

1 **A *ZIP1* Separation-of-Function Allele Reveals that Meiotic Centromere Pairing Drives**  
2 **Meiotic Segregation of Achiasmate Chromosomes in Budding Yeast**

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5 Emily L. Kurdzo\*, Hoa H Chuong, and Dean S. Dawson\*

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7  
8 Program in Cell Cycle and Cancer Biology  
9 Oklahoma Medical Research Foundation

10 and

11 \*Department of Cell Biology  
12 University of Oklahoma Health Sciences Center  
13 Oklahoma City, OK 73104

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16 **Short Title: Meiotic centromere pairing**  
17

18 **ABSTRACT**

19

20 In meiosis I, homologous chromosomes segregate away from each other – the first of two rounds  
21 of chromosome segregation that allow the formation of haploid gametes. In prophase I,  
22 homologous partners become joined along their length by the synaptonemal complex (SC) and  
23 crossovers form between the homologs to generate links called chiasmata. The chiasmata allow  
24 the homologs to act as a single unit, called a bivalent, as the chromosomes attach to the  
25 microtubules that will ultimately pull them away from each other at anaphase I. Recent studies,  
26 in several organisms, have shown that when the SC disassembles at the end of prophase, residual  
27 SC proteins remain at the homologous centromeres providing an additional link between the  
28 homologs. In budding yeast, this centromere pairing is correlated with improved segregation of  
29 the paired partners in anaphase. However, the causal relationship of prophase centromere pairing  
30 and subsequent disjunction in anaphase has been difficult to demonstrate as has been the  
31 relationship between SC assembly and the assembly of the centromere pairing apparatus. Here, a  
32 series of in-frame deletion mutants of the SC component Zip1 were used to address these  
33 questions. The identification of separation-of-function alleles that disrupt centromere pairing, but  
34 not SC assembly, have made it possible to demonstrate that centromere pairing and SC assembly  
35 have mechanistically distinct features and that prophase centromere pairing function of Zip1  
36 drives disjunction of the paired partners in anaphase I.

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## 40 **AUTHOR SUMMARY**

41  
42 The generation of gametes requires the completion of a specialized cell division called meiosis.  
43 This division is unique in that it produces cells (gametes) with half the normal number of  
44 chromosomes (such that when two gametes fuse the normal chromosome number is restored).  
45 Chromosome number is reduced in meiosis by following a single round of chromosome  
46 duplication with two rounds of segregation. In the first round, meiosis I, homologous  
47 chromosomes first pair with each other, then attach to cellular cables, called microtubules, that  
48 pull them to opposite sides of the cell. It has long been known that the homologous partners  
49 become linked to each other by genetic recombination in a way that helps them behave as a  
50 single unit when they attach to the microtubules that will ultimately pull them apart. Recently, it  
51 was shown, in budding yeast and other organisms, that homologous partners can also pair at their  
52 centromeres. Here we show that this centromere pairing also contributes to proper segregation of  
53 the partners away from each other at meiosis I, and demonstrate that one protein involved in this  
54 process is able to participate in multiple mechanisms that help homologous chromosomes to pair  
55 with each other before being segregated in meiosis I.  
56

## 57 **INTRODUCTION**

58 In meiosis I, homologous chromosomes segregate away from each other – the first of two  
59 rounds of segregation that allow the formation of haploid gametes. In order to segregate from  
60 one another the homologs must first become tethered together as a unit, called a bivalent. As a  
61 single bivalent, the partners can attach to microtubules such that the centromeres of the  
62 homologs will be pulled towards opposite poles of the spindle at the first meiotic division.  
63 Crossovers between the aligned homologs provide critical links, called chiasmata, which allow  
64 the homologs to form a stable bivalent (reviewed in (1)). Failures in crossing-over are associated  
65 with elevated levels of meiotic segregation errors in many organisms, including humans  
66 (reviewed in (2)). However, there are mechanisms, other than crossing-over, that can also tether  
67 partner chromosomes. Notably, studies in yeast and mouse spermatocytes have revealed that the  
68 centromeres of partner chromosomes pair in prophase of meiosis I (3-6). In budding yeast, it has  
69 been shown that this centromere pairing is correlated with the proper segregation of chromosome  
70 pairs that have failed to form chiasmata. But the formal demonstration that centromere pairing in  
71 prophase directly drives disjunction in anaphase has been difficult, because the mutations that  
72 disrupt centromere pairing also disrupt other critical meiotic processes (7, 8).

73 The protein Zip1 in budding yeast localizes to paired centromeres in meiotic prophase  
74 and is necessary for centromere pairing (Fig. 1 A) (7-10), and similar observations have been  
75 made in *Drosophila* oocytes and mouse spermatocytes (3, 6, 11). Zip1 is expressed early in  
76 meiosis and first appears as dispersed punctate foci in the nucleus. Some, but not all, of these  
77 foci co-localize with centromeres, and indeed, Zip1 mediates the homology-independent pairing  
78 of centromeres at this stage of meiosis, a phenomenon called centromere-coupling (Fig. 1 A,  
79 green arrowhead) (10, 12). Zip1 later acts as a component of the synaptonemal complex (SC) –  
80 a proteinaceous structure that assembles between the axes of the homologous partners as they  
81 become aligned in meiotic prophase (Fig. 1 A, blue arrowhead) (13). In budding yeast and mouse  
82 spermatocytes, when the SC disassembles in late prophase Zip1/SYCP1 remains at the paired  
83 centromeres, leaving the homologous partners only visibly joined by chiasmata and centromere-  
84 pairing (Fig. 1 A) (3, 6-8). Most Zip1/SYCP1 appears to have left the chromosomes by the time  
85 they begin attaching to the meiotic spindles. The prophase association promoted by Zip1 is

86 correlated with proper segregation, as *zip1* deletion mutants have no centromere pairing and also  
87 segregate achiasmate partners randomly (Fig. 1A) (7, 8).

88 A critical study by Tung & Roeder identified functional domains of Zip1 that contribute  
89 to SC assembly, and contributed to the current model for the structure of the SC (14). This and  
90 other studies (15) have suggested that in the SC, Zip1 is in the form of head-to-head dimers (Fig.  
91 1 B). These dimers, in turn are thought to assemble in a ladder-like structure with the N-termini  
92 in the center of the SC and the C-termini associated with the axes of the homologous partners  
93 (Fig. 1 B). This model has been extrapolated to other organisms because the basic structure of  
94 transverse filament components, like Zip1, are believed to be conserved even though their amino  
95 acid sequences have diverged (reviewed in (16)).

96 Tung and Roeder (1998) used an ordered series of in-frame deletions of *ZIP1* to identify  
97 ways in which different regions of the protein contributed to SC structure and function (Fig. 1  
98 C). This was before the discovery that Zip1 is also involved in promoting centromere coupling  
99 and centromere pairing. We have re-constructed this deletion series to evaluate the ways in  
100 which different regions of Zip1 contribute to these centromere-associated functions. This  
101 information could be used to reveal relationships in the underlying mechanisms of centromere  
102 coupling, centromere pairing and SC assembly, and identify to separation-of-function alleles that  
103 would reveal more specifically contributions made to these processes by Zip1. These approaches  
104 make clear that centromere coupling, centromere pairing, and SC assembly all require certain  
105 parts of the Zip1 protein that are not required by the others –suggesting mechanistic differences  
106 in these phenomena. Second, they provide a clear demonstration that centromere pairing in  
107 prophase, distinct from other SC-related functions of Zip1, drives disjunction of achiasmate  
108 partner chromosomes in anaphase I.  
109

## 110 RESULTS

### 111 The N and C terminal globular domains of Zip1 are essential for centromere coupling.

112 A series of nine in-frame deletion mutants (Fig. 1 C) were tested to determine which  
113 regions of the *ZIP1* coding sequence are essential for the homology independent centromere  
114 coupling that occurs in early meiotic prophase. Centromere coupling was assayed by monitoring  
115 the numbers of kinetochore foci (Mtw1-MYC) in chromosome spreads from prophase meiotic  
116 cells (10, 12) (Fig. 2 A). Diploid yeast have sixteen pairs of homologous chromosomes. When  
117 the centromeres of the thirty-two chromosomes are coupled they form on average sixteen Mtw1-  
118 MYC foci (Fig. 2 B, *ZIP1*, blue line). Mutants that are defective in coupling exhibit higher  
119 numbers of Mtw1-MYC foci (Fig. 2 B, *zip1*Δ, red line). The experiment was done in strains  
120 lacking *SPO11*, which encodes the endonuclease responsible for creating programmed double  
121 strand DNA (17)). This blocks meiotic progression beyond the coupling stage and prevents the  
122 homologous alignment of chromosomes (12, 18). The strains also featured GFP-tagged copies of  
123 the centromeres of chromosome I. Briefly, 256 repeats of the *lac* operon sequence was inserted  
124 adjacent to the centromere of chromosome I (*CEN1*) and the cells were engineered to express  
125 lacI-GFP, which localizes to the lacO array (19). In the centromere coupling stage, the two  
126 CEN1-GFP foci are nearly always separate because coupling is usually between non-  
127 homologous partner chromosomes (Fig. 2 A) (10).

