Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis

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Faithful chromosome segregation depends on the ability of sister kinetochores to attach to spindle microtubules. The outer layer of kinetochores transiently expands in early mitosis to form a fibrous corona, and compacts following microtubule capture. Here we show that the dynein adaptor Spindly and the RZZ (ROD–Zwilch–ZW10) complex drive kinetochore expansion in a dynein-independent manner. C-terminal farnesylation and MPS1 kinase activity cause conformational changes of Spindly that promote oligomerization of RZZ-Spindly complexes into a filamentous meshwork in cells and in vitro. Concurrent with kinetochore expansion, Spindly potentiates kinetochore compaction by recruiting dynein via three conserved short linear motifs. Expanded kinetochores unable to compact engage in extensive, long-lived lateral microtubule interactions that persist to metaphase, and result in merotelic attachments and chromosome segregation errors in anaphase. Thus, dynamic kinetochore size regulation in mitosis is coordinated by a single, Spindly-based mechanism that promotes initial microtubule capture and subsequent correct maturation of attachments.

Fidelity of chromosome segregation in mitosis requires chromosomes to capture microtubules and achieve stable bioriented attachments before anaphase. To achieve this, chromosomes use an intricate kinetochore machinery. The core of the kinetochore in animals and fungi is composed of the CCAN and the KMN networks, which associate with centromeric chromatin and microtubules, respectively. The core kinetochore module is supplemented with proteins whose quantity at kinetochores changes during mitotic progression. These include proteins of the spindle assembly checkpoint (SAC), dynein recruiters (ROD–Zwilch–ZW10 (RZZ) and Spindly), dynein regulators (CENP-F/Nude1/Nde1/CLIP-170), the kinesins CENP-E and Kif2b, and modifiers of microtubule dynamics such as CLASPs.

The mammalian kinetochore exhibits great morphological plasticity during mitosis. While the core kinetochore module expands little, the more centromere-distal modules expand into large crescent shapes in early prometaphase and collapse into compact spherical structures in metaphase. Recent computational modelling suggested that expanded kinetochores promote spindle assembly, and that correct rotation of the sister kinetochores followed by their compaction reduces the risk of erroneous attachments. Electron micrographs of expanded kinetochores show a halo of low-density material referred to as the fibrous corona, which is absent from kinetochores that are bound by microtubules. Although immuno electron microscopy (EM) studies have shown several proteins to reside at the corona, it is unknown what proteins create the fibrous meshwork, impeding experimental interrogation of its functions. The dynamic behaviour of the fibrous corona correlates with that of the proteins that display early mitotic expansion and subsequent compaction. It is therefore likely that one or more of them can assemble into a fibrous meshwork and that regulation of the meshwork’s expansion/compaction will hinge on these components. A candidate is the RZZ complex. RZZ shows expected expansion/compaction behaviour, is required for recruitment of several outer-kinetochore components, and has a molecular architecture resembling that of membrane-coating proteins known to form polymeric states.

Results
Spindly recruits dynein to compact kinetochores after microtubule attachment. In agreement with previous observations, kinetochores expanded in nocodazole-treated cells shortly after nuclear envelope breakdown, as evidenced by the formation of ZW10-positive crescents (Fig. 1a, examples 1 to 3). Crescents grew during mitosis, and those of sister kinetochores nearly connected to form large, ring-shaped structures (Fig. 1a, example 4). Rings were absent from cells in which a spindle was allowed to form, and an attached kinetochore on a mono-oriented chromosome was more compact than its unattached sister kinetochore (Fig. 1a, example 5). In Xenopus laevis egg extracts, kinetochore size is regulated by mitotic phosphorylation. However, small-molecule inhibitors of Aurora B, PLK1 or MPS1, when added after expansion, did not cause compaction of kinetochores (Supplementary Fig. 1).

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Dynein drives poleward transport of kinetochore proteins\textsuperscript{28–31}. To investigate if dynein is required for kinetochore compaction, we examined its kinetochore adaptor Spindly\textsuperscript{32–35}. Spindly localizes to kinetochores through interaction with the RZZ complex via its farnesylated C-terminal CAAX box\textsuperscript{21,26,36–38} (Fig. 1b). Spindly in turn recruits dynein/dynactin through Spindly box (SB) and CC1 motif (Fig. 1b and Supplementary Fig. 2)\textsuperscript{21,26,36–38}. Our ConFeaX pipeline\textsuperscript{40} additionally identified a Qxx[HY] motif close to the CC1 box, which we here named the CC2 box (Fig. 1b). CC2-like boxes are also found in other dynein/dynactin adaptors (Supplementary Fig. 2). Recruitment of the dynactin subunit p150\textsuperscript{Glu}\textsuperscript{40} to kinetochores was compromised in cells expressing GFP-Spindly mutated in any of the three motifs (A24V (Spindly\textsuperscript{SB}), Y60A (Spindly\textsuperscript{CC1}) or F258A (Spindly\textsuperscript{CC2})) (Fig. 1c and Supplementary Fig. 3c), and was nearly

