

# 1 Centromere clustering stabilizes 2 meiotic homolog pairing

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## 21 ABSTRACT

22 During meiosis, each chromosome must selectively pair and synapse with its own unique  
23 homolog to enable crossover formation and subsequent segregation. How homolog  
24 pairing is maintained in early meiosis to ensure synapsis occurs exclusively between  
25 homologs is unknown. We aimed to further understand this process by utilizing a unique  
26 *Drosophila* meiotic mutant, *Mcm5<sup>A7</sup>*. We found that *Mcm5<sup>A7</sup>* mutants are proficient in  
27 homolog pairing at meiotic onset yet fail to maintain pairing as meiotic synapsis ensues,  
28 causing seemingly-normal synapsis between non-homologous loci. This pairing defect  
29 corresponds with a reduction of SMC1-dependent centromere clustering at meiotic onset.  
30 Overexpressing SMC1 in this mutant significantly restores centromere clustering,  
31 homolog pairing, and crossover formation. These data indicate that the initial meiotic  
32 pairing of homologs is not sufficient to yield synapsis between exclusively between  
33 homologs and provide a model in which meiotic homolog pairing must be stabilized by  
34 SMC1-dependent centromere clustering to ensure proper synapsis.

35

## 36 INTRODUCTION

37

38 Accurate segregation of homologous chromosomes during the first meiotic division  
39 is essential to reestablish the diploid genome upon sexual fertilization. To ensure faithful  
40 meiosis I chromosomal segregation, homologs must become physically connected in part  
41 through crossover formation. To enable homolog crossover events, a series of

42 chromosomal and cellular events occur in early meiotic prophase I (Lake and Hawley  
43 2012) (Figure 1a).

44         During or just prior to the onset of meiosis, homologous chromosomes pair along  
45 their entire lengths (reviewed in Denise Zickler and Kleckner 2015). Between paired  
46 homologs, synapsis, the formation of the synaptonemal complex (SC), ensues. The SC  
47 is a tripartite scaffold built between homologs extending the length of the chromosomes  
48 and consists of a central region (CR) that is nestled between two lateral elements (LEs),  
49 which are successors of cohesin-based chromosome axes formed between sister  
50 chromatids. Coincidentally with synapsis, DSBs are formed and repaired using a  
51 homologous template via homologous recombination (HR), resulting in crossover  
52 formation between homologs (reviewed in Page and Hawley 2004).

53         Perhaps the most enigmatic event within early meiosis is the mechanism by which  
54 a meiotic chromosome selectively pairs and synapses with its unique homologous  
55 partner. Initial homolog pairing is believed to be facilitated through early meiotic  
56 chromosome movement and telomere or the centromere clustering (for reviews, see  
57 Denise Zickler and Kleckner 2015; Alleva and Smolikove 2017; Klutstein and Cooper  
58 2014). However, how homologous pairing is maintained during synapsis to ensure the  
59 SC is formed exclusively between homologs is unknown.

60         The model organism *Drosophila melanogaster* has been used to uncover meiotic  
61 mechanisms for over a century (Morgan 1910). In *Drosophila*, prior to meiosis,  
62 chromosomes enter the germline unpaired (Figure 1a); throughout the pre-meiotic region,  
63 homologous chromosomes gradually pair. In the nuclei at the last mitotic division prior to  
64 meiotic onset (in the 8-cell cyst), centromere-directed chromosomal movements occur,

65 presumably ensuring complete homologous pairing (Christophorou et al. 2015; Joyce et  
66 al. 2013). Also during pre-meiotic mitotic cycles, meiotic proteins, including the cohesin  
67 SMC1, are enriched at the centromere (Khetani and Bickel 2007; Christophorou, Rubin,  
68 and Huynh 2013). The onset of meiotic prophase I occurs in the 16-cell cyst. At zygotene,  
69 the first cytologically resolved stage of prophase, centromeres are clustered into 1 or 2  
70 groups (Takeo et al. 2011), and the SC nucleates in patches along chromosome arms  
71 (Tanneti et al. 2011). As zygotene proceeds into early pachytene, the SC extends  
72 between paired chromosomes, yielding full-length SC exclusively between homologs.  
73 How these early meiotic events, particularly centromere clustering, contribute to meiotic  
74 homologous pairing and synapsis in *Drosophila* is largely unknown.