128 The mutants could be assigned to one of three groups based on their coupling phenotypes  
129 (Fig. 2 B and Supplemental Table 2), indistinguishable from *ZIP1* (proficient for coupling; blue  
130 histograms), indistinguishable from *zip1*Δ (loss of coupling; red and orange histograms), or  
131 intermediate (green histogram) (Fig. 2 B). The results make it possible to assign functional roles

132 to several portions of Zip1. First, a portion of the N-terminus and adjacent coiled-coil (NM1  
133 region, amino acids 164-242) is critical for centromere coupling. This region was shown to be  
134 largely dispensable for SC assembly and sporulation in previous work (14). Second, a portion of  
135 the C-terminus (C1 region, amino acids 791-824) shown previously to be essential for SC  
136 assembly (14), is also critical for centromere coupling. Third, two mutants that are unable to  
137 assemble SC (*zip1-C2* and *zip1-M1*; (14)) are indistinguishable from wild-type cells for  
138 centromere coupling. We conclude that Zip1 contains some regions that are critical for  
139 centromere coupling but not SC formation and vice versa.

140

### 141 **The N-terminus of Zip1 is essential for promoting the segregation of achiasmate partners**

142 Though centromere coupling and centromere pairing both require Zip1, they have distinct  
143 genetic requirements suggesting they may operate by (at least partially) different mechanisms  
144 (20). To determine the regions of Zip1 that are required for achiasmate segregation we monitored  
145 the meiotic segregation of a pair of centromere plasmids that act as achiasmate partners in  
146 meiosis. Each plasmid carries an origin of DNA replication and the centromere of chromosome  
147 *III*, allowing the plasmids to behave as single copy mini-chromosomes in yeast. One plasmid is  
148 tagged with tdTomato-tetR hybrid proteins at a *tet* operon operator array (21), the other is tagged  
149 with GFP, as described above for chromosome I. Previous work has shown that such achiasmate  
150 model chromosomes disjoin properly in most meioses (22-24) and this segregation at anaphase I  
151 is correlated with the ability of their centromeres to pair late in prophase (5). To increase the  
152 synchrony of meiotic progression in this experiment *NDT80*, which promotes the transition out  
153 of prophase and into pro-metaphase, was placed under the control of an estradiol-inducible  
154 promotor (25-27). Meiotic cells were allowed to accumulate in pachytene of prophase, then  
155 induced to synchronously exit pachytene and enter pro-metaphase. We scored segregation of the  
156 plasmids in the first meiotic division by monitoring the location of their GFP and tdTomato-  
157 tagged centromeres in anaphase I cells, identified by their two separated chromatin masses (Fig 3  
158 A).

159 Wild-type cells, under these conditions, exhibited 28% non-disjunction of the CEN  
160 plasmid pair (Fig. 3 B). The loss of Zip1 function can result in a pachytene arrest in some strain  
161 backgrounds (28) including the strain used in these experiments. Reducing the sporulation  
162 temperature to 23°C, as was done here, can permit a partial bypass of the arrest (28). Still several  
163 of the mutations (*zip1Δ*, *zip1-C2*, *zip1-C1*, and *zip1-NM2*) yielded very few anaphase cells, and  
164 failed to sporulate, presumably due to the pachytene arrest. These observations are consistent  
165 with previously published work (14). Of the remaining mutants, the *zip1-N1* mutant showed  
166 significantly elevated non-disjunction of the centromere plasmids (Fig. 3 B). The *zip1-N1* mutant  
167 exhibits only mild defects in progression through meiosis, SC formation, sporulation efficiency,  
168 and the segregation of chiasmate chromosomes (14) and Figure S1), suggesting that amino acids  
169 23-163 are more critical for mediating the segregation of achiasmate partners than for SC  
170 assembly and function.

171 Because achiasmate segregation is correlated with prior centromere pairing (7, 8), we  
172 tested whether the *zip1-N1* mutants were proficient in centromere pairing. Wild-type and *zip1-N1*  
173 cells containing the GFP and tdTomato tagged centromere plasmids were induced to sporulate  
174 and harvested five - seven hours later when pachytene cells are prevalent. Chromosome spreads  
175 were then prepared and the distance between the tdTomato and GFP foci were measured in  
176 spreads exhibiting the condensed chromatin typical of pachytene cells (Fig. 4 A). The average  
177 centromere-centromere distance was significantly greater in *zip1-N1* mutants (Fig. 4 B)

178 consistent with a loss of pairing. When spreads with an inter-centromere distance of less than 0.6  
179  $\mu\text{m}$  were scored as “paired” (see example in Fig. 4 A), the *zip1-N1* mutation was found to exhibit  
180 a significant reduction in the frequency centromere pairing between the achiasmate plasmids  
181 (Fig. 4 C).

182

### 183 **The N-terminus of Zip1 is necessary for efficient localization to kinetochores**

184 Failure of centromere pairing in the *zip1-N1* mutant could be due to a failure of Zip1 to  
185 associate with centromeres. To test this, we analyzed the co-localization of the Zip protein with  
186 kinetochores in *ZIP1* and *zip1-N1* strains. The experiments were done in a *zip4 $\Delta$*  strain  
187 background to allow visualization of Zip1 localization independently of an SC structure. Images  
188 were collected using structured illumination microscopy and the level of co-localization was  
189 determined using ImageJ software (see Materials and Methods). Every *ZIP1* spread analyzed  
190 showed significantly more co-localization of Zip1 and Mtw1 than was found in a randomized  
191 sample (Fig. 5 A), consistent with earlier work (9, 10, 12), while many of the *zip1-N1* spreads  
192 showed no significant co-localization above the randomized control (Fig. 5 B). Consistent with  
193 these results, *zip1-N1* strains showed significantly lower levels of co-localization with Zip1 than  
194 was seen in *ZIP1* strains (Fig. 5 C).

195

### 196 **The N-terminus of Zip1 is necessary for the pairing of natural chromosomes**

197 The reduced localization of Zip1-N1 protein to natural centromeres, above, and the  
198 failure of pairing of plasmid centromeres in *zip1-N1* strains (Fig. 4) raised the question of  
199 whether the *zip1-N1* mutation compromises the pairing of natural chromosomes. To assay  
200 centromere pairing we counted the numbers of kinetochore foci (Mtw1-GFP) in chromosome  
201 spreads from *ZIP1*, *zip1-N1* and *zip1 $\Delta$*  cells, in the above experiment (Fig. 5) using structured  
202 illumination microscopy. Prior work had shown that in *zip4* mutants, with no SC, kinetochores  
203 are held in close proximity by centromere pairing. When *ZIP1* is deleted, the centromeres can  
204 resolve into two foci in chromosome spreads (29). The *ZIP1* strain gave an average of 13.9  
205 kinetochore foci per spread, consistent with pairing of the 32 kinetochores. The *zip1-N1* mutant  
206 gave significantly higher numbers of kinetochore foci (average 16.4;  $p < 0.01$ ) signifying a loss of  
207 centromere pairing but not as dramatic a loss was observed in the *zip1* strain (average 21.3;  
208  $p < 0.0001$ ).

209

## 210 **DISCUSSION**

211 Our analysis of a set of in-frame Zip1 deletions has added to our understanding of the  
212 functional domains of the Zip1 protein, helping to ascribe particular Zip1 functions to specific  
213 regions of the protein. Zip1 is critical for SC assembly and processes that depend on SC  
214 assembly, including crossover formation and progression through pachytene (28). More recently  
215 it has become clear that Zip1 acts at centromeres both early in prophase, where centromeres  
216 become associated in a homology-independent fashion (centromere coupling), and later when  
217 homologous centromeres, or the centromeres of achiasmate chromosomes, become associated by  
218 remnants of the SC that remain at the centromeres after SC disassembly (reviewed in (30)). The  
219 experiments here were intended to clarify whether SC assembly, centromere coupling, and  
220 centromere pairing incorporate Zip1 in the same or different mechanisms, and if there are  
221 differences in the regions of Zip1 that are critical to each function.

