Shugoshin protects centromere pairing and promotes segregation of non-exchange partner chromosomes in meiosis

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ABSTRACT

Faithful chromosome segregation during meiosis I depends upon the formation of connections between homologous chromosome pairs. For most chromosome pairs a connection is provided by crossovers. The crossover creates a link that allows the pair to attach to the meiotic spindle as a unit, such that at anaphase I, the partners will migrate away from one another. Recent studies have shown that some chromosome pairs that fail to experience crossovers become paired at their centromeres in meiotic prophase, and in some organisms, this pairing has been shown to promote proper segregation of the partners, later, at anaphase I. Centromere pairing is mediated by synaptonemal complex (SC) proteins that persist at the centromere when the SC disassembles. Here, using experiments in mouse and yeast model systems, we tested the role of shugoshin in promoting meiotic centromere pairing by protecting centromeric synaptonemal components from disassembly. The results show that shugoshin protects centromeric SC in meiotic prophase and also promotes the proper segregation in anaphase of partner chromosomes that are not joined by a crossover.

INTRODUCTION

Faithful chromosome segregation during meiosis depends upon the formation of connections between homologous chromosome pairs. Crossovers, also called exchanges, are the basis these connections. Chiamata, the cytological manifestation of crossovers, in conjunction with sister chromatid cohesion, create the physical links that hold the maternal and paternal homologous chromosomes in pairs called bivalents (reviewed in (1)). The linkage allows the bivalent to attach to the meiotic spindle as a single unit, such that at anaphase I, the partners will migrate away from one another to opposite poles of the spindle. In some instances, even in the absence of exchanges, proper meiotic chromosome segregation can be achieved (reviewed in (2-4)). In yeast and *Drosophila* when a single chromosome pair does not

experience an exchange, the pair still segregates correctly in most meioses (5-11). Although the behavior of non-exchange chromosomes has been difficult to study in mammalian models, there are indications that here too, there may be mechanisms beyond crossing-over that help to direct their behavior in meiosis I. For example, in mice, the majority of chromosomes in oocytes from Mlh1 recombination-deficient mutant appeared to be spatially balanced on the spindle (12), and in humans, while smaller chromosomes (21 and 22) fail to experience crossovers in about 5% of meioses (13-15), they are estimated to non-disjoin in less than 1% of meioses (14-17).

In yeast and *Drosophila*, the centromeres of non-exchange partners pair or interact in meiotic prophase (18-22). Similar centromere pairing is also seen in mouse spermatocytes (23, 24). Meiotic centromere pairing (or clustering in *Drosophila* females) is mediated by proteins that are components of the synaptonemal complex (SC) (20, 22-25). The SC zippers the axes of homologous chromosomes along their lengths in mid-meiotic prophase (pachytene) and disassembles in late prophase (diplotene). However, SC proteins persist at centromeres, holding them together in pairs (yeast and mouse spermatocytes; (20, 22, 23)) or clusters (*Drosophila* females; (25)). In budding yeast, this centromere pairing has been shown to be correlated with proper disjunction of the non-exchange pair (20, 22).

Important questions remain unanswered regarding the mechanism and function of centromere pairing. First, how does centromere pairing by SC components in prophase ensure disjunction in anaphase? This is especially curious as the SC components at the centromeres are largely gone by meiotic metaphase when the centromeres begin attaching to the microtubules of the spindle (20, 22-24). Second, what enables SC proteins to persist at the paired centromeres when the SC disassembles? The collection of SC components at the paired centromeres appears no different than the rest of the SC (20, 22-24). This suggests the model that there is a mechanism that protects a conventional SC structure at centromeres from the disassembly process.

The persistence of centromeric SC in late prophase, when the chromosomal arm SC

disassembles, is reminiscent of the protection of meiotic cohesins at centromeres at the metaphase I to anaphase I transition when arm cohesion is lost (reviewed in (26)). Protection of centromeric cohesin in meiosis I is mediated by shugoshin – a function first revealed by studies of the *mei-S322* gene in *Drosophila* (27, 28) (reviewed in (29)). In yeasts and mouse oocytes, shugoshin has been shown to recruit forms of PP2A phosphatase to centromeres, rendering the centromeric cohesin refractory to cleavage by the protease separase at the metaphase I to anaphase I transition (30-34). In budding and fission yeast, shugoshin acts by protecting the Rec8 component of cohesin from phosphorylation by casein kinase, and also in budding yeast by the Dbf4 kinase (DDK). In other organisms the identities of the kinases that prepare cohesins for separase cleavage have not been determined (35-37)(reviewed in (29)).

Phosphorylation also promotes SC disassembly and degradation, but at the pachytene to diplotene transition (reviewed in (38)). Studies in rats and mice have correlated the phosphorylation of SC components (SYCP1, SYCP3, TEX12, and SYCE1) with pachytene exit and SC disassembly (39, 40) and the Polo-like kinase PLK1 localizes to the SC central element in pachytene and can phosphorylate SYCP1 and TEX12 *in vitro* (39). Similarly, in budding yeast, Polo-like kinase (Cdc5) expression is central in promoting SC disassembly (41), but it works in a network with other kinases; Dbf-4 kinase and cyclin-dependent kinase (CDK) (39, 42-45). In mice, CDK has also implicated in promoting SC removal.

The parallels between the protection of cohesins and SC components at the centromeres compelled us to explore whether shugoshin is responsible for protecting the centromeric SC components. Our cytological experiments with mouse spermatocytes revealed that this is the case, while genetic approaches with budding yeast revealed that shugoshin is necessary for mediating the segregation of non-exchange chromosome pairs that depend upon centromere pairing for their meiotic segregation fidelity.

RESULTS AND DISCUSSION

Shugoshin 2 is necessary for centromere pairing in mice

Shugoshin 2 (SGO2) localizes to the centromeres of chromosomes in mouse spermatocytes (46). We compared the timing of SGO2 localization to centromeres with the timing of SC protection at centromeres in mouse spermatocytes (Fig. 1 A). The axial/lateral element component SYCP3 was used as a marker for the SC. SGO2 is first detected at centromeres in early diplotene cells (Fig. 1 A and B), and remains there thru mid- and late diplotene. This corresponds with the time at which SC components are removed from chromosome arms but not from centromeres (23, 24). Thus, these data are consistent with the model that SGO2 protects the centromeric SC from the disassembly process.

