Published in final edited form as:
Science. 2014 October 10; 346(6206): 248–251. doi:10.1126/science.1256729.

Sister kinetochores are mechanically fused during meiosis I in yeast

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Abstract

Production of healthy gametes requires a reductional meiosis I division in which replicated sister chromatids co-migrate, rather than separating as in mitosis or meiosis II. Fusion of sister kinetochores during meiosis I may underlie sister chromatid co-migration in diverse organisms, but direct evidence for such fusion has been lacking. Here we studied native kinetochore particles isolated from yeast using laser trapping and quantitative fluorescence microscopy. Meiosis I kinetochores formed stronger attachments and carried more microtubule-binding elements than kinetochores isolated from cells in mitosis or meiosis II. The meiosis I-specific monopolin complex was both necessary and sufficient to drive these modifications. Thus, kinetochore fusion directs sister chromatid co-migration, a conserved feature of meiosis that is fundamental to Mendelian inheritance.

The hallmark of meiosis is a two-fold reduction in ploidy, which occurs because one round of DNA replication is followed by two rounds of chromosome segregation. During meiosis

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I, sister chromatids uniquely co-migrate, thereby enabling segregation of homologous chromosomes. During meiosis II, which resembles mitosis, the sister chromatids separate (Fig. S1A and S1B). The co-migration of sister chromatids during meiosis I has been suggested to depend on fusion of sister kinetochores in a range of organisms (1–4) (Fig. S1C). Because fused sister kinetochore pairs would contain more microtubule-binding elements than individual kinetochores, we reasoned that they might form stronger attachments to microtubules. Alternatively, if one kinetochore within each sister pair were selectively inactivated during meiosis I (5, 6), then the remaining active kinetochores would likely form attachments with similar strength relative to individual mitotic and meiosis II kinetochores.

To distinguish between the ‘fusion’ and ‘one sister shut-off’ mechanisms, we purified native kinetochore particles from yeast cells arrested in metaphase of meiosis I (via meiosis-specific depletion of Cdc20) (7) using methods developed for the isolation of mitotic particles (8, 9). The purified material contained essentially all known kinetochore components (Table S1), and its bulk composition was very similar to material isolated from mitosis (Figs. 1A, S2A and S2B, Table S1). We used fluorescence- and laser trap-based assays to determine whether the meiosis I kinetochore particles remained functional in vitro. As shown previously for mitotic particles (8), fluorescently-labeled particles isolated from meiosis I cultures bound specifically to microtubules and tracked processively with disassembling microtubule tips (Fig. 1B and Movie S1). Furthermore, meiosis I kinetochore particles formed load-bearing attachments to microtubule tips, supporting forces up to 15 pN and persisting through ‘catastrophe’ and ‘rescue’ events, where the filament switched from assembly to disassembly and vice versa (Fig. 1C). Thus, native kinetochore particles isolated from meiotic cultures are functional. The meiotic particles formed very long-lived tip attachments, with a mean lifetime of 52 ± 23 min at 7 pN of tension, double the lifetime measured previously for mitotic particles, 26 ± 6 min, at a similar level of tension, 7.2 pN (8).

The long lifetimes of attachments formed by meiosis I kinetochore particles suggested that they may be stronger than particles from mitotic cells. To assess their strength directly, we attached them to growing microtubule tips and tested them using a force ramp, where force was increased at a constant rate until the attachments ruptured (Fig. 1D). Control kinetochore particles isolated from metaphase-arrested mitotic cells ruptured at an average force of 9.4 ± 0.4 pN (Fig. 2B), which is indistinguishable from the strength of particles harvested during vegetative (asynchronous mitotic) growth (8). Rupture strengths were unaffected by differences in ploidy and relatively insensitive to the method of mitotic cell cycle arrest (Fig. S3). Meiosis I particles, however, formed significantly stronger attachments, rupturing at forces ranging from 6.5 to 22 pN (i.e., up to the load limit of our laser trap) with an average of 13.1 ± 0.3 pN (Figs. 2A and 2B; Table S2). Mean rupture forces for both meiosis I and mitotic particles remained invariant as the density of particles on the beads was reduced below the single particle limit (Fig. S4), indicating that higher strength is an intrinsic property of individual meiosis I kinetochore particles.