222

### 223 **Centromere coupling and SC assembly**

224 Prior work has shown convincingly that the structure that mediates centromere coupling  
225 is distinct from mature SC (9, 10, 20, 31). Several proteins (Zip2, Zip3, Zip4, Ecm11, Gmc2, and  
226 Red1) known to be essential for SC assembly are not required for centromere coupling. But the  
227 domains of Zip1 that are required for centromere coupling have not been defined. The  
228 experiments here reinforce that the requirements for Zip1 for centromere coupling and SC  
229 assembly are quite different. First, centromere coupling was proficient in *zip1-C2* mutants, which  
230 have severe defects in SC assembly. But these mutants exhibit little Zip1 expression, which may  
231 be due to the lack of a nuclear localization signal (32). Thus, this result is difficult to interpret  
232 other than to suggest that centromere coupling may require far less Zip1 than does SC assembly.  
233 Notably, the *zip1-M1* mutation, which also blocks SC assembly, is proficient in centromere  
234 coupling. The *zip1-M1* mutation, which eliminates amino acids 244-511, has a unique SC defect.  
235 The Zip1-M1 protein efficiently localizes to the axes of the homologous partners, but does not  
236 efficiently cross-bridge the axes (Fig. 1 C; (14)). This defect may reflect an inability of Zip1  
237 molecules from opposite axes to associate with one another (as in Fig.1 B) or may reflect an  
238 inability of Zip1 to associate with central element proteins that promote or stabilize the cross-  
239 bridging of axes by Zip1. In either case, such cross-bridging must not be important for  
240 centromere coupling, and is consistent with the finding that the central element proteins Ecm11  
241 and Gmc2 are also not required for centromere coupling (31). Together these findings suggest  
242 that centromere coupling is probably not mediated by a structure that includes SC-like cross-  
243 bridging. The only protein, beyond Zip1, that is known to be required for centromere coupling is  
244 the cohesin component Rec8 (9) (the requirements for the other cohesin subunits have yet to be  
245 reported). It may be that centromere coupling is mediated by the cohesin-dependent  
246 accumulation of Zip1 at early prophase centromeres (9, 29), followed by interactions between  
247 Zip1 molecules that promote the association of centromere pairs.

## 248 **Centromere pairing and SC assembly**

249 Experiments performed mainly in a mouse spermatocyte model (3, 6) suggest that the SYCP1  
250 (the functional homolog of Zip1) that persists at paired centromeres, after SC disassembly, is  
251 accompanied by other SC proteins. This suggests that centromere pairing could be mediated by a  
252 conventional SC structure. But the identity of regions of Zip1 that are critical for centromere  
253 pairing, and whether they are distinct from the regions necessary for SC assembly, have not been  
254 addressed. Our work suggests that there are significant differences in the requirements for Zip1  
255 function in centromere pairing and SC assembly. We arrive at this conclusion following an  
256 evaluation of the centromere pairing phenotypes of the *zip1-N1* in-frame deletion. Prior work had  
257 shown this allele had no measurable differences from the wild-type *ZIP1* allele in spore viability,  
258 crossover frequency, and genetic interference, and a slight defect in the continuity of mature  
259 linear SC structures (14). In our strain background the *zip1-N1* mutation also exhibited wild-type  
260 levels of spore viability, and structured illumination microscopy confirmed the slight  
261 discontinuity in some SC structures in the *zip1-N1* background (Fig. S1). However, in  
262 centromere pairing assays the *zip1-N1* mutants showed major defects. In the *zip1-N1* mutant the  
263 centromeres of natural chromosome bivalents were more likely to become disengaged in  
264 chromosome spreads than was seen with wild-type controls, but the defect was not as severe as is  
265 seen in *zip1Δ* strains – suggesting that there are regions outside of the N1 region that also  
266 promote association of the bivalent centromeres. It could be that these other regions are  
267 influencing things like cross-over frequency or distribution, that along with centromere-pairing

268 help keep bivalent centromeres associated in the natural chromosome pairing assays. When we  
269 used achiasmate centromere plasmids, in which such functions cannot contribute to centromere  
270 association, then the *zip1-N1* phenotype becomes severe. The *zip1-N1* mutant showed a dramatic  
271 reduction in the pairing of plasmid centromeres. The fact that the Zip1-N1 protein is proficient  
272 for SC assembly but highly defective in centromere pairing suggests that the N-terminus imbues  
273 functions on the protein that are specifically required for centromere pairing. The mechanism of  
274 centromere pairing remains unclear as does the role of the Zip1 N-terminus, but kinetochore co-  
275 localization experiments suggest that this region of Zip1 promotes localization to, or  
276 maintenance of, Zip1 at the centromeres in late prophase. The fact that early prophase  
277 centromere coupling is normal in *zip1-N1* mutants reinforces that coupling and pairing are  
278 fundamentally distinct processes and that the N1 region is not necessary for localization of Zip1  
279 to centromeres in early prophase when coupling occurs.

280

## 281 **Meiotic prophase centromere pairing drives achiasmate disjunction**

282 Experiments in yeast, *Drosophila* and mice have shown that SC-related proteins persist at paired  
283 centromeres after SC disassembly (3, 7, 8, 11). These observations have been the foundation for  
284 the model that centromere pairing promotes subsequent disjunction, especially of achiasmate  
285 chromosomes that are only connected at their centromeres. Demonstrating that this model is  
286 correct has been complicated by the fact that the SC is a central player in controlling meiotic  
287 progression. Thus, deletion of SC components, which eliminates centromere pairing, also  
288 impacts other processes such as synapsis, crossover formation, genetic interference, and the  
289 pachytene checkpoint, making it impossible to formally name centromere pairing, and not some  
290 other SC-related function as the driver of achiasmate segregation. The *zip1-N1* separation-of-  
291 function allele, because it is largely wild-type for these other functions of Zip1, has made it  
292 possible to demonstrate in a compelling way that centromere-pairing in prophase is a requisite  
293 step in a process that mediates the segregation of achiasmate partners in anaphase.

294 The mechanistic question of how prophase centromere pairing drives disjunction remains  
295 to be answered. The fact that in yeast, mice and *Drosophila*, the majority of the centromeric SC  
296 components have been lost from the centromeres well before the partners begin to attach to  
297 microtubules makes this even more mysterious. The *zip1-N1* allele, which specifically targets  
298 centromere associations of Zip1, and the centromere pairing process, will be an important tool  
299 for addressing these questions.

300

## 301 **MATERIALS AND METHODS**

### 302 Strains

303 We created the same nine deletion mutants of *ZIP1* that Tung and Roeder had studied for their  
304 work in SC formation (14) by using standard PCR and two-step-gene-replacement methods (33,  
305 34). All mutant versions of *ZIP1* were confirmed by PCR and sequencing. The native *ZIP1*  
306 promoter was unaltered in these strains allowing each mutant protein to be expressed at the  
307 appropriate level and time. Culturing of strains was as described previously (20). Strain  
308 genotypes are listed in Table S1.



309

### 310 Centromere coupling assay

311 Centromere coupling was monitored largely as described previously (12). Cells were  
312 harvested five hours after shifting cultures to sporulation medium at 30°C. Meiotic nuclear  
313 spreads were prepared according to (35) with minor modifications. Cells were spheroplasted  
314 using 20 mg/ml zymolyase 100T for approximately 30 minutes. Spheroplasts were briefly  
315 suspended in MEM (100mM MES, 10mM EDTA, 500µM MgCl<sub>2</sub>) containing 1mM PMSF  
316 (phenylmethylsulfonyl fluoride), fixed with 4% paraformaldehyde plus 0.1% Tween20 and  
317 spread onto poly-L-lysine-coated slides (Fisherbrand Superfrost Plus). Slides were blocked with  
318 4% non-fat dry milk in phosphate buffered saline for at least 30 minutes, and incubated overnight  
319 at 4°C with primary antibodies. Primary antibodies were mouse anti-Zip1 (used at 1:1000  
320 dilution), rabbit anti-Zip1 (used at 1:1000 dilution; Santa Cruz y-300 SC-33733), rabbit anti-  
321 MYC (1:400; Bethyl Laboratories A190-105A), mouse anti-MYC (used at 1:1000 dilution; gift  
322 from S. Rankin), chicken anti-GFP (used at 1:500 dilution; Millipore AB16901), rabbit anti-  
323 DsRed (used at 1:1000-1:2000 dilution; Clontech 632496), and rabbit anti-RFP (1:500; Thermo  
324 Scientific 600-401-379). Secondary antibodies were obtained from Thermo Fisher: Alexa Fluor  
325 488-conjugated goat anti-chicken IgG (used at 1:1200 dilution), Alexa Fluor 568-conjugated  
326 goat anti-mouse IgG (1:1000), Alexa Fluor 647 conjugated goat anti-rabbit IgG (used at 1:1200  
327 dilution), and Alexa Fluor 568-conjugated goat anti-rabbit IgG (used at 1:1000 dilution).

328 Mtw1 (an inner kinetochore protein) foci (Mtw1-13xMYC) were quantified in spreads with  
329 an area of 15 µm<sup>2</sup> or more to ensure centromeres were spread enough to assay. Centromere  
330 coupling would theoretically yield 16 kinetochore (Mtw1) foci while complete absence of  
331 coupling would yield 32 kinetochore foci. All strains were *spo11Δ/spo11Δ* to block progression  
332 beyond the coupling stage (12, 18). The individual performing the scoring was blinded to the  
333 identity of the mutation. The average number of Mtw1 foci seen in the chromosome spreads of  
334 each in-frame deletion strain was compared to the values obtained from the *ZIP1* and *zip1Δ*  
335 control strains, using the Kruskal-Wallis test, performed using Prism 6.0. The statistical data for  
336 the experiment are reported in Table S2.