To test whether SGO2 is necessary for the protection of centromeric SC, we monitored the persistence of centromeric SYCP1 in chromosome spreads from *Sgo2*$^{-/-}$ spermatocytes and wild-type controls (Fig. 2). In pachytene cells the SC of the *Sgo2*$^{-/-}$ mutants was indistinguishable from wild-type control (Fig. 2 A). In early diplotene, the SYCP1 signal was visible at paired centromeres of both the wild-type control chromosome spreads and in the *Sgo2*$^{-/-}$ mutants. In wild-type spreads the SYCP1 persisted at the centromeres through diplotene, but by late diplotene, SYCP1 was significantly reduced at centromeres when SGO2 was not present (Fig. 2A-C).

The loss of SYCP1 from centromeres would predict that *Sgo2*$^{-/-}$ spermatocytes would also have a defect in homologous centromere pairing. Indeed, as the *Sgo2*$^{-/-}$ cells proceeded through diplotene they lost considerable centromere pairing, whereas it persisted to a greater extent in wild type cells (Fig. 2 A, B, and D). In both the wild-type control and the *Sgo2*$^{-/-}$ chromosome spreads, the unpaired centromeres have significantly less SYCP1 staining than do the paired centromeres (Fig. 2 E), supporting the notion that it is the protection of SYCP1 that allows centromere pairing the persist.

Together these results suggest that wild-type and *Sgo2*$^{-/-}$ spermatocytes have similar SC structure in pachytene. When the SC disassembles, centromere pairing, and SYCP1 at the paired centromeres, can be observed in the *Sgo2*$^{-/-}$ mutant chromosome spreads, demonstrating that SGO2 is not necessary for establishing centromere pairing. However, centromere pairing rapidly disappears in the *Sgo2*$^{-/-}$ spermatocytes suggesting that SGO2 is necessary to maintain centromere pairing in diplotene.

PP2A promotes centromere pairing in mouse spermatocytes

SGO2 could be protecting centromeric SC through the recruitment of one of its effector proteins to the centromere (reviewed in (29)). Shugoshins are known to recruit PP2A phosphatase to meiotic centromeres in germ cells, where the PP2A opposes the phosphorylation of centromeric cohesins (30, 31, 47). To test whether this same mechanism is being used to protect SC at centromeres from disassembly in diplotene, we evaluated the persistence of centromeric SC and centromere pairing in spermatocytes when phosphatase activity was inhibited (Fig. 3). In these experiments we evaluated chromosome spreads from cultured spermatocytes (48) treated with the phosphatase (PP1, PP2) inhibitors cantharidin (49) and okadaic acid (50), which at lower concentrations preferentially inhibit PP2A over other phosphatases. Treatment with either inhibitor significantly reduced the retention of SYCP1 at the centromeres of late diplotene chromosome spreads and resulted in a substantial loss of centromere pairing (Fig. 3 A-D). Recent studies in *Drosophila* have suggested that PP2A and shugoshin might each act to promote localization of the other to the centromeres (39), but in our experiments no reduction in SGO2 localization was seen at the centromeres following addition of the phosphatase inhibitors (Fig. 3 A and D). In sum, our results support the model that PP2A, recruited by SGO2, opposes kinase activities that promote SC disassembly upon pachytene exit.

Shugoshin promotes disjunction of non-exchange chromosomes

Is shugoshin important for promoting the meiotic segregation of non-exchange partner chromosomes? In yeast, it has been possible to test the role of centromere-pairing in meiosis, and it has been shown that non-exchange chromosomes depend on centromere pairing for their segregation fidelity (20, 22). In the mouse model, there is no established system for following the fate of non-exchange partner chromosomes, so we addressed this question using budding yeast, which has a single shugoshin gene, *SGO1*. Yeast *sgo1Δ* (deletion) mutants show only low levels of meiosis I non-disjunction, but severe defects in meiosis II (51). As was first shown in *Drosophila* (27, 28), the meiosis II defect is due to a failure to protect centromeric cohesion at the metaphase I to anaphase I transition. We first monitored the requirement for *sgo1* for centromere pairing of a pair of centromere plasmids that act as non-exchange mini-chromosome partners in meiosis (52). These mini-chromosomes do not experience exchanges, yet they disjoin properly in most meioses (6, 9, 53). Cells bearing the mini-chromosomes were induced to enter meiosis, chromosome spreads were prepared, and pachytene spreads (as judged by Zip1 morphology) were scored for the association of the two mini-chromosomes, which are tagged at their centromeres with GFP and tdTomato (Fig. 4 A). The distances between the red and green foci marking the mini-chromosomes was measured in wild-type (*SGO1*) cells or cells that do not express *SGO1* in meiosis (*sgo1-md*) (54). Foci with center-to-center distances of less than 0.6 μm were as scored as "paired" (as in Fig. 4 A top panel) while those farther apart were scored as "unpaired" (as in Fig. 4 A, bottom panel). The *sgo1-md* mutants show considerable centromere pairing in pachytene, though at slightly lower levels than the control (Fig. 4 B). Deletion of the SC gene *ZIP1*, whose protein is thought to mediate centromere pairing, reduces pairing to a few percent in these assays (52). Thus, as was seen in mice (Fig. 2) shugoshin is not essential for establishing centromere paring.

To test whether the centromere pairing in *sgo1-md* mutants is able to promote

disjunction of the mini-chromosomes we monitored their segregation in anaphase I (Fig. 4 C). Cells were harvested from meiotic time courses, and fluorescence microscopy was used to determine whether the plasmids (marked by red and green foci) segregated to opposite poles in anaphase I cells. In the wild-type control, the mini-chromosomes non-disjoined in about 29% of meioses while in the *sgo1-md* mutants they non-disjoined in 50% of meioses – consistent with random segregation (Fig. 4 D). From this we conclude that although the mini-chromosomes pair at their centromeres in pachytene, the pairing does not ensure disjunction.

