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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	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	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)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), 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	Commercially available software (LAS4000 Image Reader (Version 2.1, FujiFilm) was used for gel image analyses. The UMEX Sample-Scan software was used only for controlling the HS-AFM instrument and acquiring raw imaging data. It was not used to perform the quantitative calculations reported in this study.
Data analysis	Quantification and data analysis of gel images were performed using commercial or publicly available software including MultiGauge (Version 3.2, FujiFilm), Fiji, DataGraph (Version 5.5, Visual Data Tools). For generating HS-AFM images and videos, UMEX Viewer.exe was used. To analyze the binding angles of molecules on DNA and the radius of gyration, UMEX Viewer-for Line Analysis.exe was employed. The generation of I-form and O-form pseudo-AFM images was performed using UMEX AFM Simulator.exe. These executable files are publicly available in the following GitHub repository and can be freely used and verified by third parties: https://github.com/HS-AFM-developer/HS-AFM-software/tree/main User manuals are also uploaded in the same repository. The source code cannot be shared due to licensing restrictions; however, the Methods section includes the algorithms necessary for implementation, making it possible to reproduce the results using general-purpose software such as ImageJ or MATLAB. Regarding the generation of the Smc5/6 I-form to O-form using the FJC model, the source code used for the calculations and the resulting O-form PDB files are available on Zenodo at https://doi.org/10.5281/zenodo.21505466. By following the attached manual

(FJC_simulation_README.docx) and installing the program, the functionality of the software can be verified.

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

All data supporting the findings of this study are included in the manuscript and its supplementary information files.

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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

Life sciences study design

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

Sample size	Number of the experimental repeats was determined based on what is common in the filed and feasibility (e.g. Baris Y. et al., Nature, 2022, 606: 204-210; Minamino M. et al, Cell, 2023, 186: 837-849, Bauer BW et al, Cell, 2021, 184, 5448-5464)
Data exclusions	For biochemical experiments, no data were excluded except when technical problems invalidated the experiment, such as failure of a positive control or damage to the gel. For HS-AFM experiments, as described in the "High-speed AFM imaging" section of the Methods, approximately 80–90% of Smc5/6 molecules adsorbed on mica were observed as intact complexes, whereas the remainder became disrupted during imaging. For the analyses shown in Figs. 1 and 3, only molecules that remained intact and could be continuously tracked for several tens of frames were included. This criterion was defined to exclude molecules whose structural dynamics could not be reliably evaluated because of imaging-induced disruption.
Replication	The exact replicated number of each experiment was included in figure legends. All HS-AFM experiments were carried out at least three times. All bulk biochemical experiments were preformed at least twice with similar results. SDS-PAGE analyses of the purified proteins shown in Supplementary Fig. 1a,b, 3b, 7a were performed once. All purified proteins used in this study were also analysed by SDS-PAGE at each protein purification.
Randomization	Randomization was not applicable, as samples were measured in a consistent order and no treatment allocation was involved.
Blinding	Blinding was not applicable, as data collection and analysis were objective measurements and did not involve subjective assessment.

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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: We used the mouse anti-Pk (V5) antibody (anti-V5, MCA1360GA, BIO-RAD) for western blotting (1:2000 dilution) and for immunoprecipitation (2 uL 1 mg/ml anti-Pk / 10 uL protein A conjugated magnet beads (ThermoFisher)).

Validation: The specificity of mouse anti-Pk (for immunoprecipitation and western blotting) was validated in Murayama et al, Nature, 2024, 626, 653-660 (immunoprecipitation and western blotting using the purified cohesin) and in Borges et al., Chromosoma, 2013, 122:121-34 (western blotting using budding yeast cell extract).

Plants

Seed stocks: n/a

Novel plant genotypes: n/a

Authentication: n/a