337

### 338 Achiasmate segregation assay

339 Non-disjunction frequencies of centromere plasmids were determined in a manner similar  
340 to previously published assays (7). Plasmids were constructed with arrays of 256 repeats of the  
341 *lac* operator or *tet* operator sequence inserted adjacent to a 5.1 kb interval from chromosome III  
342 that includes *CEN3*. These cells expressed a *GFP-lacI* hybrid gene under the control of a meiotic  
343 promoter and a *tetR-tdTomato* hybrid gene under the control of the *URA3* promoter. This  
344 produced fluorescent foci at the operator arrays (33, 34). Cells were sporulated at 23°C (rather  
345 than 30°C) as this has been shown to allow by-pass of the pachytene arrests triggered by some  
346 *ZIP1* mutations (28). Even at this temperature cells with the *zip1-C1*, *zip1-C2*, *zip1-NM2* and  
347 *zip1Δ* mutations mainly arrested in pachytene, so no anaphase segregation data were gathered for  
348 these strains. Harvested cells were either assayed fresh, or were frozen in 15% glycerol and 1%  
349 potassium acetate until the time at which they were assayed. Preparation for assaying the cells  
350 included staining the cells with DAPI and then mounting the cells on agarose pads for viewing as  
351 described previously (36). Anaphase I cells were identified by the presence of two DAPI masses  
352 on either side of elongated cells, indicating that the chromosomes had segregated. To avoid  
353 scoring cells with duplicated or lost *CEN* plasmids, only cells with one GFP focus and one  
354 tdTomato focus were assayed. Images were collected using the 100X objective lens of a Zeiss

355 AxioImager microscope with band-pass emission filters, a Roper HQ2 CCD, and AxioVision  
356 software.

357

### 358 Plasmid centromere pairing assay

359 Centromere pairing in pachytene was assessed using published methods (7) but with the  
360 centromere plasmids described above. Sporulation was done at 30°C. Chromosome spreads were  
361 prepared as described in (37), with the following modifications: Cells were harvested 5-7 hours  
362 after induction of sporulation at 30°C. After chromosome spreads were created and dried  
363 overnight, the slides were rinsed gently with 0.4% Photoflo (Kodak). Each slide was then  
364 incubated with PBS/4% milk at room temperature for 30 minutes in a wet chamber. Milk was  
365 drained off of the slide, and primary antibody diluted in PBS/4% milk was incubated on the slide  
366 overnight at 4°C. A control slide with PBS/4% milk was used for each experiment. The  
367 following day, the slides were washed in PBS, and incubated with secondary antibody diluted in  
368 PBS/4% milk for 2 hours in a wet chamber at room temperature. The slides were gently washed  
369 in PBS. DAPI (4',6-diamidino-2-phenylindole, used at 1µg/ml) was added to each slide and  
370 allowed to incubate at room temperature for 10 minutes. Slides were then washed gently in PBS  
371 and 0.4% Photoflo, then allowed to dry completely before a coverslip was mounted. Antibodies  
372 are described in the previous section. Only cells that exhibited “ropey” DAPI staining were  
373 scored in this assay, and were disqualified for assessment if there was more than one GFP focus  
374 or more than one tdTomato focus. In these cells, the distance between the center of the green  
375 focus and the center of the red focus was measured using AxioVision software. The distributions  
376 of distances in the *ZIP1* and *zip1-N1* strains were determined to be significantly different with  
377 the Kolmogorov-Smirnov test (Kolmogorov-Smirnov D=0.4032; P=0.0002) using the Prism 6.0  
378 software package. As in previous work (7), foci with center-to-center distances less than or  
379 equal to 0.6 µm were scored as paired (these foci are typically touching or overlapping). The  
380 frequency of pairing (distance less than 0.6 µm) in the *ZIP1* (32 of 50) and *zip1-N1* (14 of 63)  
381 chromosome spreads was found to be significantly different (p<0.0001) using Fisher’s Exact test  
382 performed with the Prism 6.0 software package.

### 383 Synaptonemal complex evaluation by structured illumination microscopy.

384 Chromosome spreads were prepared according to the protocol of Grubb and colleagues  
385 (37) as described above, and harvested from sporulation cultures five hours after placing cells in  
386 sporulation medium at 30°C. To visualize the axial elements (Red1) and transverse elements  
387 (Zip1) of the SC by indirect fluorescence microscopy, chromosome spreads were stained with  
388 following primary and secondary antibodies: guinea pig anti-Red1 antibody (1:1000), goat anti-  
389 Guinea pig Alexa 488 antibody (Invitrogen) (1:1000), and rabbit anti-Zip1 antibody (1:800),  
390 donkey anti-rabbit Alexa 568 antibody (Invitrogen) (1:1000). Chromosome spreads were imaged  
391 with a Deltavision OMX-SR structured illumination microscope (SIM).

### 392 Mtw1-Zip1 co-localization assay

393 Chromosome spreads were prepared according to the protocol of (37) as described above.  
394 All strains carried the *zip4Δ* to prevent SC assembly. Chromosomes were stained with primary  
395 antibodies: mouse anti-MYC (Mtw1-13xMYC) (Developmental Studies Hybridoma Bank) at  
396 1:20 dilution and rabbit anti-Zip1 antibody at 1:1000 dilution and secondary antibodies Alexa  
397 488 donkey anti-mouse (Invitrogen) at 1:1000 dilution and Alexa 568 goat anti-rabbit  
398 (Invitrogen) at 1:1000 dilution. Chromosome spreads were imaged with a Deltavision OMX-SR  
399 structured illumination microscope (SIM). Acquired images were converted to binary images  
400 using ImageJ software and the number of overlapping Mtw1-13xMYC and Zip1 foci were

401 scored using the imageJ plugin, JACoP. To determine whether co-localization occurred at  
402 frequencies that were significantly higher than expected for random overlaps given the number  
403 of Mtw1 and Zip1 foci in each image, the foci in each image were randomized in one thousand  
404 simulations, then the frequency of random overlaps was determined and compared to the  
405 observed overlap. Costes' P-value was then calculated to evaluate the statistical significance of  
406 the difference between the frequency of observed versus random overlap (38). In addition, the  
407 average co-localization observed for all of the *ZIP1* spreads (26 spreads, 238 Mtw1 foci, 33 co-  
408 localized with Zip1) and all of the *zip1-N1* spreads (18 spreads, 279 Mtw1 foci, 12 co-localized  
409 with Zip1) was determined and the statistical significance of the difference determined using  
410 Fisher's two-tailed exact test (p=0.0001). The experiment presented is one of two performed,  
411 both with the same outcome (significantly reduced Mtw1-Zip1 co-localization in the *zip1-N1*  
412 mutant).

#### 413 Centromere pairing of natural chromosomes

414 The chromosome spreads used in the experiment above were used to assay the number of  
415 distinct Mtw1-13xMYC foci in *ZIP1*, *zip1-N1* and *zip1Δ* chromosome spreads. With complete  
416 pairing of the homologous chromosomes, the thirty-two kinetochores should appear as sixteen  
417 Mtw1-13xMYC foci. In the absence of pairing, kinetochores from the paired homologs can  
418 sometimes separate far enough to be resolved as individual foci (the homologs remain tethered  
419 by crossovers and probably other constraints), thus giving higher numbers of Mtw1-13xMYC  
420 foci – in theory up to thirty-two foci. The SIM images described in the preceding section were  
421 converted to binary images using ImageJ software and the number of Mtw1-13xMYC foci tallied  
422 for each spread using the Analyze Particles function in ImageJ. The average number of Mtw1-  
423 13xMYC foci per spread was determined for each genotype (*ZIP1*, *zip1-N1*, and *zip1Δ*) and the  
424 statistical significance of the observed differences between the genotypes was calculated with  
425 one-way ANOVA and multiplicity adjusted P values were obtained with Sidak's multiple  
426 comparisons testing using Prism 7.0.

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434

435 **References**

- 436 1. Duro E, Marston AL. From equator to pole: splitting chromosomes in mitosis and meiosis.  
437 Genes Dev. 2015;29(2):109-22.
- 438 2. Kurdzo EL, Dawson DS. Centromere pairing--tethering partner chromosomes in meiosis I.  
439 FEBS J. 2015;282(13):2458-70.
- 440 3. Bisig CG, Guiraldelli MF, Kouznetsova A, Scherthan H, Hoog C, Dawson DS, et al.  
441 Synaptonemal complex components persist at centromeres and are required for  
442 homologous centromere pairing in mouse spermatocytes. PLoS Genet.  
443 2012;8(6):e1002701.
- 444 4. Ding DQ, Yamamoto A, Haraguchi T, Hiraoka Y. Dynamics of homologous chromosome  
445 pairing during meiotic prophase in fission yeast. Dev Cell. 2004;6(3):329-41.
- 446 5. Kemp B, Boumil RM, Stewart MN, Dawson DS. A role for centromere pairing in meiotic  
447 chromosome segregation. Genes Dev. 2004;18(16):1946-51.
- 448 6. Qiao H, Chen JK, Reynolds A, Hoog C, Paddy M, Hunter N. Interplay between  
449 synaptonemal complex, homologous recombination, and centromeres during mammalian  
450 meiosis. PLoS Genet. 2012;8(6):e1002790.
- 451 7. Gladstone MN, Obeso D, Chuong H, Dawson DS. The synaptonemal complex protein Zip1  
452 promotes bi-orientation of centromeres at meiosis I. PLoS Genet. 2009;5(12):e1000771.
- 453 8. Newnham L, Jordan P, Rockmill B, Roeder GS, Hoffmann E. The synaptonemal complex  
454 protein, Zip1, promotes the segregation of nonexchange chromosomes at meiosis I. Proc  
455 Natl Acad Sci U S A. 2010;107(2):781-5.
- 456 9. Chuong H, Dawson DS. Meiotic cohesin promotes pairing of nonhomologous centromeres  
457 in early meiotic prophase. Mol Biol Cell. 2010;21(11):1799-809.
- 458 10. Tsubouchi T, Roeder GS. A synaptonemal complex protein promotes homology-  
459 independent centromere coupling. Science. 2005;308(5723):870-3.