As a second test of the role of Sgo1 in promoting the disjunction of non-exchange partners we monitored the segregation of authentic yeast chromosomes. In these experiments yeast carried either a normal chromosome *V* pair, with each homolog tagged at its centromere with GFP, or alternatively, a pair of homeologous chromosomes *V*s (one from *S. cerevisiae* and one from *S. bayanus*) that do not experience crossovers in meiosis because of sequence divergence along the homeologous partners (55, 56). Despite this sequence divergence, the *S. bayanus* chromosome *V* supports viability of *S. cerevisae* (55, 57). Deletion of *SGO1* (*sgo1Δ*) resulted in a small increase in non-disjunction frequency of the homologous chromosome *V*s (Fig. 4 E), consistent with earlier studies (51). In contrast, the non-exchange pair exhibited a significant increase in non-disjunction when *SGO1* was deleted consistent with nearly random segregation (Fig. 4 F)

The results of these experiments show that shugoshin acts at centromeres to protect centromeric SC in diplotene, much like it protects centromeric cohesins at anaphase I. While previous work demonstrated that prophase centromere pairing is essential for non-exchange disjunction in yeast (20, 22), these results reveal that centromere pairing is not sufficient to ensure disjunction. Earlier studies found that in wild-type cells, centromeric SC proteins disappear before chromosomes begin to orient on the spindle in early metaphase (20, 22-24). This observation, coupled with our findings, suggests that centromere pairing might be a pre-condition for setting up the mechanism that later promotes bi-orientation in anaphase. The

observation that non-exchange partners in *Drosophila* appear to be tethered by threads of peri-centromeric heterochromatin during the metaphase bi-orientation process (58) suggests the model that prophase centromere pairing could provide a platform for the establishment of centromeric connections between non-exchange partners. The fact that non-exchange segregation is randomized in yeast shugoshin mutants raises the possibility that shugoshin is not essential for centromere pairing, *per se*, but instead the formation or maintenance of a structure or process that promotes bi-orientation. By this model, the low levels of meiosis I non-disjunction of native chromosomes in shugoshin mutants may reflect the times at which these chromosomes fail to experience exchanges and rely upon a centromere pairing based mechanism to ensure their bi-orientation in meiosis I.

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MATERIALS AND METHODS

Yeast Strains

Yeast culturing and sporulation was as described previously (52). Strains are isogenic derivatives of rapidly sporulating strains of primarily SK1 and W303 ancestry, derived in the RE Esposito laboratory (59).

Mouse Strains

The Oklahoma Medical Research Foundation Animal Care and Use Committee approved all animal protocols. The following mice were used in this study: C57BL/6, Shugoshin $2^{-/-}$ (47).

Spermatocyte Cytology

Established approaches were employed for visualizing chromosomes in surface spreads (23, 60). Fixed spermatocyte images were analyzed using AxioVision software (Zeiss). Statistical tests were as described in the figure legends or text. For staging spermatocyte chromosome spreads, those with continuous SYCP1 signal and completely synapsed SYCP3 staining lateral elements were scored as pachytene. Staging of chromosome spreads in diplotene was based on the extent of SYCP1 staining. Those with five or more stretches of SYCP1 5 μm or longer were classified as early diplotene, those with two-to-four such SYCP1 stretches were scored as middle diplotene, and those with one or fewer 5 μm runs of SYCP1 were classified as late diplotene. The percent of centromere pairing (Fig. 2 and 3) was tabulated for chromosome ends that could clearly be resolved in the chromosome spread. The percent SYCP1 staining of centromeres was scored for pairs of centromeres (Fig. 2 and 3). In this assay, for unpaired centromeres, if either of the centromeres exhibited overlapping SYCP1 staining the pair was scored as SYCP1 positive.

Spermatocyte culture and chemical inhibition

Short-term culture of spermatocytes was performed essentially as described (48, 61-63). Inhibitors were added at the indicated concentrations. Equivalent volumes of DMSO or ethanol alone were added to “no treatment” control cultures. In all cell culture experiments, cell viability was quantified using the Trypan Blue exclusion assay.

Imaging

All images were collected using the 100X objective lens of a Zeiss AxioImager microscope with band-pass emission filters, a Roper HQ2 CCD. Image processing and measurements of image features were performed with, and AxioVision software.

Yeast Centromere Pairing Assay

Chromosome V centromere pairing in pachytene was evaluated using published methods (64) in which a *lac* operator array was inserted adjacent to the centromere of two chromosomes and a lacI-GFP hybrid protein was expressed under the control of a meiotic promoter to produce a focus of GFP at the *lac* operator arrays (65). Chromosome spreads were prepared and stained DAPI (4,6-Diamidino-2-phenylindole, dihydrochloride) to allow visualization of chromatin and with the primary antibodies mouse anti-Zip1p (a gift from Rebecca Maxfield), and chicken anti-GFP (Chemicon AB16901). Secondary antibodies were Alexa Fluor 488-conjugated goat anti-chicken IgG and Alexa Fluor 546-conjugated goat anti-mouse IgG (all from Molecular Probes). All were used at 1:1000 dilution. Immunofluorescence microscopy was used to identify those spreads with the condensed chromosomes typical of late meiotic prophase and the number of GFP and proximity of foci was used as a measure of pairing (64). Spreads with one focus, or two foci within 0.6 microns, were scored as paired. Those in which the foci were separated by a larger distance were scored as unpaired. Measurements were performed with AxioVision software. Pairing between centromere plasmids was performed similarly. Each plasmid carried an origin of replication (*ARS1*) and a 5.1 kb EcoRI fragment from chromosome *III* that includes the centromere. One plasmid was tagged with tdTomato-tetR hybrid proteins that localize to a *tet* operon operator array adjacent to the centromere (66), the other is tagged with GFP-lacI hybrid proteins that localize to a *lac* operon operator array adjacent to its centromere (65). Chromosome spreads were prepared as described in (67), with the following modifications: Cells were harvested 5-7 hours after induction of sporulation at 30°C. Primary antibodies were

mouse anti-Zip1 (used at 1:1000 dilution), chicken anti-GFP (used at 1:500 dilution; Millipore AB16901), rabbit anti-DsRed (used at 1:1000-1:2000 dilution; Clontech 632496). Secondary antibodies were obtained from Thermo Fisher: Alexa Fluor 488-conjugated goat anti-chicken IgG (used at 1:1200 dilution), Alexa Fluor 568-conjugated goat anti-mouse IgG (1:1000), Alexa Fluor 647 conjugated goat anti-rabbit IgG (used at 1:1200 dilution). Only cells that exhibited "ropey" DAPI staining were scored in this assay, and were disqualified for assessment if there was more than one GFP focus or more than one tdTomato focus. In these cells, the distance between the center of the green focus and the center of the red focus was measured using AxioVision software.

