in conflict with previous studies reporting that reverse transcription of sufficiently long cDNA is needed for efficient uncoating. We specifically state in our manuscript that the process of reverse transcription appears to be necessary, but - based on the data shown in the current report - may not be sufficient to induce immediate uncoating. In the Discussion section we point out the similarities between our work and the previous report by Ay et al. *"The observation of broken capsids without interior density is consistent with prior results in different cell lines that also reported incomplete capsid lattice remnants inside the nucleus of HIV-1 infected cells (Müller et al, 2021; Ay et al, 2025)."* For the discussion of reverse transcription of a sufficiently long dsDNA molecule being necessary for uncoating, we are referencing the report by Burdick et al., which provided direct and functional evidence analyzing HIV-1-based vectors of different genome lengths.

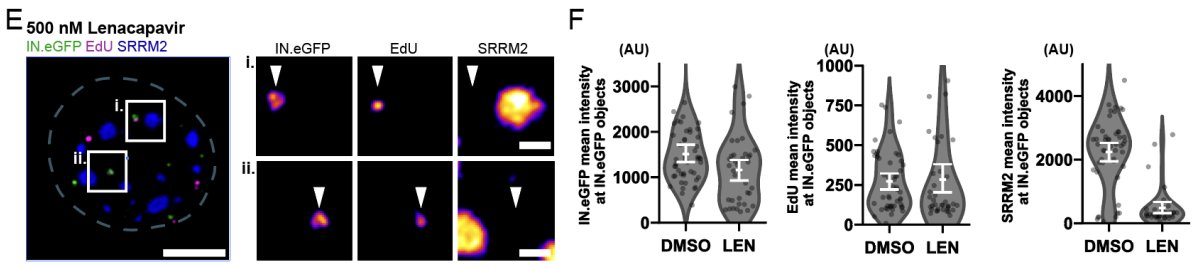
3. Can the results (more cores with positive labelling for late DNA transcripts) also be interpreted to suggest that breaking of the cores promotes more efficient reverse transcription?

We tested this hypothesis experimentally in Figure 4. The increased number of dsDNA/ANCHOR signals upon LEN treatment was also observed when further reverse transcription during treatment with capsid-targeting drugs was blocked by addition of an RT inhibitor, indicating that this increase was not due to promotion of reverse transcription. We have tried to make this clearer in the revised version by more explicitly stating that this hypothesis was experimentally tested and added the following sentence to the Results section.

"The appearance of new OR3 signals colocalizing with HIV-1 subviral complexes could also be explained if capsid breakage would promote more efficient reverse transcription, or if previously stalled reverse transcription complexes would resume reverse transcription to complete dsDNA upon opening of the capsid." This is then followed by the experimental result.

4. Figure 2e. It would be helpful to show the quantification of these images showing displacement of cores from speckels.

Following this suggestion by the reviewer, we have performed additional analyses and present the quantification in panel F of Fig. 2 in the revised version of the manuscript.



Dr. Thorsten G. Müller
Heidelberg University
Department of Infectious Diseases, Virology
Heidelberg 69120
Germany

7th Nov 2025

Re: EMBOJ-2025-121832R
Lenacapavir-induced capsid damage exposes HIV-1 genomes from nuclear speckles.

Dear Drs. Müller and Kräusslich,

Thank you for submitting your revised manuscript to The EMBO Journal. It has now been re-reviewed by the two original referees, who were both fully satisfied with the revisions. We shall therefore be happy to proceed with acceptance and publication of the study, as soon as the following editorial issues have also been adequately dealt with:

- Please consider a more accessible title for our broad general readership. In particular, I am not sure that one can "expose (something) from..." in English. A clearer phrasing may be e.g.
"Lenacapavir-induced capsid damage uncovers HIV-1 genomes emanating from nuclear speckles"?
Happy to discuss!

- On the abstract page, please pre-face the keywords with the header "Keywords", and please rename the Material and Methods into simply "Methods"

- Please carefully go through the reference list and make sure that each reference is complete with citation year, volume, and page/elocator numbers - such information is currently missing for some of them. Also, please adjust the format for citation of preprints as specified in our author guidelines:
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- Please upload each EV movie file packed together with its respective EV Movie title/legend text file into individual ZIP archives, one per movie.

- Finally, during routine pre-acceptance checks, our data editors have raised the following queries regarding figures, data, and legends; I would appreciate if you briefly answered to them in the cover letter of your final submission, and made the requested text modifications with changes/additions highlighted via the "Track changes" option, to facilitate our final checking.
1. Please note that the measure of center for the error bars needs to be defined in the legends of figures 4D-G
2. Please note that the dashed lines are not defined in the legend of figure 1B. This needs to be rectified.
3. Please note that the white arrow heads are not defined in the legend of figure EV1, EV2 A, E, F; EV3 A, C, E, F. This needs to be rectified.
4. Please note that the circles are not defined in the legend of figure 3E, G. This needs to be rectified.

I am returning the manuscript to you for a final round of minor revision, solely to allow you to make these modifications and upload the revised files. Once we will have received them, we should be ready to swiftly proceed with formal acceptance and production of the manuscript.

Yours sincerely,

Hartmut Vodermaier

Hartmut Vodermaier, PhD
Senior Editor, The EMBO Journal
h.vodermaier@embojournal.org

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2) Each figure legend must specify
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In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (5th Feb 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

Link Not Available

Referee #2:

All my comments have been fully addressed. It is an exciting study.

Referee #3:

The authors have addressed my concerns and have improved the paper. They have added data showing that LEN treatment stimulates integration by triggering the release of integration competent complexes from CPSF6 clusters. They have also made it clearer that EFV was used to block reverse transcription in certain experiments. The rebuttal letter was convincing.

EMBO Press Author Checklist

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Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2025-121832R

USEFUL LINKS FOR COMPLETING THIS FORM
The EMBO Journal - Author Guidelines
EMBO Reports - Author Guidelines
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Reporting Checklist for Life Science Articles (updated January
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures
1. Data
The data shown in figures should satisfy the following conditions:
- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple 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, Table 1 in 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	Reagents and Tools Table

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
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Reagents and Tools Table
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Material and Methods

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	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

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.	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.	Not Applicable	
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?	Not Applicable	

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	
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	