- 460 11. Takeo S, Lake CM, Morais-de-Sa E, Sunkel CE, Hawley RS. Synaptonemal complex-  
461 dependent centromeric clustering and the initiation of synapsis in *Drosophila* oocytes. *Curr*  
462 *Biol.* 2011;21(21):1845-51.
- 463 12. Obeso D, Dawson DS. Temporal characterization of homology-independent centromere  
464 coupling in meiotic prophase. *PLoS One.* 2010;5(4):e10336.
- 465 13. Sym M, Engebrecht JA, Roeder GS. ZIP1 is a synaptonemal complex protein required for  
466 meiotic chromosome synapsis. *Cell.* 1993;72(3):365-78.
- 467 14. Tung KS, Roeder GS. Meiotic chromosome morphology and behavior in *zip1* mutants of  
468 *Saccharomyces cerevisiae*. *Genetics.* 1998;149(2):817-32.
- 469 15. Dong H, Roeder GS. Organization of the yeast Zip1 protein within the central region of the  
470 synaptonemal complex. *J Cell Biol.* 2000;148(3):417-26.
- 471 16. Watts FZ, Hoffmann E. SUMO meets meiosis: an encounter at the synaptonemal complex:  
472 SUMO chains and sumoylated proteins suggest that heterogeneous and complex  
473 interactions lie at the centre of the synaptonemal complex. *Bioessays.* 2011;33(7):529-37.
- 474 17. Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are  
475 catalyzed by Spo11, a member of a widely conserved protein family. *Cell.* 1997;88(3):375-  
476 84.
- 477 18. Falk JE, Chan AC, Hoffmann E, Hochwagen A. A Mec1- and PP4-dependent checkpoint  
478 couples centromere pairing to meiotic recombination. *Dev Cell.* 2010;19(4):599-611.
- 479 19. Straight AF, Belmont AS, Robinett CC, Murray AW. GFP tagging of budding yeast  
480 chromosomes reveals that protein-protein interactions can mediate sister chromatid  
481 cohesion. *Curr Biol.* 1996;6(12):1599-608.
- 482 20. Kurdzo EL, Obeso D, Chuong H, Dawson DS. Meiotic Centromere Coupling and Pairing  
483 Function by Two Separate Mechanisms in *Saccharomyces cerevisiae*. *Genetics.*  
484 2017;205(2):657-71.

- 485 21. Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevent premature  
486 separation of sister chromatids. *Cell*. 1997;91(1):35-45.
- 487 22. Dawson DS, Murray AW, Szostak JW. An alternative pathway for meiotic chromosome  
488 segregation in yeast. *Science*. 1986;234(4777):713-7.
- 489 23. Mann C, Davis RW. Meiotic disjunction of circular minichromosomes in yeast does not  
490 require DNA homology. *Proc Natl Acad Sci U S A*. 1986;83(16):6017-9.
- 491 24. Sears DD, Hegemann JH, Hieter P. Meiotic recombination and segregation of human-  
492 derived artificial chromosomes in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*.  
493 1992;89(12):5296-300.
- 494 25. Benjamin KR, Zhang C, Shokat KM, Herskowitz I. Control of landmark events in meiosis by  
495 the CDK Cdc28 and the meiosis-specific kinase Ime2. *Genes Dev*. 2003;17(12):1524-39.
- 496 26. Meyer RE, Kim S, Obeso D, Straight PD, Winey M, Dawson DS. Mps1 and Ipl1/Aurora B  
497 act sequentially to correctly orient chromosomes on the meiotic spindle of budding yeast.  
498 *Science*. 2013;339(6123):1071-4.
- 499 27. Carlile TM, Amon A. Meiosis I Is Established through Division-Specific Translational Control  
500 of a Cyclin. *Cell*. 2008;133(2):280-91.
- 501 28. Borner GV, Kleckner N, Hunter N. Crossover/noncrossover differentiation, synaptonemal  
502 complex formation, and regulatory surveillance at the leptotene/zygotene transition of  
503 meiosis. *Cell*. 2004;117(1):29-45.
- 504 29. Tsubouchi T, Macqueen AJ, Roeder GS. Initiation of meiotic chromosome synapsis at  
505 centromeres in budding yeast. *Genes Dev*. 2008;22(22):3217-26.
- 506 30. Obeso D, Pezza RJ, Dawson D. Couples, pairs, and clusters: mechanisms and implications  
507 of centromere associations in meiosis. *Chromosoma*. 2014;123(1-2):43-55.
- 508 31. Humphryes N, Leung WK, Argunhan B, Terentyev Y, Dvorackova M, Tsubouchi H. The  
509 Ecm11-Gmc2 complex promotes synaptonemal complex formation through assembly of  
510 transverse filaments in budding yeast. *PLoS Genet*. 2013;9(1):e1003194.

- 511 32. Burns N, Grimwade B, Ross-Macdonald PB, Choi EY, Finberg K, Roeder GS, et al. Large-  
512 scale analysis of gene expression, protein localization, and gene disruption in  
513 *Saccharomyces cerevisiae*. *Genes Dev.* 1994;8(9):1087-105.
- 514 33. Longtine MS, III AM, Demarini DJ, Shah NG, Wach A, Brachat A, et al. Additional Modules  
515 for Versatile and Economical PCR-based Gene Deletion and Modification in  
516 *Saccharomyces cerevisiae*. *Yeast.* 1998;14:953–61.
- 517 34. Janke C, Magiera MM, Rathfelder N, Taxis C, Reber S, Maekawa H, et al. A versatile  
518 toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers  
519 and promoter substitution cassettes. *Yeast.* 2004;21(11):947-62.
- 520 35. Dresser ME, Giroux CN. Meiotic chromosome behavior in spread preparations of yeast. *J*  
521 *Cell Biol.* 1988;106(3):567-73.
- 522 36. Kim S, Meyer R, Chuong H, Dawson DS. Dual mechanisms prevent premature  
523 chromosome segregation during meiosis. *Genes & development.* 2013;27(19):2139-46.
- 524 37. Grubb J, Brown MS, Bishop DK. Surface Spreading and Immunostaining of Yeast  
525 Chromosomes. *J Vis Exp.* 2015(102):e53081.
- 526 38. Costes SV, Daelemans D, Cho EH, Dobbin Z, Pavlakis G, Lockett S. Automatic and  
527 quantitative measurement of protein-protein colocalization in live cells. *Biophys J.*  
528 2004;86(6):3993-4003.

529  
530

531 **Supporting Information Legends**

532

533 **Figure S1. *zip1-N1* cells assemble synaptonemal complexes and exhibit high**

534 **spore viability.** Chromosome spreads were prepared from cells 5 hours after placing

535 the cultures in sporulation medium and stained as described in Materials and Methods.

536 The axial element protein is shown in green and Zip1 is shown in Red. Each panel

537 presents representative spreads from A. ZIP1, B. *zip1D* and C. *zip1-N1* strains.

538 Panels to the right are larger images of individual chromosomes. The results in our

539 strains are in keeping with the more comprehensive previous study of SC assembly in

540 *zip1-N1* mutants (Tung and Roeder, 1998) in that the *zip1-N1* strain exhibited slightly

541 less continuous Zip1 staining in pachytene-like spreads than was observed with the

542 wild-type control strain. It is not clear if this reflects a slight reduction in assembly

543 kinetics, or reduced continuity of the Zip1 in the mature SC of the *zip1-N1* strain. D.

544 Tetrads were dissected to assess spore viability in ZIP1 and *zip1-N1* strains. Though in

545 this sample set the *zip1-N1* exhibited slightly lower spore viability than the wild-type

546 control, as in prior studies (Tung and Roeder, 1998) there was no significant difference

547 (Fisher's exact test,  $P=0.83$ ).