75 In this study, we used the *Drosophila* early meiotic program and a unique genetic  
76 mutant to investigate how homolog pairing is maintained during meiotic synapsis. We  
77 discovered that meiotic homologs in a previously described *Drosophila* mutant, *Mcm5<sup>A7</sup>*  
78 (Lake et al. 2007), initially pair, but are unable to maintain pairing during synapsis,  
79 suggesting that initial meiotic pairing must be subsequently stabilized by an unknown  
80 mechanism to ensure proper synapsis. Using *Mcm5<sup>A7</sup>* as a genetic tool to interrogate  
81 pairing stabilization mechanism(s), we show that the meiotic pairing defect and resulting  
82 heterosynapsis are due to a lack of SMC1-dependent centromere clustering at meiotic  
83 onset. From our results, we suggest a model for proper synapsis in which initial meiotic  
84 pairing must be stabilized by centromere clustering, a meiotic event produced by SMC1-  
85 enrichment at the centromere and dynamic chromosome movements.

86

## 87 RESULTS

88

89 *Mcm5<sup>A7</sup> mutants are proficient in initial meiotic pairing but deficient in pairing*  
90 *maintenance*

91

92 The *Mcm5<sup>A7</sup>* allele, discovered in a meiotic mutant screen, is a missense mutation that  
93 changes a conserved aspartic acid residue at the C-terminus, adjacent to the AAA<sup>+</sup>  
94 ATPase domain. *Mcm5<sup>A7</sup>* mutants have an X-NDJ rate of ~25% that is accompanied with  
95 a 90% decrease of crossovers on the X chromosome (Lake et al. 2007). Interestingly,  
96 the SC, as shown through staining of the central region (CR) protein C(3)G, appears  
97 normal, and DSBs are created and repaired with normal kinetics (Lake et al. 2007). The  
98 reason as to why crossovers were severely decreased in *Mcm5<sup>A7</sup>* mutants was unknown  
99 at the time of this study.

100 We hypothesized that a lack of meiotic homolog pairing could result in the severe  
101 loss of meiotic crossovers in *Mcm5<sup>A7</sup>* mutants. To test this, we examined the frequencies  
102 of X and Chromosome 3R homolog pairing in zygotene, early pachytene, and mid-  
103 pachytene meiotic cells using IF/FISH. Zygotene is the earliest cytologically resolved  
104 meiotic stage in the *Drosophila* germarium and is defined by the presence of SC patches  
105 in the 16-cell cyst. Early pachytene is defined by full-length SC in the early 16-cell cysts  
106 (Region 2A of the germarium), and mid-pachytene is defined as the most posterior  
107 nucleus in the germarium that expresses full-length SC (Region 3) (Lake and Hawley  
108 2012).

109 At the *X* locus, wild-type meiotic cells exhibit one focus throughout zygotene, early  
110 pachytene, and mid-pachytene (Z, EP, and MP, respectively, Figure 1b). In *Mcm5<sup>A7</sup>*  
111 mutants, we observed one focus at 100% frequency in zygotene. Strikingly, we can  
112 resolve two foci in approximately half of the nuclei in *Mcm5<sup>A7</sup>* mutants during early  
113 pachytene ( $***p < 0.0001$ ) and mid-pachytene ( $*p = 0.01$ , respectively).

114 Similarly, at the 3R locus wild-type homologous chromosomes are paired at 100%  
115 frequency in zygotene, early pachytene, and mid-pachytene (Figure 1c). However, in  
116 *Mcm5<sup>A7</sup>* mutants, the homologs of chromosome 3R in zygotene are paired at nearly 100%  
117 frequency, yet we can resolve two 3R foci in 35% of early pachytene nuclei ( $***p < 0.0001$ )  
118 and 78% of mid-pachytene nuclei ( $**p = 0.0002$ ).

119 The above results show that in *Mcm5<sup>A7</sup>* mutants, meiotic chromosomes enter  
120 meiosis paired, but as the meiotic nuclei proceed through meiosis, homologous pairing  
121 cannot be maintained. This suggests that homolog pairing must be stabilized in early  
122 meiosis by an unknown mechanism to ensure accurate synapsis, and in *Mcm5<sup>A7</sup>* mutants,  
123 this mechanism is perturbed. Therefore, we reasoned that *Mcm5<sup>A7</sup>* can be used as a  
124 genetic tool to interrogate the mechanism that stabilizes meiotic pairing.