Achiasmate segregation assay

Non-disjunction frequencies of centromere plasmids were determined using published assays (20). Harvested cells were either assayed fresh or were frozen in 15% glycerol and 1% potassium acetate until the time at which they were assayed. Preparation for assaying the cells included staining the cells with DAPI and then mounting the cells on agarose pads for viewing as described previously (68). Anaphase I cells were identified by the presence of two DAPI masses on either side of elongated cells, indicating that the chromosomes had segregated. To avoid scoring cells with duplicated or lost CEN plasmids, only cells with one GFP focus and one tdTomato focus were assayed. Segregation of the GFP-tagged chromosome V's was done using similar methods following a previously described protocol (64)

References

1. Hunter N. Meiotic Recombination: The Essence of Heredity. Cold Spring Harb Perspect Biol. 2015;7(12).
2. Kurdzo EL, Dawson DS. Centromere pairing--tethering partner chromosomes in meiosis I. FEBS J. 2015;282(13):2458-70.
3. Obeso D, Pezza RJ, Dawson D. Couples, pairs, and clusters: mechanisms and implications of centromere associations in meiosis. Chromosoma. 2014;123(1-2):43-55.
4. Wolf KW. How meiotic cells deal with non-exchange chromosomes. Bioessays. 1994;16(2):107-14.
5. Carpenter AT. A meiotic mutant defective in distributive disjunction in Drosophila melanogaster. Genetics. 1973;73(3):393-428.
6. Dawson DS, Murray AW, Szostak JW. An alternative pathway for meiotic chromosome segregation in yeast. Science. 1986;234(4777):713-7.
7. Guacci V, Kaback DB. Distributive disjunction of authentic chromosomes in Saccharomyces cerevisiae. Genetics. 1991;127(3):475-88.
8. Hawley RS, Irick H, Zitron AE, Haddox DA, Lohe A, New C, et al. There are two mechanisms of achiasmate segregation in Drosophila females, one of which requires heterochromatic homology. Dev Genet. 1992;13(6):440-67.
9. Mann C, Davis RW. Meiotic disjunction of circular minichromosomes in yeast does not require DNA homology. Proc Natl Acad Sci U S A. 1986;83(16):6017-9.
10. Sturtevant AH, Beadle GW. The Relations of Inversions in the X Chromosome of Drosophila Melanogaster to Crossing over and Disjunction. Genetics. 1936;21(5):554-604.
11. Weinstein A. The Theory of Multiple-Strand Crossing over. Genetics. 1936;21(3):155-99.
12. Woods LM, Hodges CA, Baart E, Baker SM, Liskay M, Hunt PA. Chromosomal influence on meiotic spindle assembly: abnormal meiosis I in female Mlh1 mutant mice. The Journal of cell biology. 1999;145(7):1395-406.
13. Cheng EY, Hunt PA, Naluai-Cecchini TA, Fligner CL, Fujimoto VY, Pasternack TL, et al. Meiotic recombination in human oocytes. PLoS Genet. 2009;5(9):e1000661.
14. Oliver TR, Feingold E, Yu K, Cheung V, Tinker S, Yadav-Shah M, et al. New insights into human nondisjunction of chromosome 21 in oocytes. PLoS Genet. 2008;4(3):e1000033.
15. Fledel-Alon A, Wilson DJ, Broman K, Wen X, Ober C, Coop G, et al. Broad-scale recombination patterns underlying proper disjunction in humans. PLoS Genet. 2009;5(9):e1000658.
16. Hassold T, Hunt PA, Sherman S. Trisomy in humans: incidence, origin and etiology. Curr Opin Genet Dev. 1993;3(3):398-403.
17. Tease C, Hartshorne GM, Hulten MA. Patterns of meiotic recombination in human fetal oocytes. American journal of human genetics. 2002;70(6):1469-79.
18. Dernburg AF, Sedat JW, Hawley RS. Direct evidence of a role for heterochromatin in meiotic chromosome segregation. Cell. 1996;86(1):135-46.
19. Ding DQ, Yamamoto A, Haraguchi T, Hiraoka Y. Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. Dev Cell. 2004;6(3):329-41.
20. Gladstone MN, Obeso D, Chuong H, Dawson DS. The synaptonemal complex protein Zip1 promotes bi-orientation of centromeres at meiosis I. PLoS Genet. 2009;5(12):e1000771.

21. Karpen GH, Le MH, Le H. Centric heterochromatin and the efficiency of achiasmate disjunction in Drosophila female meiosis. Science. 1996;273(5271):118-22.
22. Newnham L, Jordan P, Rockmill B, Roeder GS, Hoffmann E. The synaptonemal complex protein, Zip1, promotes the segregation of nonexchange chromosomes at meiosis I. Proc Natl Acad Sci U S A. 2010;107(2):781-5.
23. Bisig CG, Guiraldelli MF, Kouznetsova A, Scherthan H, Hoog C, Dawson DS, et al. Synaptonemal complex components persist at centromeres and are required for homologous centromere pairing in mouse spermatocytes. PLoS Genet. 2012;8(6):e1002701.
24. Qiao H, Chen JK, Reynolds A, Hoog C, Paddy M, Hunter N. Interplay between synaptonemal complex, homologous recombination, and centromeres during mammalian meiosis. PLoS Genet. 2012;8(6):e1002790.
25. Takeo S, Lake CM, Morais-de-Sa E, Sunkel CE, Hawley RS. Synaptonemal complex-dependent centromeric clustering and the initiation of synapsis in Drosophila oocytes. Curr Biol. 2011;21(21):1845-51.
26. Rankin S. Complex elaboration: making sense of meiotic cohesin dynamics. FEBS J. 2015;282(13):2426-43.
27. Kerrebrock AW, Miyazaki WY, Birnby D, Orr-Weaver TL. The Drosophila mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. Genetics. 1992;130(4):827-41.
28. Kerrebrock AW, Moore DP, Wu JS, Orr-Weaver TL. Mei-S332, a Drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. Cell. 1995;83(2):247-56.
29. Marston AL. Shugoshins: tension-sensitive pericentromeric adaptors safeguarding chromosome segregation. Mol Cell Biol. 2015;35(4):634-48.
30. Kitajima TS, Sakuno T, Ishiguro K, Iemura S, Natsume T, Kawashima SA, et al. Shugoshin collaborates with protein phosphatase 2A to protect cohesin. Nature. 2006;441(7089):46-52.
31. Lee J, Kitajima TS, Tanno Y, Yoshida K, Morita T, Miyano T, et al. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. Nat Cell Biol. 2008;10(1):42-52.
32. Rattani A, Wolna M, Ploquin M, Helmhart W, Morrone S, Mayer B, et al. Sgol2 provides a regulatory platform that coordinates essential cell cycle processes during meiosis I in oocytes. Elife. 2013;2:e01133.
33. Riedel CG, Katis VL, Katou Y, Mori S, Itoh T, Helmhart W, et al. Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I. Nature. 2006;441(7089):53-61.
34. Xu Z, Cetin B, Anger M, Cho US, Helmhart W, Nasmyth K, et al. Structure and function of the PP2A-shugoshin interaction. Mol Cell. 2009;35(4):426-41.
35. Ishiguro T, Tanaka K, Sakuno T, Watanabe Y. Shugoshin-PP2A counteracts casein-kinase-1-dependent cleavage of Rec8 by separase. Nat Cell Biol. 2010;12(5):500-6.
36. Katis VL, Lipp JJ, Imre R, Bogdanova A, Okaz E, Habermann B, et al. Rec8 phosphorylation by casein kinase 1 and Cdc7-Dbf4 kinase regulates cohesin cleavage by separase during meiosis. Dev Cell. 2010;18(3):397-409.