To determine whether the higher kinetochore attachment strength is specific to meiosis I or persists into meiosis II, we prepared synchronized meiotic cultures by releasing cells from a prophase I block (10, 11). Particles harvested from synchronized metaphase I cells formed attachments that ruptured at 13.1 ± 0.6 pN on average (Figs. 2A and 2B, meiosis I*). However, particles from synchronized metaphase II cells ruptured at 9.3 ± 0.7 pN on average (Figs. 2A and 2B, meiosis II*). Thus, the higher intrinsic strength of kinetochore particles occurs specifically during meiosis I and returns to mitotic-like levels as cells progress into meiosis II.

If the particles isolated from meiosis I cells are fused sister kinetochore pairs, they should contain more microtubule binding elements than mitotic particles. We purified fluorescent particles doubly-tagged with SNAP-549 on Nuf2 (a subunit of the microtubule-binding Ndc80 complex) and CLIP-647 on Mif2 (an inner kinetochore component orthologous to CENP-C). Spore viability was unaffected and rupture strengths for the fluorescent particles were indistinguishable from untagged particles (Fig. S5 and Table S2), indicating no loss of functionality. The purified kinetochore material contained a mixture of dual-color particles carrying both Nuf2 and Mif2, plus subcomplexes lacking Nuf2 or lacking Mif2 (Fig. 2C). Subcomplexes with just one detectable Nuf2 (identifiable by their single-step photobleaching behavior; Fig. S6) served as internal controls, allowing normalization of particle brightnesses into estimates for the approximate numbers of Nuf2 molecules associated with each particle. Dual-color particles from meiosis I cells carried more Nuf2 molecules on average, 6.5 ± 2.8 (mean ± s.d. from $N = 4$ preparations), than those from vegetatively growing cells, which had 3.8 ± 1.3 ($N = 4$) (Figs. 2D and 2E). The apparent Nuf2 content was variable and lower than in vivo estimates (which suggest 8 to 20 copies per mitotic kinetochore (12, 13)). However, consistent with the fusion model, Nuf2 content was significantly higher for dual-color meiosis I particles than for vegetative particles prepared in tandem, by a factor of 1.66 ± 0.26 (mean ± s.d., $N = 4$ tandem pairs; $p = 0.014$ by *t*-test).

If fusion of sister kinetochore pairs underlies the increased strength of meiosis I kinetochore particles, the increase should vanish if the particles are harvested from cells in which every kinetochore lacks a sister. We engineered cells to undergo meiosis without replicating their DNA (via meiosis-specific depletion of Cdc6 (14), part of the pre-replicative complex (15)). Because the lack of sister chromatids precludes homology-based DNA repair (16), we also deleted the Spo11 endonuclease, thereby avoiding high levels of DNA damage that might interfere with meiotic progression. Spo11 catalyzes formation of the chiasmata that link homologous chromosomes (17), so its deletion (*spo11*Δ) enabled us to test whether tension across homologs is required for sister kinetochore fusion (18). Kinetochore particles from *spo11*Δ cells were identical in strength to those from wild-type cells, indicating that linkage between homologs (and thus spindle-generated tension across them) was dispensable for the high attachment strength of meiosis I kinetochores (Figs. 2A and 2B, *no chiasmata*). However, high strength was lost when meiosis I particles were harvested from *cdc6-meiotic-null* cells (Figs. 2A and 2B, *no sisters*, or *no chiasmata & no sisters*). Thus, sister kinetochores are required for the increased strength of meiosis I kinetochore particles, as predicted by the fusion model.

