

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

|                                     |                                                                                                                                                                                                                                                                                                |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated                                                                                                                                                          |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | Image Lab Bio-rad <a href="https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z">https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z</a><br>Slidebook 6, 3i (Intelligent Imaging Innovations)<br>softWoRx (GE Healthcare)<br>Clariostar Control (BMG Labtech)                                                                                                                                                                                                                                                                        |
| Data analysis   | AlphaFold-Multimer v3.2.1 (Evans et al., 2021)<br>ChimeraX-1.5 (Pettersen et al., 2021)<br>GraphPad Prism Version 10 (GraphPad Software Inc <a href="http://www.graphpad.com">http://www.graphpad.com</a> )<br>Fiji Version 2.0.0-rc-69/1.52n (Schindelin et al., 2012 <a href="http://imagej.nih.gov/ij/">http://imagej.nih.gov/ij/</a> )<br>Image Lab (Bio-rad, <a href="https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z">https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z</a> )<br>Clariostar Data Analysis (BMG Labtech) |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

*Provide your data availability statement here.*

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | <input type="text" value="The relevant figure legends report the number of kinetochores or cells for each condition in which fluorescence values were collected. We did not predetermine sample size, nor we calculated sample size. The choice of sample size was based on previous examples in the field, and are consistent with extensive previous experimentation determining epistatic relationships within kinetochores."/> |
| Data exclusions | <input type="text" value="No data were excluded from the analysis"/>                                                                                                                                                                                                                                                                                                                                                               |
| Replication     | <input type="text" value="We indicate the number of biological/technical replicates of each experiment in the relevant figure legends"/>                                                                                                                                                                                                                                                                                           |
| Randomization   | <input type="text" value="For each immuno-fluorescence analysis, cells were chosen randomly for each quantification"/>                                                                                                                                                                                                                                                                                                             |
| Blinding        | <input type="text" value="The investigators were not blinded during data collection. The same investigators carried out the data collection and data analysis processes"/>                                                                                                                                                                                                                                                         |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

|                                     |                                                           |
|-------------------------------------|-----------------------------------------------------------|
| n/a                                 | Involved in the study                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies            |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                           |

## Methods

|                                     |                                                 |
|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

## Antibodies used

CREST/anti-centromere antibody (Antibodies, Inc., 1:200)  
 anti-CENP-C (guinea pig polyclonal, MBL-PD030, MBL, 1:1000)  
 anti-NDC80(HEC1) (mouse, clone 9G3, Gene-Tex, Inc., 1:1000)  
 anti-GFP (rabbit, made in-house, 1:1,000)  
 anti-tubulin (mouse monoclonal, Sigma-Aldrich, 1:8,000)  
 anti-MAD1 (mouse monoclonal, made in house, clone BB3-8, 1:100)  
 anti-GAPDH (rabbit, Sigma-Aldrich, 1:1000)  
 anti-MBP (mouse, NEB, 1:10000)  
 anti-BUB1 (rabbit, Abcam #9000, 1:2000)  
 anti-KNL1pMELT (rabbit, 1:1000, kindly shared by the Kops group, Hubrecht Institute, Utrecht)  
 anti-MAD1pT716 (rabbit, 1:1000, kindly shared by the Saurin group, University of Dundee)  
 anti-MAD1 labelled with DyLight550 (mouse, made in-house, Clone BB3-8, 1:200)

anti-human Rodamine Red (goat, Jackson ImmunoResearch, 1:200)  
 anti-mouse Alexa Fluor 488 (goat, Invitrogen A11001, 1:200)  
 anti-guinea pig Alexa Fluor 647 (goat, Invitrogen A-21450, 1:200)

anti-mouse HRP-conjugated (NXA931, Amersham, 1:5000)  
 anti-rabbit HRP-conjugated (NA934V, Amersham, 1:5000)

## Validation

Primary antibodies used for IF recognized a signal that disappeared upon RNAi depletion or in immunoblot on purified recombinant proteins.