**Fig. 1 | Spindly recruits dynein to compact kinetochores after microtubule attachment.** a, Immunofluorescence images of ZW10 and HEC1 in HeLa cells treated with nocodazole or S-trityl-L-cysteine (STLC) (A, attached; U, unattached). The experiment was repeated at least three times with similar results. NEB, nuclear envelope breakdown. b, Overview of the secondary structure of human Spindly with predicted coiled-coils (grey bars) and disordered regions, and with sequence logos of four conserved motifs. See also Supplementary Fig. 2. Asterisks indicate residues mutated in this study. c, Quantification of the kinetochore levels of p150\textsuperscript{Glu}\textsuperscript{40} in nocodazole-treated HeLa cells transfected with siRNAs to Spindly and expressing the indicated GFP-Spindly variants. The graph shows the mean kinetochore intensity (± s.d.) normalized to the values of Spindly\textsuperscript{FL}. Each dot represents one cell: FL (n = 206 cells), ΔCC1 (n = 200 cells), ΔSB (n = 192 cells), ΔCC2 (n = 194 cells) pooled from seven independent experiments. ΔCC1, n = 122 cells pooled from four independent experiments. Asterisks indicate significance (one-way ANOVA followed by Tukey’s test; F (4, 909) = 238.5). ****P < 0.001. Representative images of cells are shown in Supplementary Fig. 3c. d,e Representative images (d) and quantification (e) of metaphase HeLa cells transfected with siRNA against Spindly and expressing the indicated GFP-Spindly variants. Stripping activity is based on the ability of Spindly variants to recruit dynein/dynactin. The experiment was repeated at least three times with similar results. See Spindly siRNA control in Supplementary Fig. 3b. g, Representative images of the morphology of Spindly and CENP-C in metaphase kinetochores expressing the indicated GFP-Spindly variants. Stripping activity is based on the ability of Spindly variants to recruit dynein/dynactin. The experiment was repeated at least three times with similar results.
abolished when all three motifs were mutated (Spindly\textsuperscript{ACC}) (Fig. 1c and Supplementary Fig. 3c). Dynein removes Spindly from attached kinetochores\textsuperscript{39,42-45,50,51}, and we found that the Spindly motif mutants defective in dynein recruitment were not removed from kinetochores of metaphase cells (Fig. 1d and Supplementary Fig. 3d). Strikingly, Spindly\textsuperscript{ACC}, which was the most compromised in recruiting dynein (Fig. 1c), frequently appeared as expanded structures that apparently bridged the two sister kinetochores (Fig. 1d). A similar phenotype was observed by deletion of the N-terminal 65 amino acids of Spindly (Spindly\textsuperscript{N50}) that contain the CC1 and CC2 boxes (Fig. 1f). These findings suggest that Spindly, via the recruitment of dynein, contributes to compaction of previously expanded kinetochores (Fig. 1g).

**Kinetochores expand by forming a structurally stable kinetochore sub-module.** We next examined the molecular basis for kinetochore expansion. Quantitative immunofluorescence imaging of kinetochores in nocodazole-treated cells showed that the extent
of expansion of different kinetochore modules inversely correlated with their proximity to the centromeric chromatin: RZZ, Spindly, and MAD1 occupied the largest volumes, followed by the KMN network (Fig. 2e). Interestingly, during analysis of expanded kinetochores under various experimental conditions, we noticed that brief (20 min) CDK1 inhibition caused the disappearance of expanded outer kinetochores and the appearance, close to kinetochores, of crescent-shaped rods or extended filaments containing Spindly, ZW10, MAD1 and CENP-E (Fig. 2c,d). Because these cells were still in mitosis (Fig. 2c), we interpreted the rods to be expanded kinetochore modules that had fully or partially detached from the core kinetochore (examples of partial detachments: bottom-right images of the HEC1 and MAD1 examples in Fig. 2d). These data suggest that the expanded kinetochore module is a relatively stable structure with a specific architecture, composition and regulation, distinct from other outer kinetochore modules such as the KMN network (Fig. 2e).

**Spindly and RZZ are essential for kinetochore expansion.** Immunofluorescence imaging and EM showed absence of kinetochore expansion and absence of a fibrous corona, respectively, in ZW10 RNAi cells (Fig. 3a,c,e,g and Supplementary Fig. 4a–c).
and all samples gave similar results. The experiment was repeated with two independent RZZS preparations, with at least three replicates, and all samples gave similar results. Control is empty beads. The intensity level of the green channel in the control was lower, GFP-Spindly immobilized on beads and incubated in the presence of mCherry-RZZ and farnesylated Spindly (RZZS). Control is empty beads. The intensity level of the green channel in the control was consistent with the position of the lysine cluster in the cross-link pattern (Fig. 5d), suggesting amino acids 259–305 have a crucial role in long-range interactions under the assembly reaction (Fig. 4a). Notably, RZZS oligomerization in the presence of GFP-Spindly-coated agarose beads resulted in association of a filamentous meshwork with the beads (Fig. 4b).

Expression of Spindly\textsubscript{FL} but not Spindly\textsubscript{FL} (full length) in interphase cells caused spontaneous formation of cytoplasmic filaments containing ZW10, Zwich and ROD (Fig. 4c). Spindly\textsubscript{FL} was abolished upon ZW10 RNAi or mutation of the Spindly CAAX box (C602A), showing it relied on RZZ and its interaction with farnesylated Spindly (Fig. 4c). Spindly\textsubscript{CCS}, which like Spindly\textsubscript{FL} is unable to bind dynin/dynactin, did not induce filament formation (Fig. 4c). Together, these results show that RZZS can polymerize in vivo and in vitro. This molecular behaviour is consistent with what is expected from proteins that are structural components of the expanded kinetochore.

**A structural conformation of Spindly prevents RZZS oligomerization.** The ability of Spindly to induce formation of a RZZS meshwork was greatly enhanced by deletion of its N-terminal 65 amino acids (Fig. 4c). To explain this, we needed to obtain a better understanding of the structure and regulation of Spindly. Residues 1–440 of Spindly are predicted to form a coiled-coil structure and the C-terminal region is probably disordered (Fig. 1b). Intramolecular dimensions of recombinant Spindly\textsuperscript{FL} as measured by small-angle X-ray scattering (SAXS) analysis were inconsistent with those of di-, tetra- or hexameric coiled-coil models (Fig. 5a). Spindly\textsubscript{FL} showed an overall shape with dimensions similar to Spindly\textsubscript{1–440} (Fig. 5a). Negative-stain EM on single Spindly\textsubscript{1–440} and Spindly\textsubscript{FL} particles showed that Spindly adopts elongated shapes with some characteristic ‘bends’ along its length (Fig. 5b). The EM analyses thus confirmed SAXS measurements, and suggested that purified Spindly may not form a typical coiled-coil. Finally, SAXS and SEC-MALLS experiments indicated that Spindly\textsubscript{1–440} and Spindly\textsubscript{FL} are dimeric and trimeric, respectively, in solution (Supplementary Fig. 6b,c).

Cross-link mass spectrometry of Spindly\textsubscript{FL} supported the possibility of a more complicated Spindly fold, and revealed the existence of various long-range intramolecular interactions (Supplementary Fig. 6d). Several of these interactions occurred around a cluster of cross-linked lysines at positions 276, 278, 283 and 284 (Supplementary Fig. 6d). Size exclusion chromatography (SEC) showed interaction between an N-terminal fragment of Spindly (Spindly\textsubscript{1–250}) and a Spindly fragment encompassing C-terminal sequences (Spindly\textsubscript{251–605}) (Fig. 5c), which was verified by surface plasmon resonance (SPR) (Fig. 5d). No interaction was observed between Spindly\textsubscript{1–250} and Spindly\textsubscript{606–605} (Fig. 5d), suggesting amino acids 259–305 have a crucial role in long-range interactions with the N-terminal Spindly helices. This was consistent with the position of the lysine cluster in the cross-link mass spectrometry analysis (Supplementary Fig. 6d). Notably, while a truncated Spindly lacking the N-terminal 274 amino acids (Spindly\textsubscript{1–274}) retained the ability to form cytoplasmic filaments in cells, this was abolished by the additional removal of 13 amino acids (Spindly\textsubscript{1–267}) (Fig. 5c). Thus, this region that is crucial for Spindly intramolecular interactions is also important for in vivo filament formation.