37. Rumpf C, Cipak L, Dudas A, Benko Z, Pozgajova M, Riedel CG, et al. Casein kinase 1 is required for efficient removal of Rec8 during meiosis I. Cell Cycle. 2010;9(13):2657-62.
38. Cahoon CK, Hawley RS. Regulating the construction and demolition of the synaptonemal complex. Nat Struct Mol Biol. 2016;23(5):369-77.
39. Jordan PW, Karppinen J, Handel MA. Polo-like kinase is required for synaptonemal complex disassembly and phosphorylation in mouse spermatocytes. J Cell Sci. 2012;125(Pt 21):5061-72.
40. Tarsounas M, Pearlman RE, Moens PB. Meiotic activation of rat pachytene spermatocytes with okadaic acid: the behaviour of synaptonemal complex components SYN1/SCP1 and COR1/SCP3. J Cell Sci. 1999;112 (Pt 4):423-34.
41. Sourirajan A, Lichten M. Polo-like kinase Cdc5 drives exit from pachytene during budding yeast meiosis. Genes Dev. 2008;22(19):2627-32.
42. Argunhan B, Leung WK, Afshar N, Terentyev Y, Subramanian VV, Murayama Y, et al. Fundamental cell cycle kinases collaborate to ensure timely destruction of the synaptonemal complex during meiosis. EMBO J. 2017;36(17):2488-509.
43. Carlile TM, Amon A. Meiosis I is established through division-specific translational control of a cyclin. Cell. 2008;133(2):280-91.
44. Matos J, Lipp JJ, Bogdanova A, Guillot S, Okaz E, Junqueira M, et al. Dbf4-dependent CDC7 kinase links DNA replication to the segregation of homologous chromosomes in meiosis I. Cell. 2008;135(4):662-78.
45. Sun F, Handel MA. Regulation of the meiotic prophase I to metaphase I transition in mouse spermatocytes. Chromosoma. 2008;117(5):471-85.
46. Gomez R, Valdeolmillos A, Parra MT, Viera A, Carreiro C, Roncal F, et al. Mammalian SGO2 appears at the inner centromere domain and redistributes depending on tension across centromeres during meiosis II and mitosis. EMBO Rep. 2007;8(2):173-80.
47. Llano E, Gomez R, Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Vazquez-Quinones L, et al. Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice. Genes Dev. 2008;22(17):2400-13.
48. La Salle S, Sun F, Handel MA. Isolation and short-term culture of mouse spermatocytes for analysis of meiosis. Methods Mol Biol. 2009;558:279-97.
49. Honkanen RE. Cantharidin, another natural toxin that inhibits the activity of serine/threonine protein phosphatases types 1 and 2A. FEBS Lett. 1993;330(3):283-6.
50. Bialojan C, Takai A. Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. Biochem J. 1988;256(1):283-90.
51. Marston AL, Tham WH, Shah H, Amon A. A genome-wide screen identifies genes required for centromeric cohesion. Science. 2004;303(5662):1367-70.
52. Kurdzo EL, Obeso D, Chuong H, Dawson DS. Meiotic Centromere Coupling and Pairing Function by Two Separate Mechanisms in Saccharomyces cerevisiae. Genetics. 2017;205(2):657-71.
53. Sears DD, Hegemann JH, Hieter P. Meiotic recombination and segregation of human-derived artificial chromosomes in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A. 1992;89(12):5296-300.
54. Monje-Casas F, Prabhu VR, Lee BH, Boselli M, Amon A. Kinetochore orientation during meiosis is controlled by Aurora B and the monopolin complex. Cell. 2007;128(3):477-90.

55. Maxfield Boumil R, Kemp B, Angelichio M, Nilsson-Tillgren T, Dawson DS. Meiotic segregation of a homeologous chromosome pair. Mol Genet Genomics. 2003;268(6):750-60.
56. Nakao Y, Kanamori T, Itoh T, Kodama Y, Rainieri S, Nakamura N, et al. Genome sequence of the lager brewing yeast, an interspecies hybrid. DNA Res. 2009;16(2):115-29.
57. Goldman AS, Lichten M. Restriction of ectopic recombination by interhomolog interactions during Saccharomyces cerevisiae meiosis. Proc Natl Acad Sci U S A. 2000;97(17):9537-42.
58. Hughes SE, Gilliland WD, Cotitta JL, Takeo S, Collins KA, Hawley RS. Heterochromatic threads connect oscillating chromosomes during prometaphase I in Drosophila oocytes. PLoS Genet. 2009;5(1):e1000348.
59. Dresser ME, Ewing DJ, Harwell SN, Coody D, Conrad MN. Nonhomologous synapsis and reduced crossing over in a heterozygous paracentric inversion in Saccharomyces cerevisiae. Genetics. 1994;138(3):633-47.
60. Guiraldelli MF, Eyster C, Wilkerson JL, Dresser ME, Pezza RJ. Mouse HFM1/Mer3 is required for crossover formation and complete synapsis of homologous chromosomes during meiosis. PLoS Genet. 2013;9(3):e1003383.
61. Handel MA, Caldwell KA, Wiltshire T. Culture of pachytene spermatocytes for analysis of meiosis. Dev Genet. 1995;16(2):128-39.
62. Rao HB, Qiao H, Bhatt SK, Bailey LR, Tran HD, Bourne SL, et al. A SUMO-ubiquitin relay recruits proteasomes to chromosome axes to regulate meiotic recombination. Science. 2017;355(6323):403-7.
63. Wiltshire T, Park C, Caldwell KA, Handel MA. Induced premature G2/M-phase transition in pachytene spermatocytes includes events unique to meiosis. Dev Biol. 1995;169(2):557-67.
64. Kemp B, Boumil RM, Stewart MN, Dawson DS. A role for centromere pairing in meiotic chromosome segregation. Genes Dev. 2004;18(16):1946-51.
65. Straight AF, Belmont AS, Robinett CC, Murray AW. GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr Biol. 1996;6(12):1599-608.
66. Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell. 1997;91(1):35-45.
67. Grubb J, Brown MS, Bishop DK. Surface Spreading and Immunostaining of Yeast Chromosomes. J Vis Exp. 2015(102):e53081.
68. Kim S, Meyer R, Chuong H, Dawson DS. Dual mechanisms prevent premature chromosome segregation during meiosis. Genes Dev. 2013;27(19):2139-46.