548

549 **Table S1. Strains Used in this Study**

550

551 **Table S2. Statistics for centromere coupling experiments**

552

553



554 **Figure Legends**

555 **Figure 1. Meiotic centromere behaviors in budding yeast. A.** In meiosis of budding yeast,  
556 Zip1 (orange) mediates centromere coupling (green arrowheads) between non-homologous  
557 partner chromosomes (light blue and purple). As the cell proceeds through later stages of  
558 meiosis, homologs pair and the mature synaptonemal complex (SC) structure zips the  
559 chromosomes together. After pachytene, the SC disassembles, except at the centromeres (blue  
560 arrowhead). **B.** The Zip1 protein is predicted to have globular domains at its ends spanning a  
561 longer coiled-coil and forms parallel dimers with N-termini in the center of the SC (denoted by  
562 N) and the C-termini along the axial elements (denoted by C). **C.** We evaluated the same nine  
563 *ZIP1* deletion mutants previously described by Tung and colleagues (Tung & Roeder, 1998). The  
564 mutations are named for their relative position along the genetic sequence – N for N-terminus, M  
565 for middle region, and C for C-terminus. The approximate SC structure formed in each mutant as  
566 described by Tung and Roeder (1998) is shown.

567  
568 **Figure 2. Centromere coupling requires parts of the N and C-termini of Zip1. A.**  
569 Centromere coupling values were obtained by scoring the number of Mtw1-GFP foci in meiotic  
570 chromosome spreads. *CEN1* loci were visualized by virtue of lacI-GFP localized to a *lac*  
571 operator array next to the centromere. **B.** Coupling data. Mutants are listed according to the  
572 severity of their coupling phenotype. The thin blue and red lines indicate average Mtw1 foci  
573 values for wild-type and *zip1Δ*, respectively. The mutants were split into three groups – like  
574 wild-type (light blue), intermediate (green), and like *zip1Δ* (orange). The “like wild-type” group  
575 had values indistinguishable from wild-type but were significantly different from *zip1Δ*  
576 ( $p < 0.05$ ); whereas the “like *zip1Δ*” group had values indistinguishable from *zip1Δ* but  
577 significantly different from wild-type ( $p < 0.05$ ). The *zip1-M2* mutant had an intermediate  
578 phenotype that was significantly different from both wild-type and *zip1Δ*. A complete list of  
579 averages and statistical values are presented in Table S2.

580  
581 **Figure 3. Centromere plasmid disjunction requires the N-terminus of Zip1. A.**  
582 Representative binucleate cells with disjoined (a *ZIP1* cell) and non-disjoined (a *zip1-N1* cell)  
583 centromere plasmids. The segregation of CEN plasmids in anaphase I was assessed by  
584 monitoring the tetR-tdTomato and lacI-GFP foci localized to *tet* and *lac* operator repeats,  
585 respectively, inserted into a plasmid that contains 5.1 kb of *CEN3* sequence. **B.** Non-disjunction  
586 frequencies for CEN plasmids in each strain. n values: *ZIP1*, 250; *zip1-N1*, 190; *zip1-NM1*, 200;  
587 *zip1-M1*, 143; *zip1-M2*, 54; *zip1-MC1*, 69; *zip1-MC2*, 55. Statistical comparisons were  
588 performed with Fisher’s exact test to compare all genotypes to WT. Bonferroni’s correction was  
589 utilized to adjust for the number of comparisons. \* $p \leq 0.05$ .; \*\*\* $p \leq 0.00625$ . Scale bars equal 2  
590  $\mu\text{m}$ .

591  
592 **Figure 4. Centromere plasmid pairing requires the N-terminus of Zip1.** Pairing of plasmid  
593 centromeres in prophase chromosome spreads was assessed by monitoring the pairing of tetR-  
594 tdTomato and lacI-GFP foci localized to *tet* operator and *lac* operator arrays on plasmids bearing  
595 a 5.1 kb region of chromosome *III* encompassing *CEN3*. **A.** An example of a spread with  
596 unpaired plasmid centromeres. **B.** Distances between the centers of the tdTomato and GFP foci  
597 in each spread (average and standard deviation). \*\*\*  $P = 0.0002$ . The grey cross-hatched region  
598 indicates separation of less-than 0.6  $\mu\text{m}$  between the centers of the foci, a distance used to infer

599 pairing of the centromeres. **C.** The percent of spreads scored as “paired” in the *ZIP1* (58%, n=50)  
600 and *zip1-N1* (22%, n=63) strains. \*\*\*\*p<0.0001. Scale bar equals 2  $\mu$ m.

601

602 **Figure 5. The Zip1 N-terminal domain is required for efficient co-localization to**  
603 **centromeres.** Chromosome spreads were prepared from prophase *ZIP1* and *zip1-N1* cells  
604 expressing Mtw1-GFP as a kinetochore marker. Indirect fluorescence structured illumination  
605 microscopy was used to visualize Mtw1-GFP and Zip1 foci. **A and B.** The overlap of Mtw1 foci  
606 with Zip1 foci (green circles) and Zip1 foci with Mtw1 foci (blue circles) was measured in each  
607 spread and the statistical significance of the difference between the observed Mtw1 co-  
608 localization with Zip1 from random simulations was evaluated with Costes’ P-value (gray  
609 triangles; greater than 95% is considered significant). Representative images from the two strains  
610 are shown. Zip1 (red), Mtw1-GFP (green), overlapping foci (white arrowhead), scale bars equal  
611 2  $\mu$ m. **C.** The average co-localization of Mtw1 foci with Zip1 across all the chromosome spreads  
612 was determined. \* p<0.05. **D.** Centromere pairing was evaluated by counting the number of  
613 Mtw1-GFP foci in the chromosome spreads. *ZIP1* (n=27), *zip1-N1* (n=22), *zip1*  $\Delta$  (n=22).  
614 \*\*P<0.01, \*\*\*\*P<0.0001.

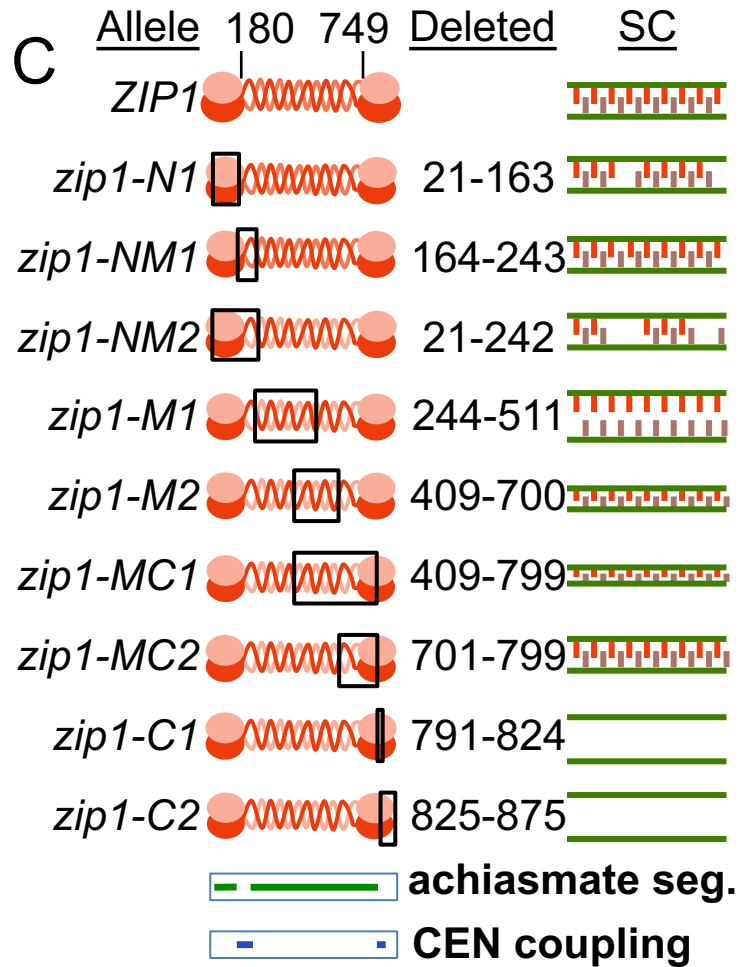
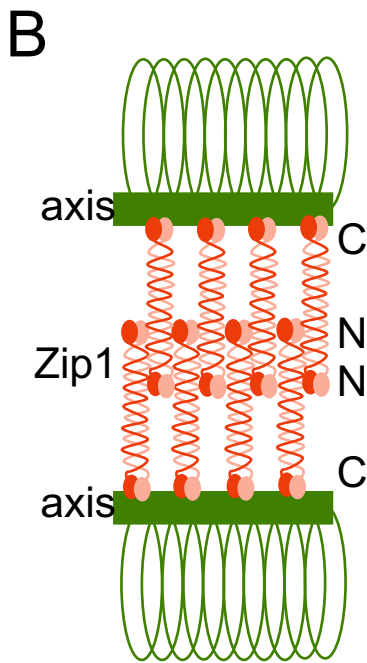
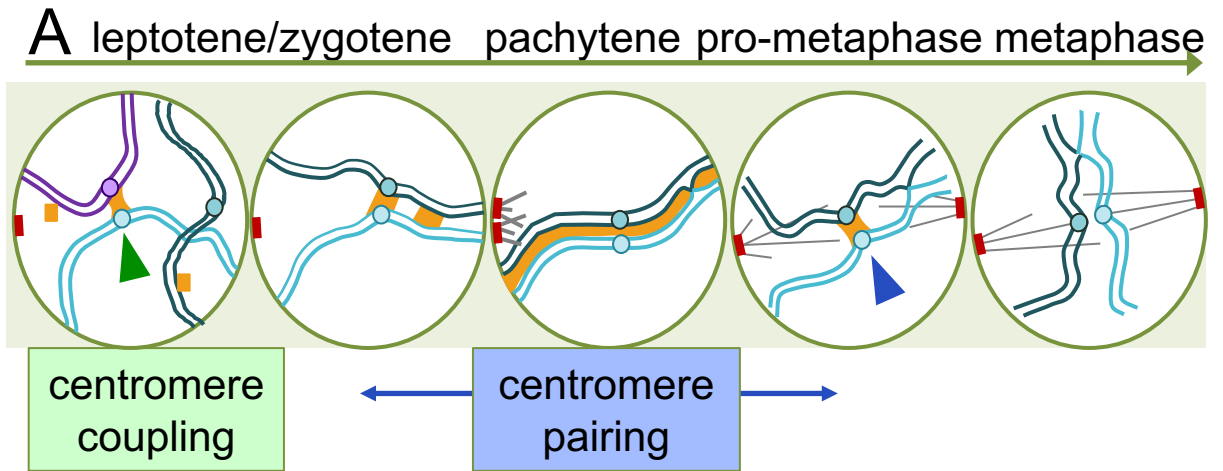


