

Nested TAD hierarchy defines cohesion zones on replicated chromosomes

Zsuzsanna Takacs, Dmitry Mylarshchikov, Sofia Kolesnikova, Christoph Langer, Anton Goloborodko, and Daniel Gerlich

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(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. Daniel W. Gerlich
Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA)
Dr. Bohrgasse 3
Vienna 1030
Austria

20th May 2026

Re: EMBOJ-2026-124333
Nested TAD hierarchy defines cohesion zones on replicated chromosomes

Dear Daniel,

Thank you for submitting your study dissecting distinct cohesin activities to The EMBO Journal, and apologies for the delay in getting back to you with a decision. We sent your study to three expert referees, but despite multiple reminders sent from our office, have still not received comments from one of them. Since the other two reports are in good agreement, I chose to contact you with a final decision now, in order to prevent unnecessary further loss of time.

As you will see, referees 2 and 3 consider your study both important and convincing, and recommend its publication pending adequate clarification of a few particular issues noted in their reports. I am therefore inviting you to incorporate these suggestions and to resubmit a revised manuscript, ideally already taking into account our specific guidelines for preparation/formatting of revisions and file uploading (as detailed below and in our Guide to Authors) - adhering to them as closely as possible shall greatly facilitate editorial processing upon resubmission. Should you have any questions in this regards, please do not hesitate to contact us directly.

Thank you again for the opportunity to consider this work for The EMBO Journal, and I look forward to your revision.

With kind regards,

Hartmut

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

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2) Each figure legend must specify

- size of the scale bars that are mandatory for all micrograph panels
- the statistical test used to generate error bars and P-values
- the type error bars (e.g., S.E.M., S.D.)
- 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:
<https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>

- 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.
- 6) Please complete our Author Checklist, and make sure that information entered into the checklist is also reflected in the manuscript; the checklist will be available to readers as part of the Review Process File.
- 7) All authors listed as (co-)corresponding need to deposit, in their respective author profiles in our submission system, a unique ORCID identifier linked to their name. Please see our Guide to Authors for detailed instructions.
- 8) Please note that supplementary information at EMBO Press has been superseded by the 'Expanded View' for inclusion of additional figures, tables, movies or datasets; with up to five EV Figures being typeset and directly accessible in the HTML version of the article.
- 9) To facilitate reproducibility and cross-laboratory adoption of methodologies, please structure the Materials & Methods section as outlined in our guide to authors, including a completed Reagents and Tools Table.
- 10) Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be clearly noted in the figure legend and/or the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure. Finally, we generally encourage uploading of numerical as well as gel/blot image source data.

At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (18th Aug 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:

In this study, Takacs et al. investigate how cells maintain 3D genome structure after DNA replication, a process essential for gene regulation and DNA repair. It resolves the apparent conflict between the two roles of cohesin: extruding DNA loops and holding sister chromatids together. Using cell cycle synchronization, G2-specific protein depletion, sister-chromatid-sensitive Hi-C, the authors show that these functions of cohesin are spatially separated within chromosomal domains. Cohesin that mediates sister-chromatid cohesion accumulates at major boundary regions, forming "cohesion zones." In contrast, cohesin responsible for dynamic loop extrusion operates at all boundaries. The authors propose that this segregation is facilitated by CTCF boundary elements, which act as semi-permeable barriers. This partitioning allows replicated chromosomes to simultaneously support the dynamic folding needed for transcription and the stable tethering required for accurate sister-chromatid-templated DNA repair.

Overall, the study addresses a major question in the chromatin field, i.e. how loop extruding cohesin cope with cohesive cohesin on replicated chromosomes in interphase. Experiments are well designed and performed to high technical quality. The paper is nicely written and a pleasure to read. Publication is recommended.

I only have two minor points that the authors may wish to address.

Minor points

(1) The authors should examine whether cohesin/sororin distributions differ between A and B compartments. Likewise, it will be interesting to check whether there are specific patterns in centromere-proximal regions.