**Spindly stimulates RZZ- Spindly polymerization in vitro and in vivo.** Dimeric RZZ structurally resembles coat scaffolds, which can self-assemble into polymeric states, and it was speculated that RZZ may be a structural fibrous corona precursor and driver of kinetochore expansion\textsuperscript{13}. Purified recombinant RZZ, which assembles with 2:2:2 (ROD:Zwich:ZW10) stoichiometry\textsuperscript{21}, did not oligomerize, as assessed by direct visualization of mCherry-ROD (Fig. 4a). Addition of purified farnesylated Spindly (Spindly\textsubscript{FAR}), however, caused spontaneous oligomerization into filamentous structures at 30°C (Fig. 4a and Supplementary Movie 1). In vitro filament formation of RZZ-Spindly (RZZS) complexes could be prevented by addition of detergent, suggesting that hydrophobic interactions underlie the assembly reaction (Fig. 4a). Notably, RZZS oligomerization in the presence of GFP-Spindly-coated agarose beads resulted in association of a filamentous meshwork with the beads (Fig. 4b).

Expression of Spindly\textsubscript{FL} but not Spindly\textsubscript{FL} (full length) in interphase cells caused spontaneous formation of cytoplasmic filaments containing ZW10, Zwich and ROD (Fig. 4c). Spindly filament formation was abolished upon ZW10 RNAi or mutation of the Spindly CAAX box (C602A), showing it relied on RZZ and its interaction with farnesylated Spindly (Fig. 4c). Spindly\textsubscript{CCS}, which like Spindly\textsubscript{FL} is unable to bind dynin/dynactin, did not induce filament formation (Fig. 4c). Together, these results show that RZZS can polymerize in vivo...
Together, these data support the hypothesis that the Spindly N-terminal region imposes an autoinhibitory configuration that precludes RZZ-Spindly oligomerization.

**Release of Spindly autoinhibition promotes its interaction with RZZ.** We next performed SPR analyses with immobilized, purified RZZ to examine interactions of recombinant Spindly versions with the RZZ scaffold. In the absence of C-terminal farnesylation, ~two molecules of SpindlyFL weakly bound one (dimeric) molecule of RZZ with a \( K_D \) of ~1 \( \mu \)M (Fig. 6a). Farnesylation had little impact on overall interaction affinity in vitro but increased the number of Spindly molecules accumulating on RZZ. Similar observations were made with an alternative source of Spindly protein (Supplementary Fig. 7a). The farnesyl group thus appeared to target Spindly to multiple sites on RZZ or to other Spindly molecules already on RZZ under these conditions. Spindly lacking the N-terminal helices (Spindly54–605) associated with RZZ with higher affinity (~0.7 \( \mu \)M) and at higher stoichiometries: at least four molecules of Spindly could associate with RZZ. Notably, farnesylation no longer impacted interactions between Spindly54–605 and RZZ (Fig. 6a).
Because farnesylation of Spindly\textsuperscript{AN} was required for cytoplasmic filament formation (see Fig. 4c) but was dispensable for RZZ-Spindly\textsuperscript{AN} interactions when RZZ was on the SPR chip, we reasoned that farnesylation might facilitate initial RZZ-Spindly interactions that then promote additional, farnesyl-independent ones. To test this, we examined if RZZ complexes concentrated on mitotic kinetochores could recruit unfarnesylated Spindly molecules. As expected, mutation of the farnesylated cysteine (C602A) or treatment with the farnesyl transferase inhibitor lonafarnib (Lon) prevented Spindly\textsuperscript{FL} localization and kinetochore expansion (Fig. 6b–e). Removal of the N-terminal helices of Spindly (Fig. 7c–f) was independent of dynein (Supplementary Fig. 7c–f).

**MPS1 promotes RZZS meshwork formation and kinetochore expansion.** Our data so far suggest a mechanism for kinetochore expansion in which release of Spindly autoinhibition enables direct interactions with RZZ and other Spindly molecules to form the RZZS meshwork. Such a release is expected to occur at or near kinetochores in mitosis, and we reasoned that kinetochore-localized kinases may cause the triggering event. Unlike inhibition of Aurora B or PLK1 (Fig. 7a,b), inhibition of MPS1 before mitotic entry substantially affected Spindly localization and kinetochore expansion to an extent similar to Spindly depletion (Fig. 7a,b,c–f). Importantly, Spindly kinetochore levels and kinetochore expansion were rescued in MPS1-inhibited cells by deletion of the N-terminal helices of Spindly (Fig. 7c–f). To examine if MPS1 is sufficient to trigger Spindly-dependent RZZ oligomerization, we targeted MPS1 to cytoplasmic Spindly by co-expressing GFP-Spindly\textsuperscript{FL} and Spindly\textsuperscript{FL FAR}. Removal of the N-terminal helices of Spindly (Fig. 7c–f) was independent of dynein (Supplementary Fig. 7c–f).
DARPInGFP-MPS1Δ200. This MPS1 variant is unable to localize to kinetochores43 but can bind to the Spindly GFP tag by virtue of a GFP-binding DARPIn moiety44. Strikingly, when bound by active but not inactive MPS1, GFP-SpindlyFL was able to induce interphase filament formation (Fig. 7g), just like SpindlyAN could in the absence of MPS1 activity (Fig. 4a). We conclude that MPS1 kinase activity triggers RZZ-Spindly oligomerization by impacting the mechanism that releases inhibitory intramolecular Spindly interactions.