125

126 *The synaptonemal complex (SC) shows no observable defects in *Mcm5<sup>A7</sup>* mutants*

127

128 Although pachytene homolog pairing is disrupted at a high frequency in *Mcm5<sup>A7</sup>* mutants,  
129 the SC, as determined by C(3)G staining, still forms (Lake et al. 2007) (Figure 2a). To  
130 explain this, we hypothesize that either (1) the unpaired loci do not correspond with linear  
131 SC, or (2) the unpaired loci are forming stable SC with non-homologous loci, creating

132 heterosynapsis. To differentiate between these two, we examined whole mount germaria  
133 with IF/FISH and super-resolution microscopy (AIRY Scan) and examined tracts of SC.  
134 In wild-type, we can discern that one linear tract of C(3)G is built between the paired *X*  
135 locus (Figure 2b). In *Mcm5<sup>A7</sup>* mutants, separate homologous loci are associated with  
136 separate linear tracts of C(3)G, indicating that the unpaired *X* loci are synapsed with non-  
137 homologous loci (see Supplemental Movies 1 and 2). From these data, we conclude that  
138 *Mcm5<sup>A7</sup>* mutants have the ability to heterologously synapse.

139 To determine the nature of heterosynapsis, we examined the localization of two  
140 SC central region (CR) proteins, C(3)G and Corolla (Figure 2a). In wild-type, Corolla co-  
141 localizes with C(3)G dimers (Collins et al. 2014), as shown in Figure 2c under structured-  
142 illumination microscopy. Under higher resolution, Corolla and C(3)G signal were found  
143 to overlap and C(3)G signal is wider, as expected due to its dimer-dimer conformation  
144 (Jeffress et al. 2007). In *Mcm5<sup>A7</sup>* mutants, Corolla and C(3)G exhibit a similar localization  
145 pattern (Figure 2d). To examine proper CR protein levels, we quantified total C(3)G  
146 nuclear signal during early and mid-pachytene in wild-type and *Mcm5<sup>A7</sup>* mutants (Figure  
147 2e). During these timepoints, we see no significant differences between wild-type and  
148 *Mcm5<sup>A7</sup>* C(3)G nuclear fluorescence intensity ( $p = 0.5601$  and  $p = 0.3993$ , respectively,  
149 unpaired T-test).

150 The chromosome axis between sister chromatids serves as the lateral element  
151 (LE) and provides a barrier to prevent inter-sister recombination (Webber, Howard, and  
152 Bickel 2004). To test the function of the LE, we examined inter-sister recombination rates  
153 using a genetic ring/rod chromosomal transmission assay (Figure 2f). *Mcm5<sup>A7</sup>* mutants  
154 exhibit no decrease in ring:rod transmission (1.1:1 ratio). However, a LE mutant (*ord<sup>10</sup>*)

155 shows a severe decrease in ring:rod transmission to a normalized ratio of 0.28:1. These  
156 results suggest that *Mcm5<sup>A7</sup>* mutants exhibit no observable defects in the SC, indicating  
157 that seemingly-normal synapsis can occur independent of pairing.

158

159 *Centromere-directed chromosome movements are normal in *Mcm5<sup>A7</sup>* mutants*

160

161 We set out to understand how initial pairing of homologs is proficient in *Mcm5<sup>A7</sup>* mutants,  
162 despite exhibiting defects in pairing maintenance. Rapid chromosome movements are  
163 thought to contribute to homolog pairing (reviewed in Alleva and Smolikove 2017). To  
164 determine whether perturbations in centromere-directed chromosome movement  
165 contribute to the observed defects in *Mcm5<sup>A7</sup>* mutants, we examined centromere  
166 dynamics in 8-cell cysts of wild-type and *Mcm5<sup>A7</sup>* mutant germaria through live cell  
167 imaging (Figure 3a, Supplemental Movies 3, 4).