(2) The authors should mention in the main text the types of cells they used for their study.

Referee #3:

The ATPase cohesin complex belongs to the SMC family and fulfills two distinct functions: it establishes sister chromatid cohesion by topologically entrapping sister DNAs, and it also promotes DNA loop formation through the process of loop

extrusion. In mammalian cells, cohesin-mediated loop extrusion is essential for partitioning the genome into topologically associated domains (TADs) and for regulating DNA-dependent processes during interphase. Nevertheless, it remains unclear whether, and how, these two distinct activities of cohesin interact and regulate one another during interphase. In the present study, Takacs et al. combine elegant approaches, including ChIP-seq, Hi-C, and sister-chromatid-sensitive Hi-C, to investigate how two distinct populations of cohesin are distributed within nested TADs. The authors show that cohesin involved in sister chromatid cohesion preferentially accumulates at high-level boundaries, whereas loop-extruding cohesin occupies boundaries across all hierarchical levels. Their findings suggest that the translocation of extruding cohesin displaces cohesion-associated cohesin, thereby confining it to high-level boundaries enriched in CTCF sites. Overall, the manuscript is clearly written, technically sound, and presents important and conceptually interesting findings. I would therefore recommend acceptance after the authors address the following minor points :

Figure 4A. If loop extrusion concentrates cohesion-associated cohesin at high-level boundaries, one would expect that NIPBL inactivation would not only impair sororin accumulation at high-level boundaries, but also promote its accumulation toward low- and middle-level boundaries. However, according to Figure 4A, NIPBL inactivation appears to reduce sororin occupancy at all loci. The authors should clarify how this observation fits with their proposed model.

Figures S3, S4, and S5. In all analyses, the authors compare wild-type and depleted conditions. Including an untreated tagged condition would strengthen the study by allowing a better assessment of the specific effects of protein depletion and by determining whether the tagged proteins themselves affect 3D genome organisation.

Takacs and Mylarshchikov et al. – response to the reviewers

We thank the reviewers for their thorough assessment of our work and the constructive suggestions how to improve the manuscript. We have addressed all concerns as explained point-by-point below.

Referee #2:

In this study, Takacs et al. investigate how cells maintain 3D genome structure after DNA replication, a process essential for gene regulation and DNA repair. It resolves the apparent conflict between the two roles of cohesin: extruding DNA loops and holding sister chromatids together. Using cell cycle synchronization, G2-specific protein depletion, sister-chromatid-sensitive Hi-C, the authors show that these functions of cohesin are spatially separated within chromosomal domains. Cohesin that mediates sister-chromatid cohesion accumulates at major boundary regions, forming "cohesion zones." In contrast, cohesin responsible for dynamic loop extrusion operates at all boundaries. The authors propose that this segregation is facilitated by CTCF boundary elements, which act as semi-permeable barriers. This partitioning allows replicated chromosomes to simultaneously support the dynamic folding needed for transcription and the stable tethering required for accurate sister-chromatid-templated DNA repair.

Overall, the study addresses a major question in the chromatin field, i.e. how loop extruding cohesin cope with cohesive cohesin on replicated chromosomes in interphase. Experiments are well designed and performed to high technical quality. The paper is nicely written and a pleasure to read. Publication is recommended.

I only have two minor points that the authors may wish to address.

Minor points

(1) The authors should examine whether cohesin/sororin distributions differ between A and B compartments. Likewise, it will be interesting to check whether there are specific patterns in centromere-proximal regions.

We thank the reviewer for suggesting that we examine these regional chromosome features. We stratified TAD boundaries by their location within A- or B-compartment and, separately, by their location on chromosome arms or in centromere-proximal regions, and then analyzed Sororin and RAD21 distributions across the boundary hierarchy.