Figure 1

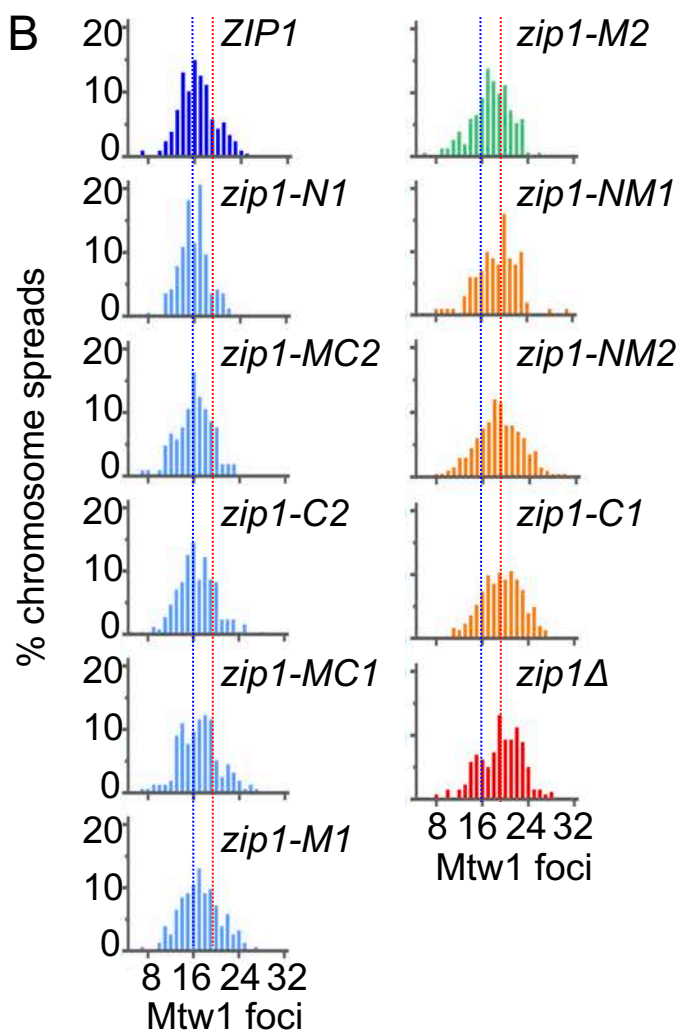
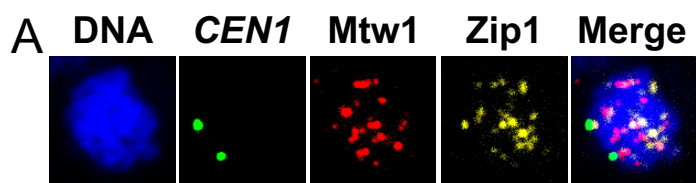


Figure 2

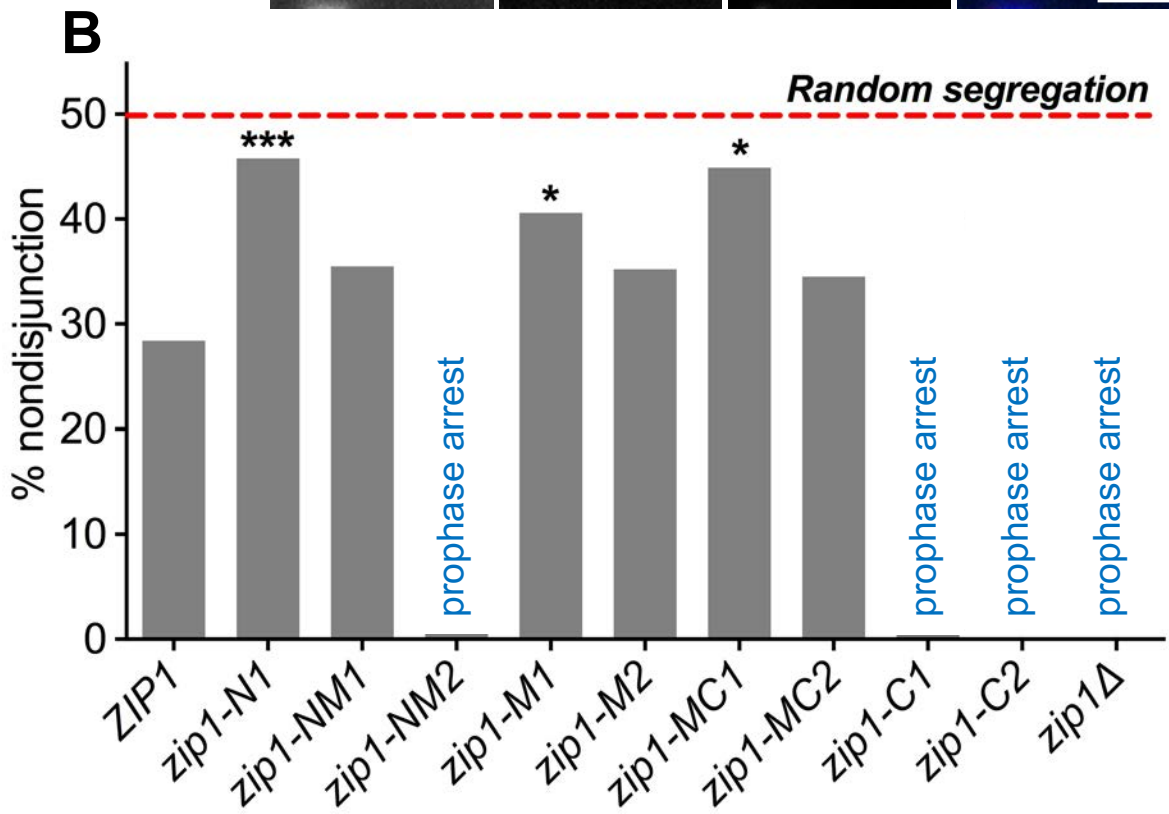
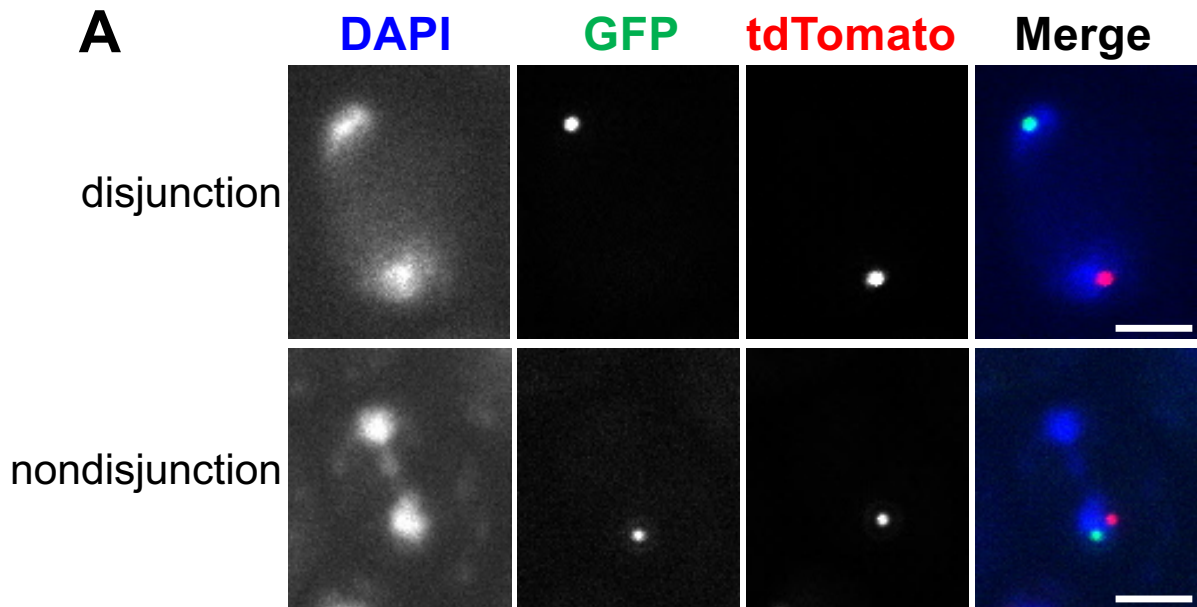