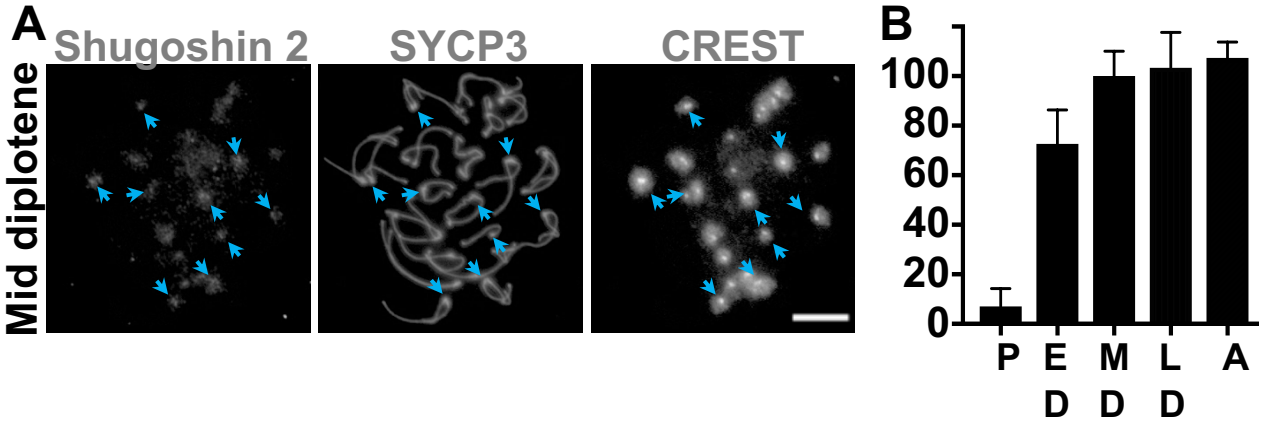
A
Shugoshin 2
SYCP3
CREST
Mid diplotene
B
100
80
60
40
20
0
P
E D
M D
L D
A

Figure 1

Figure Legends

Figure 1. SGO2 co-localizes with persisting synaptonemal complex components at centromeres in prophase of meiosis I. (A) Indirect immunofluorescence was used to evaluate localization SGO2 on chromosomes at stages of diplotene. Staging of chromosome spreads was by the extent of separation of the chromosome axes which were labelled with antibodies against SYCP3; early diplotene spreads feature still-synapsed regions, while later diplotene spreads feature chromosome axes are only joined at chiasmata. Staining with CREST antibody was used to identify centromere regions. Arrowheads indicate examples of paired centromeres. Scale bar represents 5 μm and applies to all images. (B) The average percent of centromeres per spread showing co-localization of SGO2. Error bars indicate standard deviation. A minimum of twenty spreads was scored for each category.

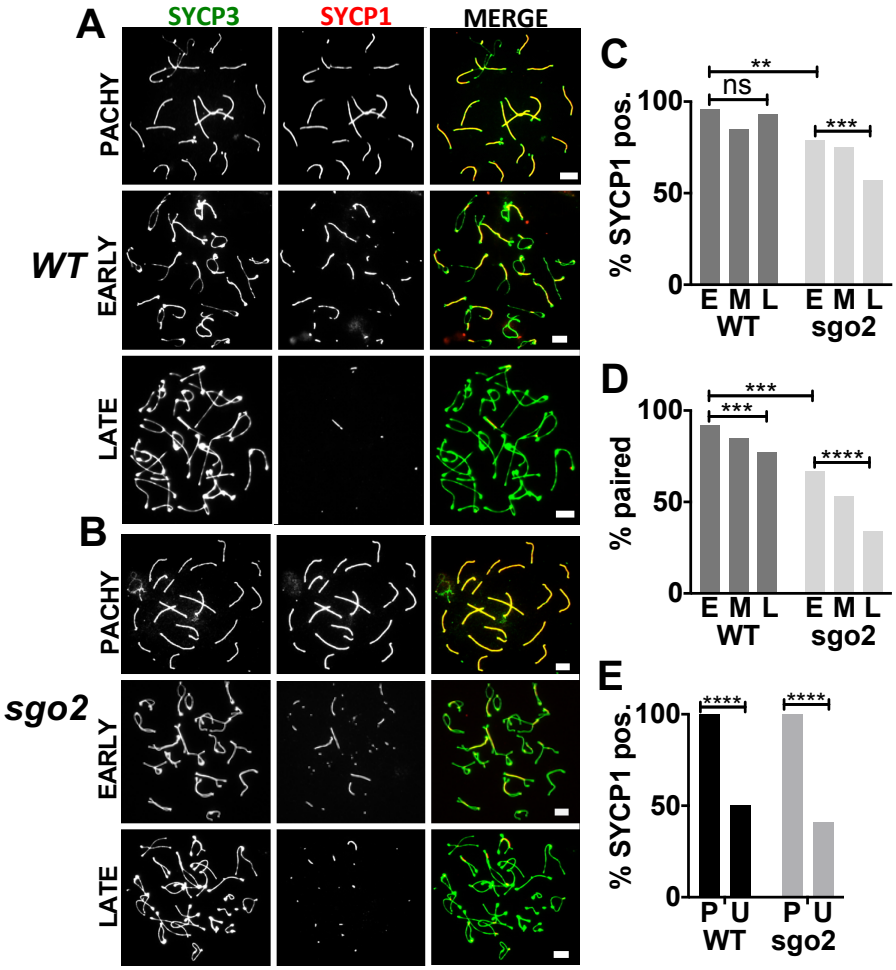
A
SYCP3
SYCP1
MERGE
PACHY
EARLY
LATE
WT
B
PACHY
EARLY
LATE
sgo2
C
% SYCP1 pos.
100
50
0
ns
**
***
E M L
WT
E M L
sgo2
D
% paired
100
50
0
***
***
****
E M L
WT
E M L
sgo2
E
% SYCP1 pos.
100
50
0
****
****
P U
WT
P U
sgo2