168 In wild-type, a representative centromere track illustrates chromosome movement  
169 around the volume of a nucleus (Figure 3b), covering a nuclear volume of 12.9  $\mu\text{m}^3$   
170 (Figure 3d, Supplemental Movie 5). A representative centromere track in *Mcm5<sup>A7</sup>*  
171 mutants shows similar chromosome movement (Figure 3c), covering a nuclear volume of  
172 15.7  $\mu\text{m}^3$  (Figure 3d, Supplemental Movie 6). Of all centromeres analyzed, *Mcm5<sup>A7</sup>*  
173 mutants show no significant difference between relative nuclear volume covered  
174 compared to wild-type (Figure 3e,  $p = 0.75$ , Kolmogorov-Smirnov test), demonstrating  
175 that *Mcm5<sup>A7</sup>* mutants exhibit centromere-directed chromosome movements similar to  
176 wild-type in the 8-cell cyst. Importantly, these data show that centromere-directed



177 chromosome movement may promote initial meiotic homolog pairing but is not sufficient  
178 for maintaining homolog pairing.

179

180 *Meiotic centromere clustering is defective in  $Mcm5^{A7}$  mutants*

181

182 In *Drosophila*, eight centromeres aggregate into one or two diffraction-limited clusters at  
183 the onset of meiosis, which is defined cytologically as zygotene. Centromeres remain  
184 clustered through pachytene (Takeo et al. 2011). To determine whether centromere  
185 clustering at the onset of meiosis is associated with initial homolog pairing, we quantified  
186 the foci number of CID, the CENP-A homolog (Henikoff et al. 2002), in zygotene nuclei in  
187 wild-type and  $Mcm5^{A7}$  mutants at zygotene (Figure 4a). We observed a mean of 2 CID  
188 foci in wild-type, demonstrating centromere clustering. In  $Mcm5^{A7}$  mutants, we see a  
189 significant increase in CID foci, with a mean of 4.8 per nucleus ( $p < 0.001$ , unpaired T-  
190 test). These results show that in  $Mcm5^{A7}$  mutants, centromeres are not heterologously  
191 clustered entering meiosis, even though chromosome arms are paired.

192 Next, we determined whether centromeres cluster in pachytene in  $Mcm5^{A7}$   
193 mutants. As shown in Figure 4b, we observe a mean of 1.7 CID foci in early pachytene  
194 nuclei of wild-type, compared to 5.6 foci in  $Mcm5^{A7}$  mutants ( $p < 0.001$ , unpaired T-test).  
195 In mid-pachytene,  $Mcm5^{A7}$  mutants exhibit a mean of 5.2 CID foci, significantly higher  
196 than wild-type (1.6 CID foci;  $p < 0.001$ , unpaired T-test) (Figure 4c). We conclude that  
197 centromere clustering is perturbed throughout early and mid-pachytene in  $Mcm5^{A7}$   
198 mutants.

199 In the regions assessed, we observed more than four CID foci in most *Mcm5<sup>A7</sup>*  
200 nuclei (Figure 4a, b, c), suggesting that homologous centromeres are unpaired. To test  
201 this, we examined the pairing frequency of the 359-bp locus, which is adjacent to the *X*  
202 centromere (Dernburg, Sedat, and Hawley 1996) (Figure 4d). As previously reported,  
203 this locus is paired in ~90% of meiotic cells (Joyce et al. 2013); however, in *Mcm5<sup>A7</sup>*  
204 meiotic nuclei, 359-bp locus pairing is significantly reduced to 61% ( $p < 0.001$ , two-tailed  
205 Fisher's exact test). From these results, we conclude that meiotic homologous  
206 centromere pairing and heterologous centromere clustering are severely decreased, if  
207 not eliminated, in *Mcm5<sup>A7</sup>* mutants. These data indicate that a decrease in meiotic  
208 centromere clustering is associated with defects in homologous chromosome pairing but  
209 not pre-meiotic pairing. Also, these results suggest that mechanisms regulating  
210 chromosome arm pairing and chromosome centromere pairing may be distinct.