The expanded kinetochore module interacts with microtubule lattices. Previous ultrastructural work has suggested that fibrous coronas facilitate lateral microtubule capture45, and mathematical modelling has predicted the same for expanded kinetochores46. The persistent presence of an expanded kinetochore in cells expressing SpindlyΔΔCS and SpindlyAN provided a means to examine the functional relevance of kinetochore expansion and subsequent compaction. Imaging of congressed chromosomes in SpindlyAN-expressing cells showed that the expanded kinetochores of sister chromatids engaged with the sides of microtubules (Fig. 8a) and had lower Astrin levels, indicative of fewer mature end-on kinetochore–microtubule interactions (Supplementary Fig. 8a,b44). The microtubules that engaged expanded kinetochores were positive for PRCl (Supplementary Fig. 8c) and thus likely were anti-parallel bridging fibres (‘B’ in Fig. 8a and Supplementary Fig. 8c)47. Cold treatment caused depolymerization of PRCl-positive bundles (Supplementary Fig. 8c) and revealed the additional presence of stable, end-on microtubule interactions (Fig. 8b and Supplementary Fig. 8c). Super-resolution imaging by expansion microscopy (ExM)48 confirmed the co-occurrence of lateral and end-on attachments on the same kinetochore (‘L’ and ‘E’ in Fig. 8c) and showed an extensive microtubule surface capable of lateral microtubule interactions (example 1 in Fig. 8c). Live imaging of mCherry-tubulin and GFP-SpindlyAN further showed that the expanded kinetochore maintained interactions with the lattices of microtubules that go through rounds of growth and shrinkage (‘Gr’ and ‘Sh’ in example 2 of Fig. 8d and Supplementary Fig. 8d, and Supplementary Movies 4 and 5), and with the lattices of microtubules that move away from the sister kinetochores (example 1 in Fig. 8d and Supplementary Movie 3). In line with a proposed role for the fibrous corona49, the expanded kinetochore thus efficiently captures and maintains interactions with the lattices of dynamic microtubules (Fig. 8e).

Persistently expanded kinetochores cause attachment errors and chromosome missegregations. We noticed that the expanded kinetochores of sister chromatids in SpindlyAN cells frequently formed merotelic attachments (‘M’ in Fig. 8f and example 2 of Fig. 8f). Live imaging captured the following events: during successive cycles of capture-release, we observed short-lived events in which persistently expanded kinetochores cause attachment errors and chromosome missegregations.

Fig. 7 | MPS1 promotes RZZS meshwork formation and kinetochore expansion. a, Timeline of the treatments with kinase inhibitors and nocodazole (Noc) of the experiments shown in the rest of the figure. b, Representative images of ZW10 immunostainings of cells treated as indicated in a. The intensity levels of the magnifications in the insets were equalized to facilitate the direct comparison of the size of the kinetochores. The experiment was repeated at least three times with similar results. c–f, Representative images of cells treated with nocodazole and Cpd-5 as indicated in a. The graph shows the mean kinetochore intensity (±s.d.) normalized to the values of SpindlyFL. Each dot represents one cell: FL in DMSO (n = 87 cells), FL in Cpd-5 (n = 92 cells), AN in DMSO (n = 87 cells) and AN in Cpd-5 (n = 93 cells), pooled from three independent experiments. Asterisks indicate significance (one-way ANOVA followed by Tukey’s test; F (3, 355) = 255.6). ****P < 0.0001. g, Representative images of HeLa cells overexpressing GFP-SpindlyFL and an active (WT) or kinase-dead (KD) version of mCherry-MPS1 targeted to GFP-Spindly by DARPinGFP. The experiment was repeated two times with similar results.
merotelic attachments (see second 15 in example 1 of Fig. 8d and Supplementary Movie 3). Merotely is an important cause of chromosome segregation errors in both healthy and cancerous cells\(^\text{[61]}\). To examine if inability to compact kinetochores increases the frequency of chromosome segregation errors, we live-imaged Spindly mutant cells undergoing anaphase, induced by treatment with an MPS1 inhibitor to bypass the SAC silencing defect resulting from the absence of kinetochore dynein\(^\text{[18,44]}\). We analysed only cells that achieved full chromosome alignment so as not to bias for alignment problems associated with persistently expanded kinetochores (Fig. 8f). In contrast to cells expressing Spindly variants that allowed kinetochore compaction (Spindly\(^\text{FL}\) and Spindly\(^\text{ASS}\)), cells expressing Spindly\(^\text{ACS}\) showed a high rate of lagging chromosomes in anaphase, indicative of persistent merotelic attachments. Kinetochore compaction is therefore important to establish proper amphitelic attachments of sister chromatids.

**Discussion**

While our understanding of the assembly mechanisms of the core kinetochore has seen great progress in recent years\(^\text{[49]}\), the molecular mechanisms and functions of kinetochore size dynamics have remained enigmatic. Our data are consistent with a model...
in which kinetochore expansion, driven by Spindly and RZZ, enables efficient capture of microtubule lattices. We propose (Supplementary Fig. 8e) that before localizing to kinetochores, Spindly exists in an autoinhibited state that masks a surface required for polymerization of RZZ. Farnesylation-dependent targeting of Spindly to kinetochores by virtue of an initial interaction with ROD then enables kinetochore-localized MPS1 activity to (directly or indirectly) release Spindly autoinhibition and stimulate RZZ-Spindly polymerization. Inhibitory intramolecular interactions have also been observed in the Spindly-related dynein adaptor BICD225. Meanwhile, Spindly-driven kinetochore expansion has set the stage for future compaction by dynein once the expanded kinetochore module interacts with microtubules. Removal of the module may then promote exposure of the KMN network, facilitating end-on microtubule interactions and chromosome biorientation (Supplementary Fig. 8e).

The expanded kinetochore can remain relatively intact when disconnected from the underlying outer kinetochore (Fig. 2), and therefore appears to be a stable kinetochore sub-module similar to the CCAN and the KMN network, albeit more transient. We speculate that the RZZS meshwork is the underlying scaffold of the expanded kinetochore module. The properties of this meshwork resemble those of other cellular polymers that assemble through a ‘collaborative’ mechanism26. Collaborative filaments form on a supporting matrix, such as DNA or membranes, and frequently use hydrophobic interactions as a driving force for their assembly. Analogously, RZZ assembles on the core kinetochore in a manner dependent on BUB1 and KNL115,32, and subsequently forms a filamentous structure involving hydrophobic interactions, which is maintained even when its association to the core kinetochore is lost after brief CDK1 inhibition.