Figure 2

Figure 2. SGO2 is required for the persistence of centromeric synaptonemal complex components in diplotene. Indirect immunofluorescence was used to monitor the morphology of chromosomes from wild-type and *SGO2* $^{-/-}$ spermatocytes. SYCP3 localizes to chromosome axes, SYCP1 localizes to the central region of the SC and CREST antibody was used to identify centromeres. Representative chromosome spreads from pachytene, early diplotene and late diplotene (A) wild-type spermatocytes and (B) *Sgo2*$^{-/-}$ spermatocytes. Scale bars represent 5 μm. (C) Histogram of SYCP1 localization at centromeres in spreads from wildtype and *Sgo2* $^{-/-}$ spermatocytes in early, middle, and late diplotene. The number of centromeres scored was: *WT* early (50/52), *WT* middle (46/54), *WT* late 88/95, *Sgo2* $^{-/-}$ (72/91) *Sgo2* $^{-/-}$ (114/151) *Sgo2* $^{-/-}$ (100/174). (D) Histogram of the percent paired centromeres on chromosomes from wildtype and *Sgo2* $^{-/-}$ spermatocyte. The number of centromeres scored was: *WT* early (48/52), *WT* middle (46/54), *WT* late (67/98), *Sgo2* $^{-/-}$ (61/91) *Sgo2* $^{-/-}$ (80/151) *Sgo2* $^{-/-}$ (59/174). (E) SYCP1 localization to paired and unpaired centromeres. (E) Paired (P) and unpaired (U) centromeres from all stages of diplotene (D above) were classified as according to their SYCP1 staining. The numbers of centromeres scored was: WT paired (167/167), WT unpaired (17/34), *Sgo2* $^{-/-}$ paired (200/200), *Sgo2* $^{-/-}$ unpaired (89/216). The significance of differences between samples was evaluated using Fisher's exact test. **p<0.01, *** p<0.001, ****p<0.0001.

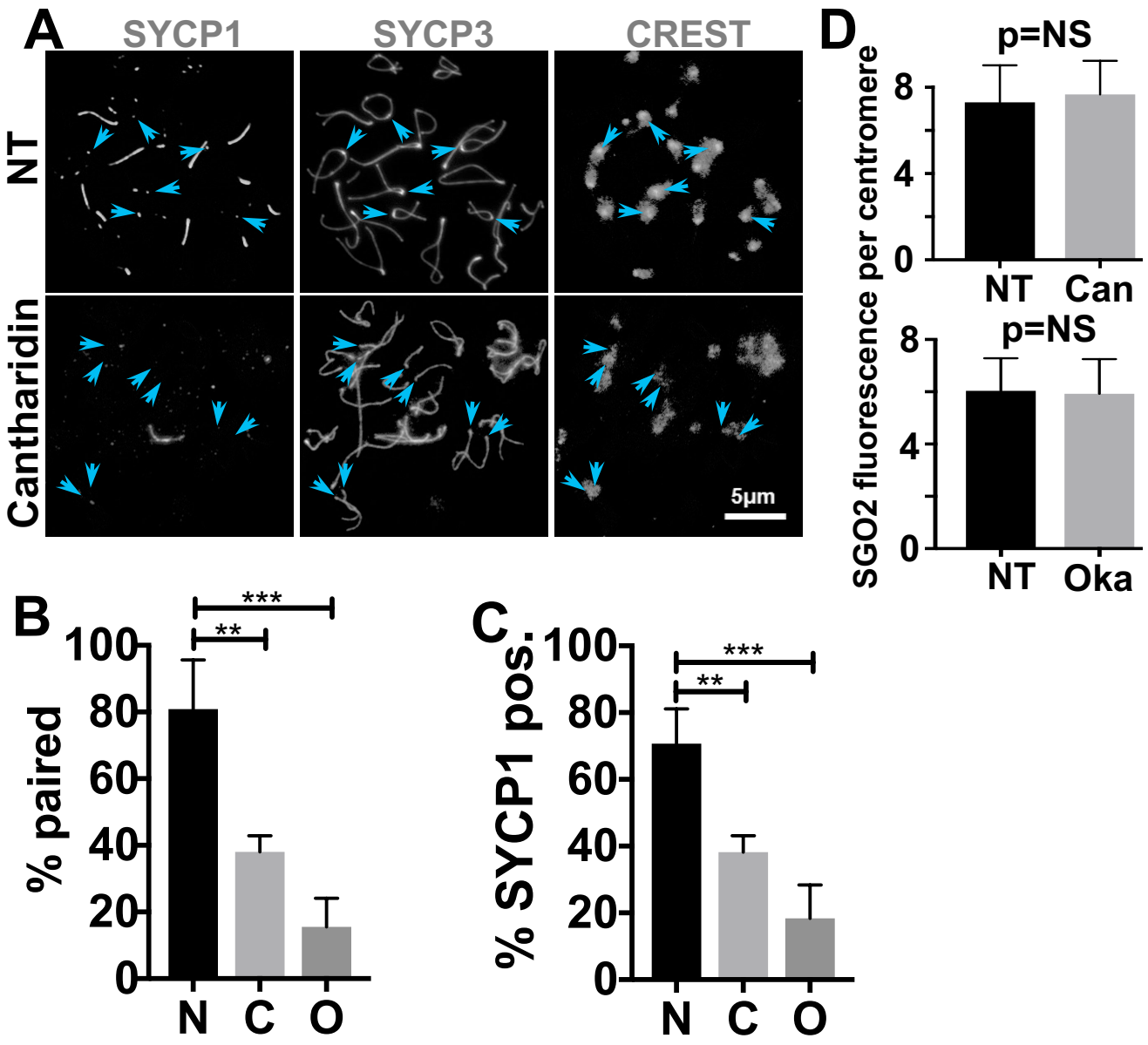
A
SYCP1
SYCP3
CREST
NT
Cantharidin
5µm
B
% paired
***
**
100
80
60
40
20
0
N
C
O
C
% SYCP1 pos.
***
**
100
80
60
40
20
0
N
C
O
D
p=NS
SGO2 fluorescence per centromere
8
4
0
NT
Can
p=NS
8
4
0
NT
Oka

Figure 3

Figure 3. Phosphatase activity is needed for SYCP1 to persist at centromeres in diplotene. Cultured spermatocytes were treated with the phosphatase inhibitors okadaic acid or cantharidin. Chromosome spreads from diplotene cells, identified by the separation of their chromosome axes (stained with antibodies against SYCP3) were then evaluated using indirect immunofluorescence microscopy. The presence of SYCP1 at centromeres and the fraction of chromosomes in each spread with paired centromeres (stained with CREST antibodies) were scored. (A) Representative images of chromosome spreads that were not treated (NT) or treated with cantharidin. Scale bar represents 5 μm and applies to all panels. Arrowheads indicate the locations of example paired centromeres in top panels and unpaired centromeres in bottom panel. For each treatment, one hundred diplotene chromosome spreads were scored for SCYP1 localization to centromeres and centromere pairing. (B) The percentage of chromosomes in each spread with SYCP1 present at the centromeres. Averages and standard deviations are: N (not treated) 80.9 +/-14.7%. C (cantharidin) 38.0+/-4.9%. O (okadaic acid) 15.5+/8.6%. (C) The percentage of chromosomes in each spread with paired centromeres. Averages and standard deviations are: N (not treated) 70.7 +/-10.4%. C (cantharidin) 38.2+/-4.9%. O (okadaic acid) 18.4+/10.0%. One hundred chromosome spreads were scored for each treatment and significance was evaluated using the student's t test. (D). Histogram showing the relative amount of SGO2 on centromeres of untreated or cantharidin or okadaic acid treated spermatocytes. ** $p<0.01$, *** $p<0.001$.