211

### 212 *SMC1 localization is reduced specifically at the centromere in Mcm5<sup>A7</sup> mutants*

213

214 Centromere clustering is perturbed in sine cohesin and SC mutants (Takeo et al. 2011;  
215 Christophorou, Rubin, and Huynh 2013; Tanneti et al. 2011), suggesting that specific  
216 proteins at the centromeres are required for the aggregation of centromeres. Since we  
217 see no decrease of C(3)G at centromeres in *Mcm5<sup>A7</sup>* mutants compared to wild-type  
218 (Supplemental Figure 1), we hypothesized that a lack of centromeric cohesion in meiosis  
219 may contribute to the decrease in centromere clustering. To test this, we investigated  
220 chromosome associated-SMC1 using meiotic chromosome spreading (Khetani and  
221 Bickel 2007) (Figure 5a). In wild-type meiotic nuclei, SMC1 is enriched at the centromere

222 (green arrowhead); because SMC1 contributes to the axial element (AE) formed between  
223 sister chromatids, which later serves as the LE of the SC, SMC1 is visualized at the arm  
224 as thread-like (yellow arrowhead, dotted line). In *Mcm5<sup>A7</sup>*, SMC1 exhibits thread-like  
225 patterning along the arms, but SMC1 enrichment at the centromere appears to be  
226 compromised.

227 We quantified SMC1 localization at the centromere and along the arms at meiotic  
228 onset (defined cytologically as zygotene and early pachytene, which cannot be  
229 distinguished based on SMC1 patterning), when we hypothesize SMC1 enrichment would  
230 be essential for centromere clustering. Strikingly, at meiotic onset, SMC1 is significantly  
231 reduced in *Mcm5<sup>A7</sup>* mutants at the centromere, but not along the arms ( $***p < 0.001$  and  
232  $p = 0.0548$ , respectively, Figures 5b, 5c). These data indicate that SMC1 enrichment  
233 specifically at the centromere is perturbed in *Mcm5<sup>A7</sup>* mutants during meiotic onset when  
234 non-homologous centromeres should cluster.

235

### 236 *Increasing centromere clustering ameliorates pairing defects*

237

238 Using *Mcm5<sup>A7</sup>* mutants, we observed that a decrease in centromeric SMC1 at meiotic  
239 onset is associated with a reduction in meiotic centromere clustering and homologous  
240 chromosome pairing in pachytene, but not chromosome pairing in zygotene. Thus, we  
241 hypothesized that the centromeric-SMC1 defect at meiotic onset causes the reduction in  
242 centromere clustering, and that centromere clustering defects cause the defect in pairing  
243 maintenance.

244 To test this hypothesis, we attempted to restore SMC1 localization at the meiotic  
245 centromere in *Mcm5<sup>A7</sup>* mutants by exogenously expressing SMC1 (Gyuricza et al. 2016)  
246 in the background of *Mcm5<sup>A7</sup>* (*nos>Smc1; Mcm5<sup>A7</sup>*) (Supplemental Figure 5). Using  
247 quantitative microscopy, we found that centromeric-SMC1 is significantly higher in  
248 *nos>Smc1; Mcm5<sup>A7</sup>* than in *Mcm5<sup>A7</sup>* mutants at meiotic onset ( $***p < 0.0001$ , unpaired T-  
249 test) (Figure 6a). We next assayed centromere clustering at early pachytene, when we  
250 first observe pairing defects in *Mcm5<sup>A7</sup>* mutants (Figure 1b); as shown in Figure 6b,  
251 centromere clustering was significantly increased in *nos>Smc1; Mcm5<sup>A7</sup>* as compared to  
252 *Mcm5<sup>A7</sup>* ( $***p < 0.0001$ , unpaired T-test), indicating that the increase in centromeric-SMC1  
253 localization at meiotic onset partially rescues the early pachytene centromere clustering  
254 deficiency in *Mcm5<sup>A7</sup>* mutants.

255 We reasoned that if SMC1-dependent centromere clustering is partially rescued at  
256 early pachytene in *nos>Smc1; Mcm5<sup>A7</sup>*, then the pairing defect at this stage will be  
257 attenuated. To test this, we examined pairing frequency of X and 3R at early pachytene  
258 in *nos>Smc1; Mcm5<sup>A7</sup>* flies (Figure 6c). We see a significant pairing increase in  
259 *nos>Smc1; Mcm5<sup>A7</sup>* mutants compared to *Mcm5<sup>A7</sup>* mutants (pairing frequency of 71% and  
260 59%, respectively,  $**p = 0.0066$ , chi-square). From these data, we propose that SMC1-  
261 dependent centromere clustering in early meiosis promotes the stabilization of meiotic  
262 homolog pairing, giving rise to homosynapsis.