The meiosis I-specific monopolin complex consists of four proteins (Mam1, Csm1, Lrs4, and Hrr25) (5, 19–21) with twin kinetochore-binding sites that have been proposed to directly cross-link sister kinetochores in budding yeast (1, 5, 20, 22). However, because the receptor for monopolin, Dsn1 (1, 23), is present in multiple copies in the kinetochore (12), another possibility is that the twin monopolin sites bind to the same kinetochore and inactivate it, thereby shutting off one of the two sister kinetochores (Fig. S1C) (5, 6).

Monopolin was detectable at low levels in kinetochore material from meiosis I cultures (Fig. S2C). To test the impact of monopolin on the behavior of kinetochore particles, we genetically disrupted its function in three ways (*mam1*Δ, *csm1-L161D*, and *dsn1*-Δ*N*), all of which disrupt sister co-migration during meiosis I (1, 21, 23). In all cases we found that the high 13 pN strength of meiosis I kinetochore particles was lost and their strength returned to mitosis-like levels, ~9 pN (Fig. 3), confirming that monopolin is required for high attachment strength. We also engineered cells to ectopically express monopolin during mitosis by inducing expression of *MAM1* together with *CDC5* (encoding Polo kinase), which caused erroneous co-orientation in 28% of cells (5). Kinetochore particles isolated from these cells gave a bimodal rupture force distribution (Fig. 3A) with an intermediate average strength, 11.2 ± 0.4 pN (Fig. 3B). This observation may be explained by the incomplete penetrance of monopolin induction in these cells (5).

To test whether kinetochores can be fused by monopolin in the absence of other cellular factors, we recombinantly expressed and purified the four-protein monopolin complex (containing a kinase-dead K38R mutant of Hrr25; Fig. S2D) (22) and incubated it with isolated kinetochore particles. Incubation with recombinant monopolin was sufficient to strengthen mitotic particles and also particles from meiosis I cells in which monopolin was disrupted (*mam1*Δ or *csm1-L161D*), raising their mean rupture forces from ~9 pN to ~13 pN (Figs. 4A and 4B) in a dose-dependent manner (Fig. 4C). However, the same treatment did not affect the strength of particles from cells lacking the monopolin binding site on Dsn1 (*dsn1*-Δ*N*) (Figs. 4A and 4B). Likewise, monopolin addition did not strengthen particles (*mam1*Δ) pre-linked to laser trapping beads (Fig. 4C), presumably because immobilization on beads prevented cross-linking of the particles. When fluorescent particles from vegetatively growing cells were incubated with increasing amounts of monopolin, their average brightness grew monotonically and the approximate number of Nuf2 molecules associated with each particle increased two-fold, from 4.8 ± 0.4 to 10.9 ± 1.5 (mean ± s.d.; $N \geq 2$ experiments) (Fig. 4D). Thus, monopolin alone is sufficient for fusion of kinetochore particles in vitro.

Sister chromatid co-migration is a universal feature of meiosis I that governs Mendelian inheritance, and its failure is a major cause of birth defects and infertility (24). Here we have shown that a meiosis I-specific factor from budding yeast, monopolin, generates kinetochores with more microtubule-binding elements and greater strength. These findings provide direct evidence that sister kinetochore fusion underlies the co-segregation of sister chromatids during meiosis I.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank A. Hoskins, M. Miller, N. Umbreit, and E. Yusko for helpful comments. We thank A. Desai for providing antibodies and A. Hoskins for SNAPf and CLIPf plasmids. The Wellcome Trust supported this work through a Sir Henry Wellcome Fellowship to E.D. (096078), by Senior Research Fellowships to A.L.M. (090903) and J.R. (084229), by two Wellcome Trust Centre Core Grants (077707, 092076) and an instrument grant (091020). The work was also supported by National Institutes of Health grants to C.L.A. (RO1GM079373, S10RR026406), to S.B. (R01GM064386), and to K.D.C. (R01GM104141), and by a Packard Fellowship to C.L.A. (2006-30521). K.D.C. also acknowledges support from the Ludwig Institute for Cancer Research and the Sidney Kimmel Foundation. Additional data described in this work can be found in the online supplementary materials. E.D., K.K.S., S.B., A.L.M., and C.L.A. conceived the experiments. E.D. generated new yeast strains and isolated kinetochore particles. K.K.S. performed laser trap experiments. Y.D. performed the fluorescence measurements. F.L.A. and J.R. performed proteomics analysis. Q.Y. and K.D.C. purified monopolin. K.N.O. and S.C. optimized fluorescent labeling of kinetochore particles. E.D., K.K.S., A.L.M., and C.L.A. prepared the manuscript.