Pioneering ultrastructural studies during the second half of the twentieth century had observed that outer plates of expanded kinetochores have a fibrous corona (original works have been reviewed elsewhere27) and that at least CENP-E, dynein and CLASP1 are part of its constituents18-20. Because of their similar localization to expanded kinetochores, CENP-F, RZZ, Spindly and MAD1 are also likely fibrous corona constituents25,26 (Figs. 2 and 3). We now show that fibrous corona formation and kinetochore expansion both rely on RZZS (Fig. 3), implying they are mechanistically similar if not identical. This is consistent with absence of RZZS from metaphase kinetochores, which are compact and devoid of a corona25. It will be important to examine how the RZZS meshwork interacts with other corona constituents and which of these, if any, additionally contribute to kinetochore expansion and corona formation. Having such insights will greatly facilitate understanding of how fibrous coronas capture the lateral sides of microtubules, how they integrate with SAC signalling, and how their disassembly enables correct interactions with microtubule plus ends.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41556-018-0130-3.

Received: 8 March 2018; Accepted: 22 May 2018;
Published online: 18 June 2018

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Acknowledgements
We thank all lab members for suggestions and discussions. We are grateful to A. Murachelli for help with EM data figure preparation; to E. von Castelmur, T. Heidebrecht and Y. Hiruma for help with Spindly structure experiments; to J. Vaughan for help with ExM; to R. Gassmann for sharing unpublished results and Spindly constructs; to I. Cheeseman, S. Lens and R. Medema for reagents; and to A. de Graaf of the Hubrecht Imaging Center. The Horizon 2020 INEXT project (653706) provided financial support and access to EM infrastructures. This work is part of the OncoCode Institute which is partly financed by the Dutch Cancer Society. This work was further supported by the Netherlands Organisation for Scientific Research (NWO) (gravitron program CancerGenomics.nl; VICI grant (865.12.004 to G.J.P.L.K.)), the Dutch Cancer Society (KWF/HU/BR-11080 to G.J.P.L.K.), and the ERC (675737 to A.M.). V.G. is supported by the Proteins@Work initiative of the Netherlands Proteomics Centre.

Author contributions
C.S. and G.J.P.L.K. conceived the project. C.S., G.J.P.L.K., M.U.D.A., A.P., J.K. and A.M designed experiments and interpreted data. C.S. performed the cell biology experiments. M.U.D.A. and J.K. performed the in vitro experiments with the help of A.F. J.F. and J.K. performed and analysed the electron microscopy experiments. V.G. performed the cross-linking experiments. E.T. performed the comparative sequence analysis. R.M. and J.M.C designed experiments and interpreted data. C.S. performed the cell biology experiments. M.U.D.A. and J.K. performed the in vitro experiments with the help of A.F. J.F. and J.K. performed and analysed the electron microscopy experiments. V.G. performed the cross-linking experiments. E.T. performed the comparative sequence analysis. R.M. and J.M.C performed the electron microscopy of Spindly. C.S. and G.J.P.L.K. wrote the manuscript with the help of A.P. and A.M. and the input of the rest of authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41556-018-0130-3.
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**Methods**

**Cell culture and generation of stable cell lines.** HeLa FlpIn cells were grown in Dulbecco's modified Eagle medium (DMEM; Sigma D6429) supplemented with 9% tetracycline-free fetal bovine serum (FBS), penicillin-streptomycin (50 μg/ml; Sigma P0781; and Ala-Gln (2 mM; Sigma G8541). Constructs were expressed by addition of 1 μg/ml doxycycline.

Plasmids were transfected into FlpIn HeLa cells using Fugene HD (Promega) according to the manufacturer's instructions. To generate stably integrated HeLa FlpIn, with LAP-tagged genes stably integrated in the FRT site and TetR inducible, pcDNA5-constructs were co-transfected with pOG44 recombinase in a 1:9 and kept in hygromycin (Roche, 10845535001) selection for three weeks. To generate stably expressing mCherry-tubulin and DARPinGfp-mCherry-Mps1, HeLa FlpIn cell lines, pcDNA4-α-tubulin-ires-puro2e (Adgene no. 21043) or pcDNA4-DARPInGfp-mCherry-Mps1 variants were transfected and selected in puromycin (Sigma, P7255) for three weeks. For generation of stably cas9 LAP-MAD1Δ28 cell lines, HeLa FlpIn cells were first infected with lentiviral iCas9 and cultured in puromycin selection for three weeks. Cells were then seeded to clonal density in 96-well plates and grown in puromycin. A colony from a single well was expanded, and validated for inducible expression of Cas9 upon doxycyclin addition. This monoclonal cell line was transfected with pcDNA5-LAP-MAD1 U6-Guide and cultured in hygromycin and Puromycin for three weeks. Cas9 and LAP-MAD1Δ28 were induced with doxycyclin addition and cells were then screened to clonal density in 96-well plates, and kept in presence of hygromycin and doxycycline. A colony from a single well was expanded, and validated for the presence of LAP-MAD1 and reduced levels of endogenous MAD1. Protein levels of the different Spindly constructs are shown in Supplementary Figs 3a and 7b.

**Plasmids, cloning and virus production.** Cloning was performed by Gibson assembly and restriction-ligation strategies. The list of plasmids and primers used in this study can be found in Supplementary Table 1. Spindly mutants derived from pcDNA5-LAP-Spindly (a gift from Reto Gassmann39) were PCR amplified from pCDNA5-LAP-Spindly using primers 35–44. The Spindly construct was amplified by GenScript and cloned into the BamH1/NotI sites of pcDNA5-LAP-MAD1Δ28. The U6 promoter-sgRNA scaffold containing the Guide A was introduced in pCDNA5 with primers 13–16. pCDNA4-DARPInGfp-mCherry-Mps1Δ200-MSD1 and pcDNA4-DARPInGfp-mCherry-Mps1Δ200-MSD1 encode a resistant version of MSD1 to Cpd-5 (C604Y) and kinase dead (D664A), respectively, both lacking the first 200 amino acids and tagged with mCherry and a DARPin that recognizes GFP (DARPin 3G86.32). The DARPin fragment was synthesized by GenScript and cloned into the pcDNA4-DARPInGfp-mCherry-Mps1Δ200-MSD1. Subsequently, mCherry amplified with primers 23/24 was introduced into the NotI site, and MSD1 was mutated with primers 25–28. Finally, DARPinGfp-mCherry-Mps1Δ200-MSD1 constructs were subcloned into the KpnI and Apal sites of pcDNA4.