263 We initially hypothesized that a lack of homolog pairing results in the loss of meiotic  
264 crossovers in *Mcm5<sup>A7</sup>* mutants. We reasoned that in the presence of heterosynapsis, as  
265 seen in *Mcm5<sup>A7</sup>* mutants, meiotic DSBs cannot be repaired into crossovers because no  
266 homologous template is available (for a review on homologous recombination, see Hunter

2015). To test this hypothesis, we measured crossovers across chromosome 2L in wild-type, *Mcm5<sup>A7</sup>*, and *nos>Smc1; Mcm5<sup>A7</sup>* mutants (Figure 6d). Wild-type flies exhibit a crossover level of 45.8 cM, while the *Mcm5<sup>A7</sup>* crossover level is significantly decreased to 12.3 cM (\*\**p* < 0.0001, chi-square). In *nos>Smc1; Mcm5<sup>A7</sup>* mutants, crossover level is significantly increased to 29.8 cM (\*\**p* < 0.0001, chi-square, as compared to *Mcm5<sup>A7</sup>* mutants). These results indicate that the pairing defect and heterosynapsis during early pachytene is, at least in part, the cause for the loss of crossovers in *Mcm5<sup>A7</sup>* mutants.

Because crossover level is partially rescued in *nos>Smc1; Mcm5<sup>A7</sup>* flies, then the high nondisjunction rate in *Mcm5<sup>A7</sup>* should be lessened when SMC1 is overexpressed in these mutants. We observe that *nos>Smc1; Mcm5<sup>A7</sup>* mutants have a significant decrease in X-NDJ as compared to *Mcm5<sup>A7</sup>* mutants (NDJ rate of 11.5% and 26.5%, respectively, Figure 6e) (\*\**p* < 0.0001). Overall, these studies show that germline overexpression of SMC1 can restore SMC1 at the centromere in *Mcm5<sup>A7</sup>* mutants in early pachytene, leading to increases in centromere clustering, homolog pairing (and homosynapsis), crossover formation, and a decrease in NDJ.

## 282 DISCUSSION

283  
284 For a successful meiosis, homolog pairing must be maintained during synapsis, but how  
285 homologs remain paired as synapsis ensues is unclear. At the beginning of this study,  
286 we hypothesized that the crossover defect in *Mcm5<sup>A7</sup>* mutants was due to a homolog  
287 pairing deficiency. Our FISH results support this hypothesis (Figure 1) and revealed that  
288 homolog pairing is reversible, and if not stabilized, can cause seemingly-normal  
289 heterosynapsis (Figure 2). Centromere-directed chromosome movements occur in

290 *Mcm5<sup>A7</sup>* mutants (Figure 3), presumably yielding initial chromosome arm pairing;  
291 however, centromere clustering is perturbed (Figure 4). SMC1 enrichment at the  
292 centromere is decreased in *Mcm5<sup>A7</sup>* mutants (Figure 5), and an increase in centromeric  
293 SMC1 rescues this deficiency and downstream meiotic defects, including centromere  
294 clustering, pairing, crossover formation, and segregation (Figure 6). From our data, we  
295 propose that meiotic centromere clustering stabilizes initial homolog pairing to give rise  
296 to secure meiotic pairing and homosynapsis (Figure 7).

297

### 298 *A centromere clustering-dependent homolog pairing model in Drosophila*

299 Prior to meiosis, cellular events occur to prepare chromosomes for meiotic pairing  
300 and synapsis. Meiotic cohesins are loaded onto centromeres (Khetani and Bickel 2007),  
301 and homologous chromosomes pair (Joyce et al. 2013; Christophorou, Rubin, and Huynh  
302 2013), partly due to centromere-directed movements in the division prior to meiotic onset  
303 (Christophorou et al. 2015). We propose a model in which initial chromosomal pairing is  
304 stabilized throughout early meiosis by SMC1-dependent centromere clustering (Figure  
305 7).