The preferential enrichment of Sororin at high-level relative to low-level boundaries was preserved in both A and B compartments and in centromere-proximal regions, while RAD21 showed only modest variation across the boundary hierarchy, consistent with our genome-wide analysis. These new data, described in the Results and presented in Fig. S2A and B, corroborate our central conclusion that cohesive cohesin preferentially accumulates at high-level boundaries.

(2) The authors should mention in the main text the types of cells they used for their study.

We thank the reviewer for pointing out this omission. All experiments in this study were performed in HeLa cells. We now state this explicitly when the experimental system is first introduced in the Results and provide the relevant details in the Methods.

Referee #3:

The ATPase cohesin complex belongs to the SMC family and fulfills two distinct functions: it establishes sister chromatid cohesion by topologically entrapping sister DNAs, and it also promotes DNA loop formation through the process of loop extrusion. In mammalian cells, cohesin-mediated loop extrusion is essential for partitioning the genome into topologically associated domains (TADs) and for

regulating DNA-dependent processes during interphase. Nevertheless, it remains unclear whether, and how, these two distinct activities of cohesin interact and regulate one another during interphase. In the present study, Takacs et al. combine elegant approaches, including ChIP-seq, Hi-C, and sister-chromatid-sensitive Hi-C, to investigate how two distinct populations of cohesin are distributed within nested TADs. The authors show that cohesin involved in sister chromatid cohesion preferentially accumulates at high-level boundaries, whereas loop-extruding cohesin occupies boundaries across all hierarchical levels. Their findings suggest that the translocation of extruding cohesin displaces cohesion-associated cohesin, thereby confining it to high-level boundaries enriched in CTCF sites. Overall, the manuscript is clearly written, technically sound, and presents important and conceptually interesting findings. I would therefore recommend acceptance after the authors address the following minor points :

Figure 4A. If loop extrusion concentrates cohesion-associated cohesin at high-level boundaries, one would expect that NIPBL inactivation would not only impair sororin accumulation at high-level boundaries, but also promote its accumulation toward low- and middle-level boundaries. However, according to Figure 4A, NIPBL inactivation appears to reduce sororin occupancy at all loci. The authors should clarify how this observation fits with their proposed model.

We thank the reviewer for this careful observation. We agree that NIPBL depletion reduces Sororin enrichment at all boundary classes rather than causing Sororin to accumulate at low- or mid-level boundaries (Fig. 4A,B). Instead, two observations indicate that Sororin-bound cohesin is redistributed away from boundary-centered peaks to become more broadly dispersed across chromatin.

First, chromatin fractionation shows that chromatin-bound Sororin remains high after NIPBL depletion (Fig. S4F), arguing against a general dissociation of Sororin-bound cohesin from chromatin. Second, the reduction in boundary-centered Sororin peaks is accompanied by an elevated baseline between boundary regions in the ChIP-seq tracks (Fig. 4A). Together, these findings indicate that the reduced Sororin signal at boundaries reflects spatial redistribution across chromatin rather than simple dissociation.

Consistent with this interpretation, Sororin enrichment becomes similar at low-, mid-, and high-level boundaries after NIPBL depletion (Fig. 4B). Thus, loss of loop extrusion abolishes the hierarchy-dependent distribution observed in wild-type cells: cohesive cohesin is no longer preferentially concentrated at high-level boundaries but is more broadly dispersed outside boundary-centered peaks. We have revised the Results to clarify this interpretation and to distinguish redistribution across chromatin from redistribution toward lower-level boundaries.

Figures S3, S4, and S5. In all analyses, the authors compare wild-type and depleted conditions. Including an untreated tagged condition would strengthen the study by allowing a better assessment of the specific effects of protein depletion and by determining whether the tagged proteins themselves affect 3D genome organisation.

We agree that untreated tagged controls are important for assessing baseline effects of the engineered cell lines. We have therefore added Hi-C contact maps from the untreated tagged lines alongside the wild-type and depletion conditions (Figs. S3J, S4E, and S5F). These data allow readers to directly evaluate the net baseline effects associated with each tagged line.