**Exochisis constructs were generated as follows:** The full-length Spindly and the 306–605 constructs were cloned in NKL LIC 1.2 and NKL LIC 1.1 vectors respectively which resulted in the introduction of a cleavable N-terminal 6His tag to these constructs. Spindly 1–40, 54–605 and 259–605 constructs were cloned in NKL LIC 1.10 vector, which resulted in the introduction of a cleavable N-terminal 6His-SUMO tag to these constructs. The genes for these constructs were PCR amplified from pcDNA4-LAP-Spindly using primers 35–44.

**Live-cell Imaging.** For live-cell imaging, cells were seeded in 8-well plates (μ-Slide 8 well, Ibidi). Imaging of mCherry-tubulin and GFP-Spindly was performed over 7 z-slices separated by 200 nm every 5–15 seconds at 1x1 binning on an Andor CSU-W1 spinning disk (50 μm disk) with 60x 1.3 NA water objective lens (Nikon) and 1.5 zoom.

**Cell treatments, transfections and reconstitution.** For knockdown experiments, siRNAs (see Supplementary Table 1 for sequences and concentrations) were transfected using HiPerfect (Qiagen) or RNAi Max (Thermo Fisher Scientific) according to manufacturer's instructions. After 16 h of siRNA treatment, cells were arrested in S-phase by addition of thymidine (2 mM; Sigma-Aldrich cat. no. T1895). For the rescue experiments, doxycycline (1 μg/ml; Sigma D9981) was also added to this point to induce the expression of the constructs. In the experiments in which farnesyl transferase activity was inhibited, lonafarnib (5 μM; Selleckchem cat. no. S2797) was added together with thymidine, and the induction of the constructs with doxycycline was performed 8 h later. After 24 h of thymidine addition, cells were released and treated with the indicated drugs. ZM-447439 (2 μM; Sigma-Aldrich, cat. no. 2458); BI-2536 (100 nM; Advanced Bioscience Laboratories); nocodazole (3 μM; Sigma-Aldrich cat. no. M1404); MG-132 (5 μM; cat. no. C2211). Cells were used for experiments between 6–10 h after thymidine release.

**Electron microscopy and quantification.** Cells were seeded in 6-well culture plates, treated with the indicated siRNAs, synchronized in thymidine and released in nocodazole. Mitotic cells were chemically fixed by adding PHEM buffer (pH 6.9)
containing 2.5% glutaraldehyde (Merck) and 2% paraformaldehyde (Sigma) to the culture medium for 10 min. The cells were replaced with fresh fixative for 2 h at room temperature after which cells were washed and pelleted in 2% low-melting point agarose (Sigma) in PBS and then lysed in PHEM buffer containing 1% osmium tetroxide and 1.5% potassium ferrocyanide for 2 h at 4°C, followed by 0.5% uranyl acetate in dH2O for 1 h at 4°C. Cells were dehydrated using a graded acetone series and then by sonication in 100 µl lysis buffer (50 mM Hepes, pH 8.5, 200 mM NaCl, 10% glycerol, 20 mM imidazole, 2 mM TCEP, 1 mM PMSF and 1 mM protease inhibitor cocktail (Serva)). The cleared lysate was loaded onto an equilibrated 5–ml HiTran Fast Flow column (GE Healthcare) using a peristaltic pump (2 ml/min ow rate). The column was washed with 500 ml wash buffer (50 mM Hepes, pH 8.5, 200 mM NaCl, 10% glycerol, 20 mM imidazole, and 2 mM TCEP). Elution was performed with wash buffer supplemented with 250 mM imidazole. The 2 ml fractions were analysed by SD–PAGE, and those containing Spindly were concentrated up to a volume of 500 µl and applied to a Superose 6 10/300 column. The peak fractions were analysed by SD–PAGE, and those containing pure Spindly proteins were concentrated up to 20 mg/ml, flash frozen in liquid nitrogen and stored at −80°C.

**Expression and purification of mCherry-RRZ.** mCherry-RRZ was produced using the biGbac system for baculovirus expression. Speciﬁcally, the coding sequence of ROD, Zwilch and ZW10 were subcloned into the multiple cloning site of pLIB with a 6-histidine tag at the N-terminus. Expression of Spindly constructs was performed with the biGbac system in TnaO38 cells. Speckled, the coding sequence of Spindly constructs were subcloned into the multiple cloning site of pLIB with a 6-histidine tag at the N-terminus. For all the constructs, a three-step purification was performed: immobilized metal affinity chromatography followed by ion-exchange and gel filtration. Except for the 259-C construct, the affinities for all the other constructs were removed. The proteins were stored in 40 mM HEPS pH 7.5, 100 mM NaCl and 2 mM TCEP. SDS–PAGE gels and Coomassie staining of the constructs can be found in Supplementary Fig. 6a.

**Expression and purification of Spindly**. Spindly was incubated with farnesyl transferase and 3 M excess of farnesyl di-phosphate for 90 min at 25 °C in the reaction buffer (50 mM Hepes, pH 8.0, 250 mM NaCl, 10 mM MgCl2, and 2 mM TCEP). The sample was then loaded onto a Superose 6 15/150 column (GE Healthcare) pre-equilibrated in the reaction buffer and the relevant fractions were pooled, concentrated, flash frozen in liquid nitrogen, and stored at −80°C.

**In vitro farnesylation of Spindly.** Spindly was incubated with farnesyl transferase and 3 M excess of farnesyl di-phosphate for 90 min at 25 °C in the reaction buffer (50 mM Hepes, pH 8.0, 250 mM NaCl, 10 mM MgCl2, and 2 mM TCEP). The sample was then loaded onto a Superose 6 15/150 column (GE Healthcare) pre-equilibrated in the reaction buffer and the relevant fractions were pooled, concentrated, flash frozen in liquid nitrogen, and stored at −80°C.

**In vitro filamentation assays.** Filamentation was performed in 10 µl of 50 mM HEPS, pH 7.5, 10 mM KCl, 1 mM MgCl2, containing 2 µM of mCherry-RZZ and 8 µM of farnesylated Spindly isolated from insect cells. Reaction was incubated at the indicated conditions for 1 hour and filaments were imaged in no. 1.5 glass bottom 96-well plates (Cellvis) on an Andor CSU-W1 spinning disk (50 µm disk) with 100x 1.45 NA objective lens (Nikon). Where indicated, filament reaction was induced in the presence of GFP-Spindly beads or empty beads at 30 °C. Before imaging, beads were diluted in 100 µl of buffer. To immobilize GFP-Spindly in agarose beads, HeLa cells expressing GFP-Spindly were arrested in nocodazole and collected by mitotic shake-off. Cells were lysed in 50 mM Hepes, pH 7.5, 10 mM KCl, 1 mM MgCl2, supplemented with 1% NP 40 and protease inhibitor cocktail (Roche). Lysates were sonicated and centrifuged for 10 min at 20,000g at 4°C. Supernatants were collected and incubated with GFP-Trap beads (Chromotek) for 2 hours at 4°C and washed 5 times in the same buffer with 0.025% Tween-20.