Figure 3

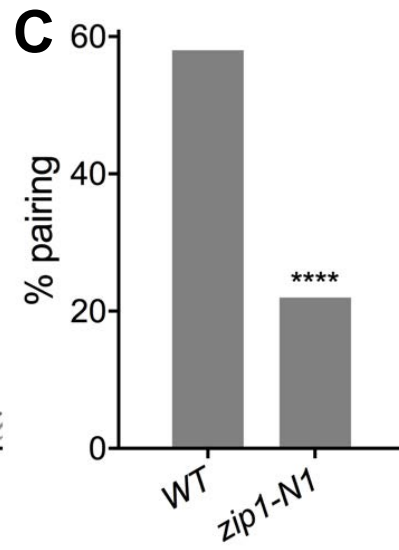
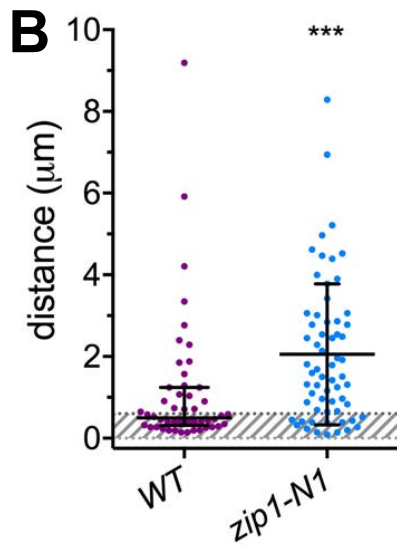
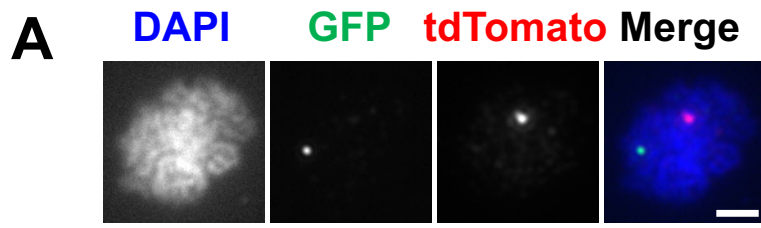


Figure 4

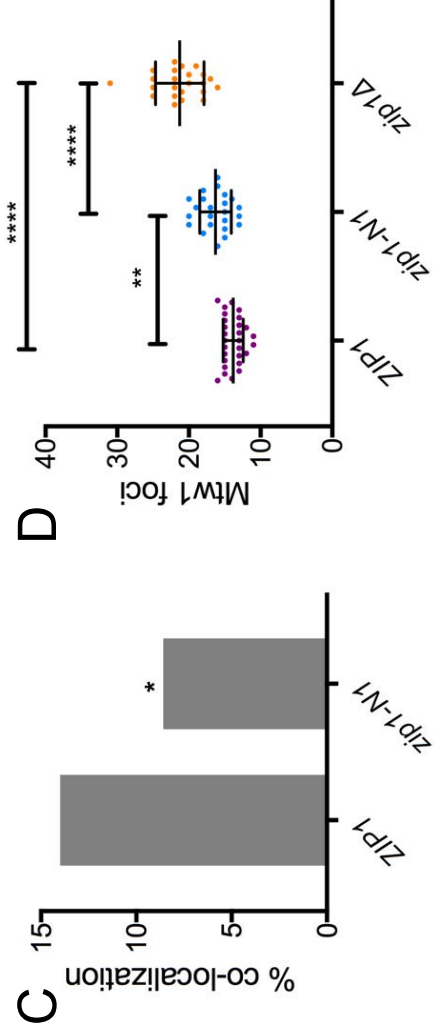
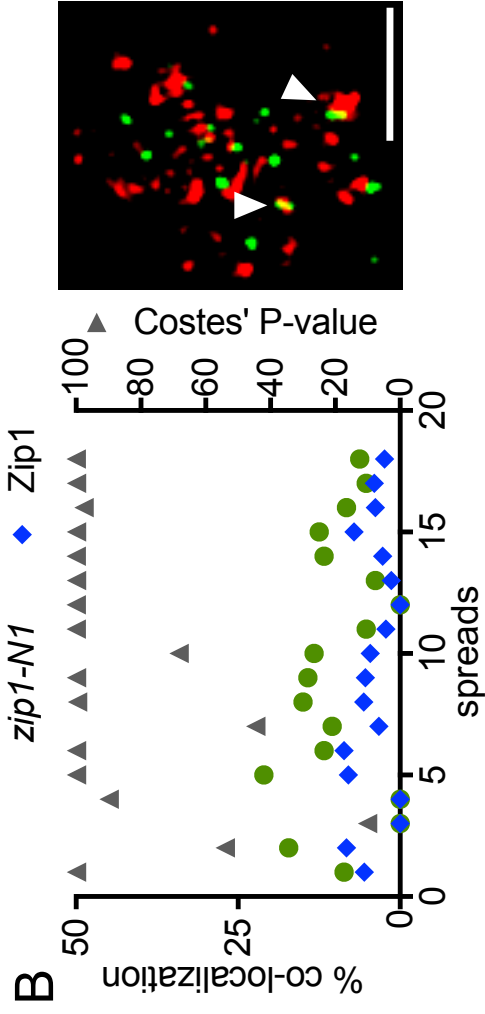
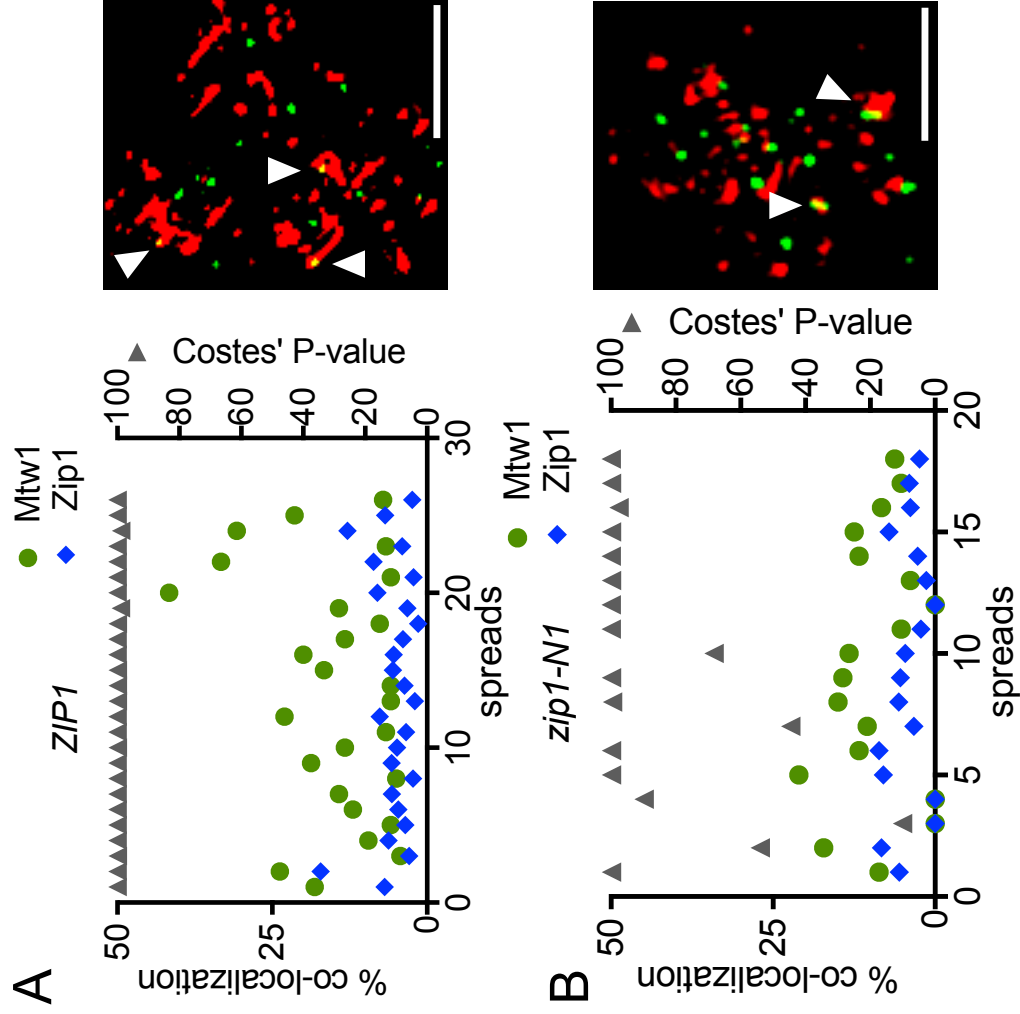


Figure 5