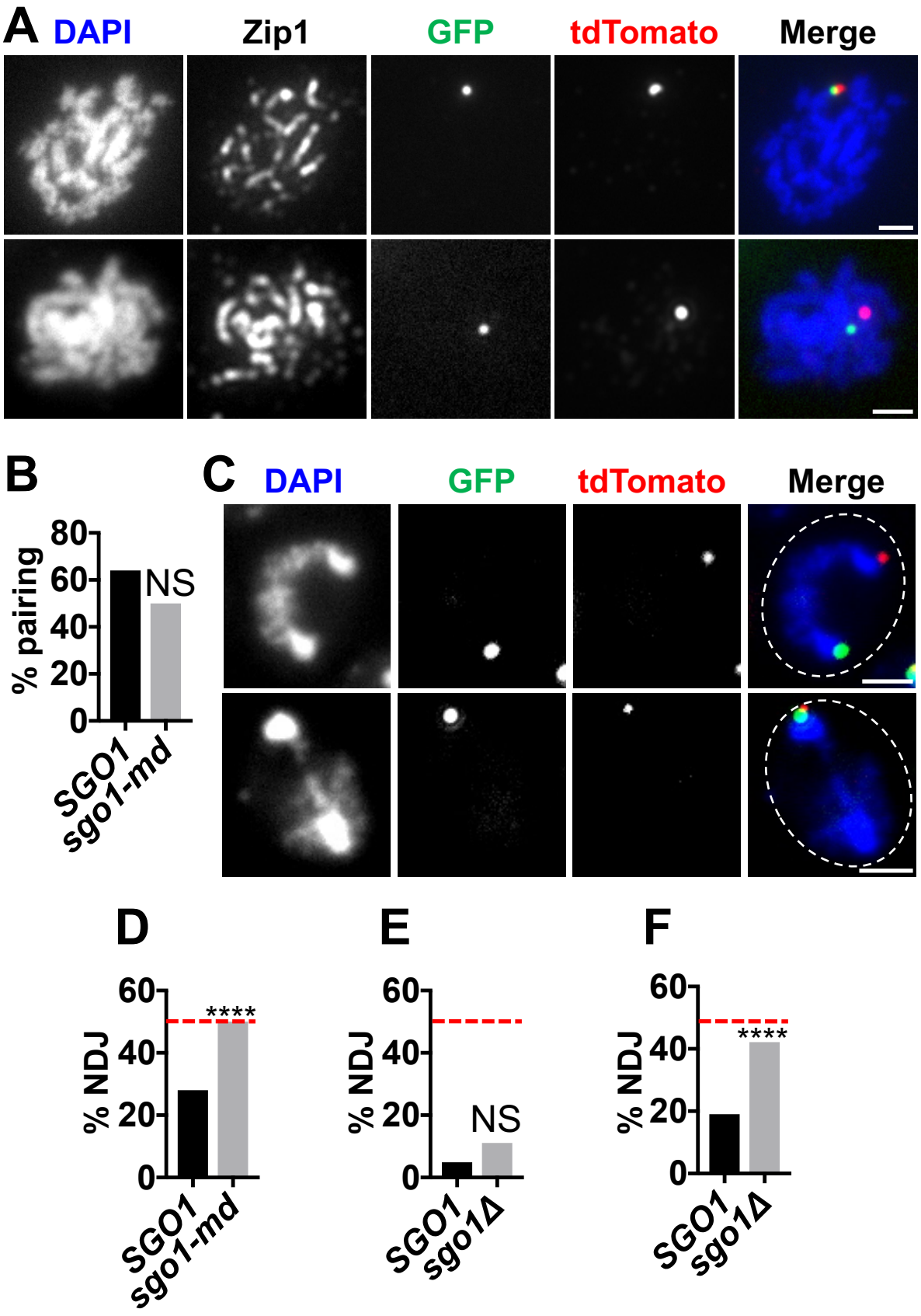
A
DAPI
Zip1
GFP
tdTomato
Merge
B
% pairing
80
60
40
20
0
NS
SGO1
sgo1-md
C
DAPI
GFP
tdTomato
Merge
D
% NDJ
60
40
20
0
****
SGO1
sgo1-md
E
% NDJ
60
40
20
0
NS
SGO1
sgo1Δ
F
% NDJ
60
40
20
0
****
SGO1
sgo1Δ

Figure 4

Figure 4. Shugoshin is not needed for centromere pairing in pachytene but is required for non-exchange segregation. (A) Representative chromosome spreads showing examples of paired (top) and unpaired (bottom) mini-chromosome centromeres (both images from the *sgo1-md* strain). Chromosome spreads were stained with DAPI to show chromatin, anti-Zip1 antibody to show the SC, and anti-GFP and DsRed to show the locations of the centromere-proximal tags. Scale bar equals 1 μm. (B) Histogram showing percent centromere pairing in each strain (*SGO1* n=50 spreads, *sgo1-md* n=100 spreads). (C) Shugoshin is required for non-exchange segregation of mini-chromosomes. Representative anaphase cells showing disjoined (top) and non-disjoined (bottom) mini-chromosomes. Cells were stained with DAPI to show chromatin. Locations of mini-chromosome centromeres were detected by GFP and tdTomato fluorescence. Scale bar equals 1 μm. (D) Histogram showing non-disjunction frequencies of mini-chromosomes in *SGO1* and *sgo1-md* cells (n=150 cells for both strains). (E) Histogram showing non-disjunction frequencies of homologous chromosomes in *SGO1* and *sgo1*Δ cells. (n=122 cells for *SGO1* and 90 for *sgo1*Δ). (F) Histogram showing non-disjunction frequencies of homeologous chromosomes in *SGO1* and *sgo1*Δ cells. (n=121 cells for both strains, non-disjunction frequencies were 19.0% vs 42.1%, P=0.0001). (B, D-F) Red line equals the level of non-disjunction expected for random segregation. Statistical comparisons were performed with Fisher's exact test. For all histograms, NS=not significant, **** $p < 0.0001$.