We retain wild-type cells as the primary reference because untreated degron-tagged cells can exhibit modest basal target degradation and/or tag-related effects and therefore do not necessarily represent an unperturbed state. Indeed, the untreated tagged lines show modest baseline differences in chromatin organization relative to wild type. Using wild type also provides a common reference across the different engineered lines and datasets. The depletion-associated phenotypes are substantially stronger than these baseline differences. We now explain this rationale in the revised manuscript.

Dr. Daniel W. Gerlich
Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA)

-
Dr. Bohrgasse 3
Vienna 1030
Austria

29th Jul 2026

Re: EMBOJ-2026-124333R
Nested TAD hierarchy defines cohesion zones on replicated chromosomes

Dear Daniel,

Thank you for submitting your revised manuscript for our consideration. I have now looked through the revision and your responses to the specific points raised by our two referees, and found the concerns well-addressed in the new version. We shall therefore be happy to accept the study for EMBO Journal publication, as soon as a few remaining editorial issues have been incorporated:

- Please list the contact email address for correspondence (i.e., your email) on the title page.
- Please carefully go through the reference list, which still contains several entries lacking full citation information such as volume or page/eLocator numbers.
- For preprint citations: Please adjust the in-text reference format according to "preprint: Miller et al, 2025"; and in the reference list, name of the platform plus DOI + preprint label - e.g. "bioRxiv doi: 1234/002.d01123 [PREPRINT]". Please make sure that the Preprint label is only used for real preprints, not for papers that have already been formally published in a classical journal.
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I am returning the manuscript to you for an ultimate round of minor revision, hoping you will be able to upload the modified final files by the end of this week, which would facilitate a swift proceeding with formal acceptance and production of the manuscript before the summer break.

With kind regards,

Hartmut

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

*** PLEASE NOTE: All revised manuscript are subject to initial checks for completeness and adherence to our formatting guidelines. Revisions may be returned to the authors and delayed in their editorial re-evaluation if they fail to comply to the following requirements. As a first step please read our guidelines for revised submissions:
<https://link.springer.com/journal/44318/submission-guidelines#cms-Revised-submissions>

1) Every manuscript requires a Data Availability section (even if only stating that no deposited datasets are included). Primary datasets or computer code produced in the current study have to be deposited in appropriate public repositories prior to resubmission, and reviewer access details provided in case that public access is not yet allowed.

2) Each figure legend must specify

- size of the scale bars that are mandatory for all micrograph panels
- the statistical test used to generate error bars and P-values
- the type error bars (e.g., S.E.M., S.D.)
- the number (n) and nature (biological or technical replicate) of independent experiments underlying each data point
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<https://media.springernature.com/original/springer-cms/rest/v1/content/27825798/data/v1>

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.

6) Please complete our Author Checklist, and make sure that information entered into the checklist is also reflected in the manuscript; the checklist will be available to readers as part of the Review Process File.

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9) To facilitate reproducibility and cross-laboratory adoption of methodologies, please structure the Materials & Methods section as outlined in our guide to authors, including a completed Reagents and Tools Table.

10) Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be clearly noted in the figure legend and/or the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure. Finally, we generally encourage uploading of numerical as well as gel/blot image source data.

At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (27th Oct 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:

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Corresponding Author Name: Daniel W. Gerlich
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2026-124333R

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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](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your

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The data shown in figures should satisfy the following conditions:

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

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
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 - 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.

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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)
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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, Methods

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Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

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, Methods
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	Methods

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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)
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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	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if external detailed step-by-step protocols are available.	Not Applicable	

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Yes	Figure legends, Methods
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?	Yes	Methods
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.		
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 legends, 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

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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 approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
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Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Not Applicable	
For tumor marker prognostic studies , we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For phase II and III randomized controlled trials , please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have primary datasets been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data Availability Section
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Yes	Data citations in the reference list