**Negative stain EM.** Concentrated protein samples were diluted to a concentration of ~30 µg/ml. Samples were applied to glow-discharged carbon-coated copper grids, washed quickly with distilled water and negatively stained with 2% (w/v) uranyl acetate and observed using a FEI-1230 operated at 100 kV. Images of single molecules were obtained automatically using a TVIPS F416 CMOS and a final magnification of 54,926.

All image processing was performed using the Scipion platform (http://scipion.cnb.csic.es), which is an image processing framework that integrates several source code packages, interfaces with ImageJ, and supports interfacing with large-scale datasets. For each sample, a total of 10,767 and 13,400 particles were extracted from 58 and 64 micrographs, respectively, using the particle picking tool of Xmipp. The particles were classified in 2D using Xmipp.

**Cross-linking and mass spectrometry.** 86 µM of recombinant Spindly purified from E. coli was cross-linked with 860 µM of freshly prepared bis(sulfo)succinimidyl 4,1′-pentafluorophenyl)borate (BSP) (ThermoFisher Scientific) for 30 minutes at room temperature. Reaction was quenched by adding Tris to a final concentration of 50 mM for 10 min at room temperature. The sample was partially dried down and denatured in 8 M urea. Disulfide bonds were reduced using 10 mM Tris(2-carboxyethyl)phosphine (TCEP) (Sigma). The sample was alkylated with 2 M chloroacetamide (Sigma). The sample was diluted to 2 M urea using 500 mM ammonium bicarbonate (Fluka) containing 3 µg of trypsin/lys-c (Promega). Protein was digested overnight at 37°C, desalted on a C-18 Zip-tip and washed with 0.1% formic acid in water. SCX fractionation was then performed using the Thermo Ultimate 3000 HPLC system equipped with a PolyLC.
Polysulfophenyl A column (100 x 1.0 mm, 3 micron, cat. no. 101ISE0303). Fractions were dried down and again desalted on C-18 zip-tips. Fractions were loaded on a 30 cm in-house prepared column containing 1.9 micron C-18 beads and measured on the Thermo Orbitrap Fusion Tribrid mass spectrometer using a Proxeon 1000 bar nano-LC system and a 150 min gradient. MS1 resolution was set at 120 K, HCD (35%) MS2 at 30 K also in the orbitrap with a max injection time of 100 ms and AGC target of 50000. MS2 was triggered for peptides with at least 3+ charges. The resulting data was analysed using the Xlink workflow in Thermo Proteome Discoverer 2.2.

SEC-SAXS data collection and analysis. Synchrotron X-ray data for Spindly\(^1\) and Spindly\(^2\)\(^{++,++}\) constructs were collected on a Pilatus 1 M detector at the ESRF beamline BM29. 50 µl of each sample, at a concentration 8 mg ml\(^{-1}\) were loaded onto a Superose 6 Increase 3.2/300 column. The flow rate for SAXS data collection was 0.2 ml min\(^{-1}\) and a scattering profile was integrated every second. Frames for each dataset were selected based on the examination of the Size Exclusion profile together with the calculated Rg and D\(_{max}\) values. At least 20 frames for each dataset were selected, scaled and averaged using PRIMUS\(^{68}\) following the standard procedures. SAXS data analysis was performed using the ScAtter software package\(^7\). The forward scattering I(0) was evaluated using the Guinier approximation\(^6\) assuming the formula

\[
I(q) = I(0) \exp(-qRg^2/3)
\]

for a very small range of momentum transfer values (q\(_R\) < 1.3). Calculation of the pair distribution function and maximum distance D\(_{max}\) was performed using ScAtter which uses modification of the Moore function for transforming the data to real space. The R\(_g\) was estimated by Guinier approximation. The molecular mass was calculated using the Porod volume, and the QR method\(^7\).

SEC-MALLS data collection and analysis. Size exclusion chromatography-multiplex angle laser light scattering (SEC-MALLS) was performed using an AKTA HPLC system connected to miniDAWN Tristar detector (Wyatt Technologies). 100 µl of purified Spindly\(^1\)\(^{+++}\) or Spindly\(^2\)\(^{++}\) at 5 mg ml\(^{-1}\) was loaded onto a Superose 6 Increase 10/300 column at a flow rate of 0.4 ml min\(^{-1}\) in a buffer containing 40 mM HEPES pH 7.5, 100 mM NaCl and 2 mM TCEP. The scattering data were analysed by ASTRA (Wyatt Technologies) software and estimation of molecular weight was done by using the refractive index signal as measure of the concentration.

Surface plasmon resonance (SPR). For binding affinity determination by SPR, a Streptavidin sensor chip (GE) was used to immobilize the Spindly constructs or RZZ. Before immobilization, the non-specific binding of the Spindly constructs or RZZ to the Streptavidin chip was determined by flowing over these constructs over the sensor chip and monitoring the response in resonance units (RUs). Constructions which did not have the non specific-binding selected as analytes. Based on these results, Spindly 259-C, 306-C and RZZ were selected as ligands and biotinylated via lysines by adding 1 µg ml\(^{-1}\) of NHS-Biotin (Thermofisher) to each of these ligands in a 2:1 molar ratio (protein: NHS-Biotin) to ensure that preferably a single Lysine per molecule is cross-linked. The biotinylation reaction was carried at room temperature for 30 min. ~80 RU’s of Spindly 259-C and 306-C were immobilized on two different channels of the streptavidin sensor chip and Spindly 1–250 flown over in subsequently increasing concentration (5 nM to 20.48 µM).

For RZZ, ~235 RUs were immobilized on the Streptavidin sensor chip and Spindly\(^2\), Spindly\(^3\) or their farnesylated forms were flown in over increasing concentrations (5 nM to 20.48 µM). The binding buffer composed of 40 mM HEPES pH 7.5, 100 mM NaCl, 2 mM TCEP supplemented with 0.05% Tween-20, 1 mg ml\(^{-1}\) BSA and 1 mg ml\(^{-1}\) CM-Dextran to reduce non-specific binding. The experiments were performed using Biacore T200 (GE Healthcare).