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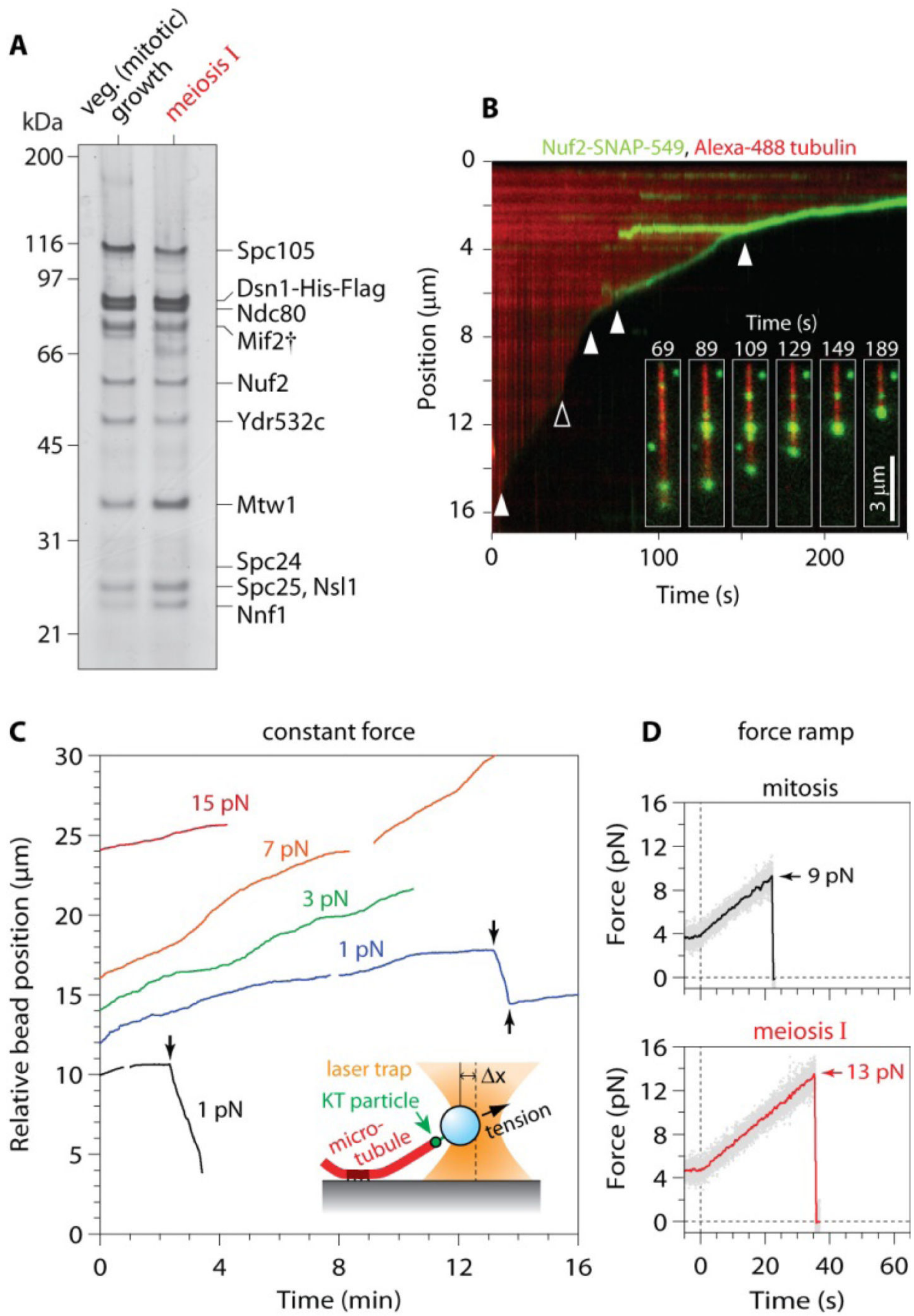