CREST/anti-centromere antibody (Antibodies, Inc., 1:200): <https://www.antibodiesinc.com/products/anti-centromere-protein-antibody-15-234?srsltid=AfmBOoqybvdvr5brtiyTbtKoqwzFpwJOVJGws0CBkhrWSo0MrIjJE0H06>  
 anti-CENP-C: discontinued  
 anti-NDC80(HEC1) (mouse, clone 9G3, Gene-Tex, Inc., 1:1000): <https://www.genetex.com/Product/Detail/Hec1-antibody-9G3-23/GTX70268?srsltid=AfmBOoBLvJZBUTrXbOPaPtCrffvoNcBfRHdVrB29a-DWsiQ3FDFDghh>  
 anti-tubulin (mouse monoclonal, Sigma-Aldrich, 1:8,000): [https://www.sigmaaldrich.com/DE/de/product/sigma/t6199?srsltid=AfmBOoqcwZDI9hjcqaVLHZbQ1QUNu9CeyFpYlnxk\\_J\\_YV\\_IRjw01gsnn](https://www.sigmaaldrich.com/DE/de/product/sigma/t6199?srsltid=AfmBOoqcwZDI9hjcqaVLHZbQ1QUNu9CeyFpYlnxk_J_YV_IRjw01gsnn)  
 anti-GAPDH (rabbit, Sigma-Aldrich, 1:1000): [https://www.sigmaaldrich.com/DE/de/product/sigma/g9545?utm\\_source=google&utm\\_medium=cpc&utm\\_id=21480163361&utm\\_campaign=%7Bcampaignname%7D&utm\\_content=165772919038&utm\\_term=sigma+anti+gapdh&gad\\_source=1&gclid=CjwKCAjwktO\\_BhBrEiwAV70jXkh3kfiy2nntIzc4I9oQkQvJnkOiYESO4jelmcpq4nTrT1MNAc2\\_wxoCLEAQA vD\\_BwE](https://www.sigmaaldrich.com/DE/de/product/sigma/g9545?utm_source=google&utm_medium=cpc&utm_id=21480163361&utm_campaign=%7Bcampaignname%7D&utm_content=165772919038&utm_term=sigma+anti+gapdh&gad_source=1&gclid=CjwKCAjwktO_BhBrEiwAV70jXkh3kfiy2nntIzc4I9oQkQvJnkOiYESO4jelmcpq4nTrT1MNAc2_wxoCLEAQA vD_BwE)  
 anti-MBP (mouse, NEB, 1:10000): [https://www.neb.com/en/products/e8032-anti-mbp-monoclonal-antibody?srsltid=AfmBOopdl8gu6\\_zvEtmUVyPqcWqRzFaPP-VSziqEaENC1IAXPhaLL\\_50](https://www.neb.com/en/products/e8032-anti-mbp-monoclonal-antibody?srsltid=AfmBOopdl8gu6_zvEtmUVyPqcWqRzFaPP-VSziqEaENC1IAXPhaLL_50)  
 anti-BUB1: discontinued

anti-human Rodamine Red (goat, Jackson ImmunoResearch, 1:200): <https://www.jacksonimmuno.com/catalog/products/109-295-003>  
 anti-guinea pig Alexa Fluor 647: <https://www.thermofisher.com/antibody/product/A-21450.html?CID=AFLCA-A-21450>  
 anti-mouse Alexa Fluor 488: <https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001>

anti-mouse HRP-conjugated (NXA931, Amersham, 1:5000): <https://www.cytivalifesciences.com/en/us/shop/protein-analysis/blotting-and-detection/blotting-standards-and-reagents/amersham-ecl-hrp-conjugated-antibodies-p-06260>  
 anti-rabbit HRP-conjugated (NA934V, Amersham, 1:5000): <https://www.cytivalifesciences.com/en/us/search?q=Amersham%20ECL%20Rabbit%20IgG&t=coveo5819fbca>

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

|                                                                      |                                                                                                                                                                                                 |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | -Sf9 cells (GibcoTMInvitrogen Corporation, Cat. No. 11496-015)<br>-HeLa cells were a gift of Sara Barozzi (Imaging Facility, IFOM-IEO Campus, Milan, Italy) and were not further authenticated. |
| Authentication                                                       | None of the cell lines used were authenticated. The original commercial source of the HeLa cell line is unknown                                                                                 |
| Mycoplasma contamination                                             | Cell lines were regularly tested for mycoplasma contamination and the test found to be negative                                                                                                 |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | We did not use any misidentified cell line                                                                                                                                                      |

## Plants

|                       |                |
|-----------------------|----------------|
| Seed stocks           | Not applicable |
| Novel plant genotypes | Not applicable |
| Authentication        | Not applicable |