Equilibrium binding responses were plotted as a function of the concentration in GraphPad PRISM v. 7.0c (GraphPad Software Inc.) and fitted using the equation for non-linear regression: \(Y = B_{\text{max}} \cdot X/(K_X + X)\) where, \(B_{\text{max}}\) is the maximum specific binding in the same units as X. \(K_X\) is the equilibrium binding constant, in the same units as X. The response plotted on the y-axis was normalized for the molecular weight of the analyte to yield the stoichiometry of binding by using the formula: \(Y = Y/K\) where \(K = (\text{RU's of ligand immobilized/mol. weight of ligand}) \cdot \) mol. weight of analyte.

Statistics and reproducibility. Representative results are displayed or all data were reported, as specified in individual figure legends. Results from immunofluorescence images from different experiments were pooled and no difference was observed between different experimental sets. The comparisons most pertinent for the conclusions and number of independent experiments are specified in the figures and legends. Two-tailed, unpaired t-tests or one-way ANOVA followed by Tukey's test were performed to compare experimental groups in immunofluorescence quantifications when \(n \geq 3\). In those cases in which \(n < 3\), no statistical analysis was performed and the source data is provided in Supplementary Table 3. Data are presented as mean ± s.d., and \(P < 0.001\) was considered statistically significant. The source data of the experiments performed with purified proteins is provided in Supplementary Table 3. All replicates showed similar results and a representative experiment was reported.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Custom ImageJ macros used in this study are available upon request.

Data availability. Sequences analysed here can be found in Supplementary Tables 4 and 5. Source data for Figs. 4c, 5d, 6a, and 5f and Supplementary Figs.4c, 6b,c,d, 7a, 7e, 7f and 8b have been provided as Supplementary Table 3. All other data supporting the findings of this study are available from the corresponding author upon request.

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| A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons | ☒         |
| A full description of the statistics 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 | ☒         |
| Clearly defined error bars | ☒         |
| State explicitly what error bars represent (e.g. SD, SE, CI) | ☒         |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Fluorescent images were collected using SoftWorx 6.0 software (Applied Precision/GE Healthcare) and Nikon Imaging Software (NIS-Elements AR 5.51.00). Western blot signals were detected using an ImageQuant LAS 4000 (GE Healthcare) imager and its control software. Electron microscopy images were collected using Tecnai Imaging & Analysis 4.7 (Thermo Scientific, Eindhoven). Mass-spectrometry data was collected using Thermo Scientific Xcalibur processing and its instrument control software. SEC data was collected using the UNICORN software (GE healthcare). Single molecule EM images were collected using JEOL-1230 and its instrument control software. SEC-MALLS: Scattering data collected using miniDAWN Tristar detector (Wyatt Technologies) and ASTRA V 5.3.2.17. SPR: Biacore T200 (GE Healthcare) and Biacore T200 Control Software Version: 2.0.1. SAXS: Synchrotron X-ray data collected on Pilatus 1M detector and BsxCuBE (doi:10.1107/S0909049513010431).

Image J v1.50e (https://imagej.nih.gov/ij/) and Fiji v2.0.0-rc-43/1.51h (https://fiji.sc/) were used to analyze image data. Proteome Discoverer™ Software v2.2 (ThermoFisher Scientific) was used to analyze MS data. CRYO-EM image processing was performed using the Scipion platform (http://scipion.cnb.csic.es). SAXS data analysis was performed using ScAtter (http://www.bioisis.net/tutorial). The SEC-MALLS data were analysed by ASTRA (Wyatt Technologies) software. Conserved Feature Extraction was performed with IUPred, MARCOL, MEME algorithm, MAFFT-LINSI, HMMER package.

Data analysis

Prism 6 and 7 and Microsoft Excel 2017 were used for statistical analysis and graphical data presentation (https://www.graphpad.com/). Image J v1.50e (https://imagej.nih.gov/ij/) and Fiji v2.0.0-rc-43/1.51h (https://fiji.sc/) were used to analyze image data. Proteome Discoverer™ Software v2.2 (ThermoFisher Scientific) was used to analyze MS data. CRYO-EM image processing was performed using the Scipion platform (http://scipion.cnb.csic.es). SAXS data analysis was performed using ScAtter (http://www.bioisis.net/tutorial). The SEC-MALLS data were analysed by ASTRA (Wyatt Technologies) software. Conserved Feature Extraction was performed with IUPred, MARCOL, MEME algorithm, MAFFT-LINSI, HMMER package.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

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

Life sciences

Study design

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

Sample size

No statistical method was used to predetermine the sample size. Sample size was chosen based on previous experience and standards in the field. Sample size and number of independent experiments are stated in the figure legend or in the Methods section. Three to more independent results were used to perform statistical analyses. If less, no statistics were performed.

Data exclusions

No data is excluded if the experiments were successfully performed.

Replication

All experiments were reliably reproduced.

Randomization

Electron microscopy pictures of cells were randomized.

Blinding

Electron microscopy pictures of cells were blinded and independently analyzed by two investigators.

Materials & experimental systems

Unique materials

Cpd-5 was obtained from Rene Medema lab (DOI:10.1038/onc.2015.319). Relevant plasmids used in this study are available upon reasonable request.

Antibodies

Information of antibodies, RRIDs, including species and dilution ratio are described in Supplementary Table 2.

Validations

Validations are based on the datasheets from the manufacturers (RRIDs of the antibodies are provided). We additionally validated the following antibodies by the use of siRNA-treated samples as negative control: ZW10, p150Glued, HEC1, KNL1, BUB1, CENP-F, MAD1, CENP-E, ROD, Spindly, Zwilch.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
HeLa Flp-in cells provided by the S. Taylor lab, University of Manchester, England, UK. The Tnao38 cells come from (Hashimoto et al, 2012. BMC Biotechnol. 12:12. doi:10.1186/1472-6750-12-12)

Authentication
Cell lines were not authenticated by ourselves.

Mycoplasma contamination
Cell lines were tested multiple times over the study for eliminating possible mycoplasma contamination

Commonly misidentified lines
No commonly misidentified cell line was used

(See ICLAC register)

Method-specific reporting

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | Magnetic resonance imaging |