Fig. 1. Native kinetochore particles from meiotic cells recapitulate tip-coupling in vitro

(**A**) Core kinetochore proteins co-purified from cells undergoing vegetative (mitotic) growth and cells arrested in metaphase I of meiosis, visualized by silver-stained SDS-PAGE (9). Mif2 (†) co-migrates with non-specific background proteins (8). (**B**) Kymograph showing movement of fluorescent meiosis I kinetochore particles (green) driven by a disassembling microtubule (red; see Movie S1). Filled arrowheads mark tip-particle encounters, open arrowhead marks particle release. Inset shows images at indicated times. (**C**) Position versus time for tip-attached meiosis I particles tested with a force clamp at indicated loads. Arrows mark catastrophes and rescue. Intervals when the laser trap was briefly shuttered (to clear debris) appear as gaps in the 1 and 7 pN traces. Inset shows schematic of assay (9). (**D**) Tensile force versus time for indicated particles bound to assembling tips and tested with a 0.25 pN s^{-1} force ramp. Gray dots show raw data. Colored traces show same data after smoothing (500–ms sliding boxcar average). Dashed vertical lines mark start of force ramp. Arrows mark rupture.

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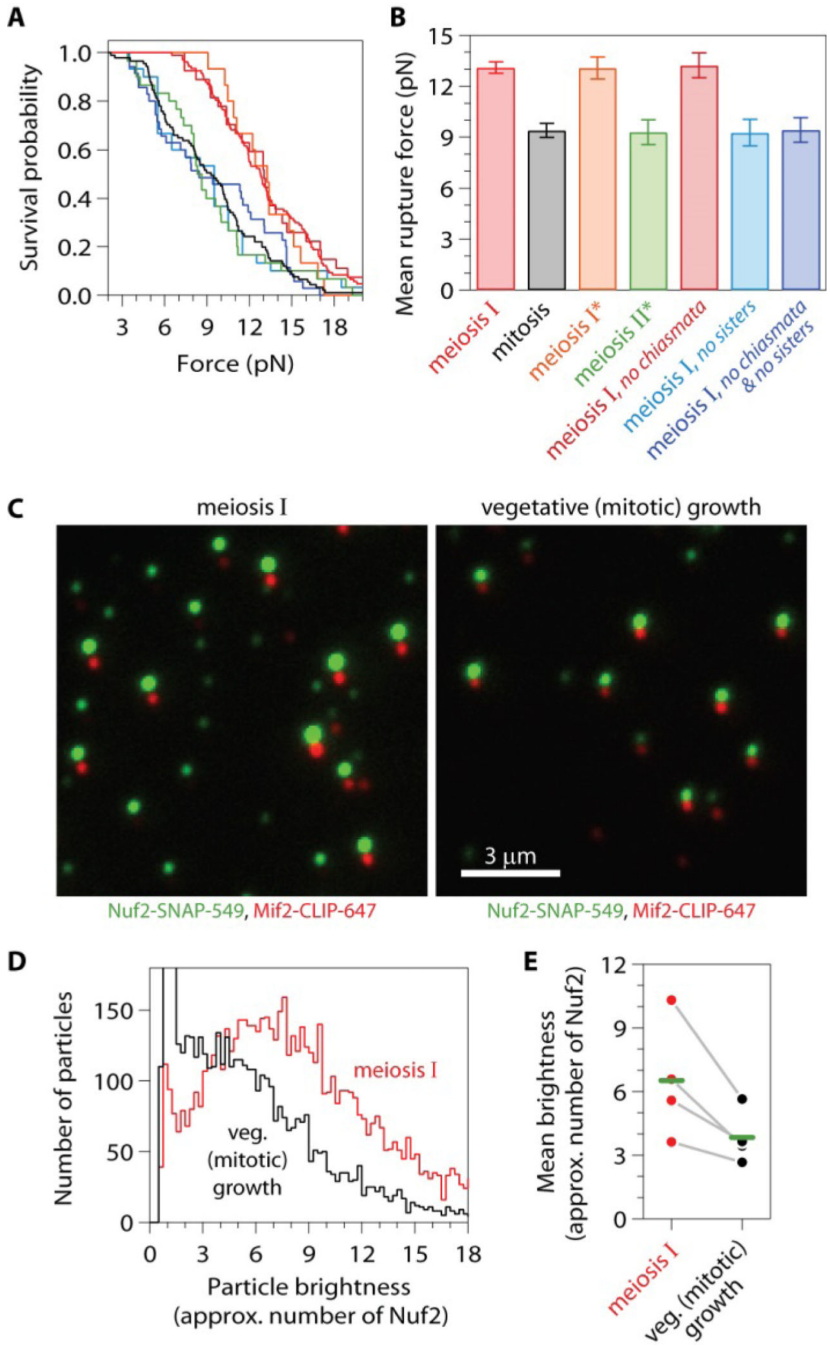

Fig. 2. Meiosis I kinetochore particles are stronger and brighter

(**A**) and (**B**) Distributions of rupture force (A) and mean rupture force values (B) for indicated kinetochore particles (color matched). Asterisks (*) indicate particles from cells undergoing meiosis synchronized by release from a prophase I block. Error bars represent s.e.m. (N = 15–107 ruptures). (**C**) Fluorescence images of particles carrying Nuf2-SNAP-549 (green) and Mif2-CLIP-647 (red) bound to coverslips. Colors are offset slightly; green/red pairs represent colocalized, dual-color particles. (**D**) Distributions of Nuf2 brightness for dual-color particles (N > 4,900) relative to the brightness of a single Nuf2. (**E**)

Mean Nuf2 brightnesses for dual-color particles from four pairs of kinetochore preparations. Points are means from individual preparations; gray lines connect means from particles prepared in tandem (9); green horizontal lines are means across all preparations.

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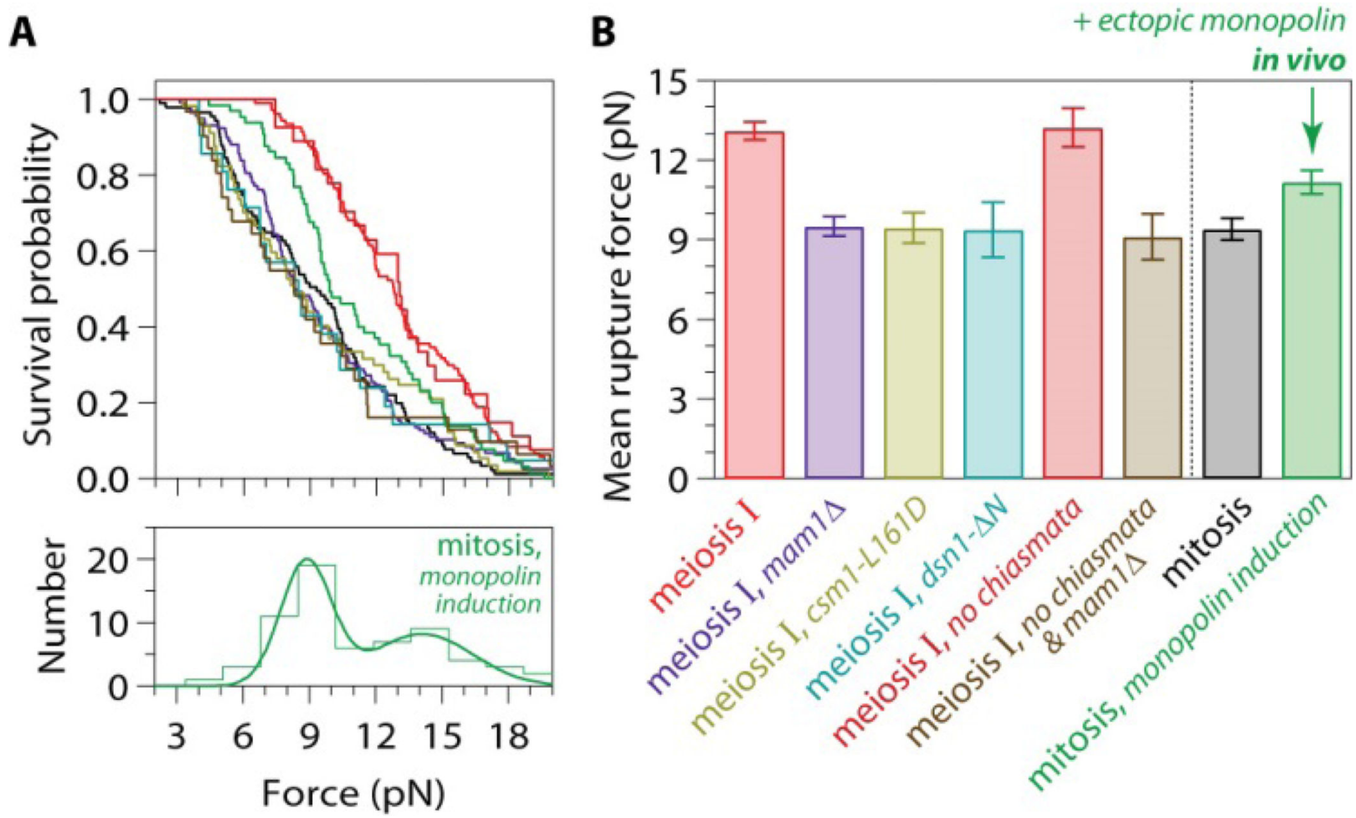

Fig. 3. Monopolin is necessary for the high strength of meiosis I kinetochore particles, and sufficient in vivo

(**A**) and (**B**) Distributions of rupture force (A) and mean rupture force values (B) for indicated kinetochore particles (color matched). Data for particles from meiosis I (red), from meiosis I without chiasmata (dark red), and from mitosis (black) are replotted from Figs. 2A and 2B for comparison. Error bars represent s.e.m. (*N* = 21–118 ruptures).

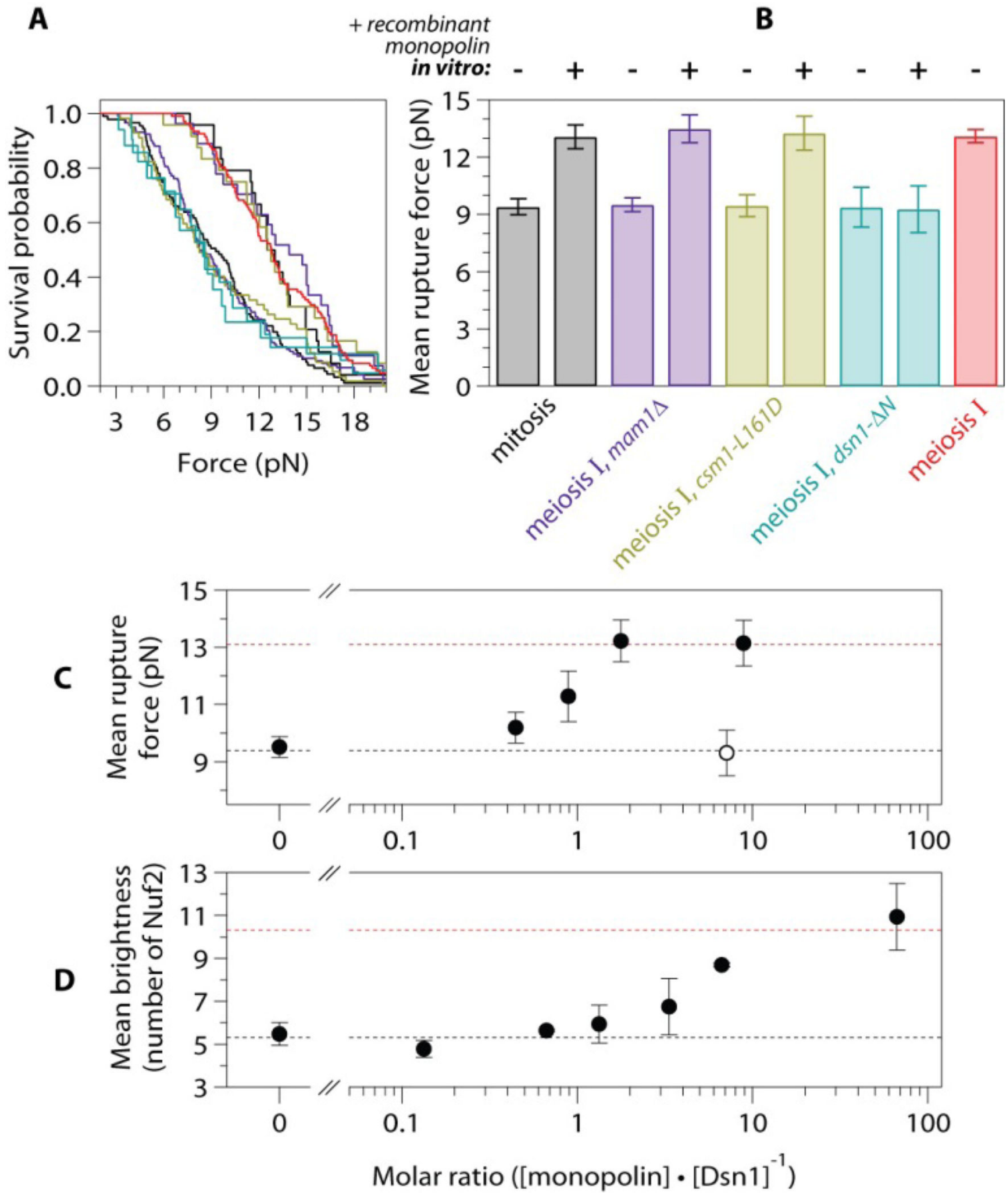

Fig. 4. Pure recombinant monopolin is sufficient to increase the strength and brightness of kinetochore particles in vitro

(**A**) and (**B**) Distributions of rupture force (A) and mean rupture force values (B) for kinetochore particles (color matched) after incubation with recombinant monopolin (at molar ratio 1.8 versus Dsn1-His-Flag; '+'). Data for particles without monopolin incubation ('−' in B) are replotted from Figs 3A and 3B for comparison. Error bars represent s.e.m. (*N* = 17–118 ruptures). (**C**) Mean rupture forces for meiosis I, *mam1*Δ kinetochore particles after incubation with indicated amounts of recombinant monopolin (9). Filled circles are data from particles pre-incubated with monopolin before linking to polystyrene laser trapping beads. Open circle shows control in which particles were first linked to trapping beads and subsequently incubated with monopolin. Error bars represent s.e.m. (*N* = 28–118 ruptures). Dashed lines are means for mitotic (black) and meiosis I (red) particles (from Fig. 2B). (**D**) Mean Nuf2 brightnesses for dual-color particles isolated from cells undergoing vegetative (mitotic) growth, incubated with indicated amounts of recombinant monopolin. Error bars represent s.d. (*N* = 2–3 experiments). Dashed lines are mean brightnesses for dual-color vegetative (black) and meiosis I (red) kinetochore particles prepared on the same day, without monopolin incubation (from Fig. 2E).