306 According to this model, the enrichment of SMC1 at the centromere and  
307 chromosome movements in pre-meiotic stages yield centromere clustering at meiotic  
308 onset. While chromosome arms and centromeres enter meiosis paired, heterologous  
309 centromere clustering serves as a mechanism to stabilize the pairing, resisting forces  
310 generated by synapsis nucleation and/or diffusion that may otherwise push paired  
311 chromosomes apart. As the SC extends between the arms of homologs, DSBs are

312 formed and subsequently repaired via HR to yield crossovers, which promote accurate  
313 disjunction at the end of meiosis.

314 In *Mcm5<sup>A7</sup>* mutants, coordinated pre-meiotic centromere-directed movements  
315 occur, yet there is not sufficient SMC1 enriched at the centromere to yield centromere  
316 clustering. Thus, at meiotic onset, arms are paired, but centromeres are not clustered.  
317 As SC nucleation occurs, the stabilization provided by centromere clustering is absent  
318 and chromosome arms move freely in response to SC nucleation and/or diffusion. As  
319 synapsis extends, the SC is formed between nearby chromosomes, regardless  
320 homology, yielding heterologous synapsis. During instances of heterosynapsis, DSBs  
321 are made but cannot be repaired via HR without a homologous template. Therefore,  
322 overall crossover levels are reduced, and nondisjunction occurs at high frequency in  
323 *Mcm5<sup>A7</sup>* mutants.

324 The centromere clustering-dependent pairing model highlights that initial meiotic  
325 pairing is not sufficient to yield homosynapsis, indicating that pairing may be a two-step  
326 process. Initial homolog pairing must occur, but a stabilization step must be enforced for  
327 proper synapsis. In *Drosophila*, this stabilization is provided by SMC1-dependent  
328 centromere clustering. We propose that, to ensure stabilization of the initial pairing event,  
329 centromere clusters act as anchors at the nuclear envelope, maintaining the rigid AE  
330 (which runs along the entire length of the arm to the centromere) of each chromosome in  
331 proximity of its homolog.

332 Although meiotic pairing programs vary among organisms, we suggest that the  
333 centromere clustering-dependent pairing model can be universally applied. In *Drosophila*  
334 and *C. elegans*, meiotic pairing is independent of meiotic recombination. In contrast,

335 meiotic pairing in organisms such as yeast, plants, and mice require DSB formation  
336 (although recombination-independent alignment is required for pairing in these  
337 organisms)(Denise Zickler and Kleckner 2015). In DSB-dependent pairing programs,  
338 homologs are considered paired at ~400 nm, where DSB-mediated interhomolog  
339 interactions can be visualized as bridges (Albini and Jones 1987). However,  
340 contemporaneous with DSB formation, centromeres are coupled or clustered (reviewed  
341 in Da Ines and White 2015). We speculate that these centromere interactions stabilize  
342 the DSB-dependent arm pairing to ensure synapsis exclusively between homologs.

343

#### 344 *Pairing and subsequent synapsis in Drosophila*

345 This study reveals the interesting phenomenon of extensive, stable  
346 heterosynapsis. Extensive heterosynapsis has been previously reported in *C. elegans*  
347 (Sato-Carlton et al. 2014.; Couteau et al. 2004; Couteau and Zetka 2005; Martinez-Perez  
348 and Villeneuve 2005) and yeast (Zickler and Kleckner 1999) with variable SC defects.  
349 Though we cannot rule out SC aberrations in *Mcm5<sup>A7</sup>* mutants, our data reveal no  
350 structural defects, supporting the notion that “normal” synapsis is largely homology-  
351 independent(Rog and Dernburg 2013). However, results from this study suggest that  
352 synapsis initiation may require homology.

353 In *Drosophila*, synapsis initiates at the arms in patches during zygotene (Tanneti  
354 et al. 2011). In *Mcm5<sup>A7</sup>* mutants, synapsis initiation between paired homologs appears  
355 normal in zygotene; rather, the elongation of SC from the presumed homologous initiation  
356 patches fails to occur between homologs. Therefore, it appears that the initiation of  
357 synapsis requires homology, unlike SC elongation. Similar to what has been observed in



358 other organisms (reviewed in Rog and Dernburg 2013), we speculate that synapsis  
359 elongation is processive, such that once nucleated, the SC central region will build  
360 between two non-homologous chromosome axes that are in close proximity. Future  
361 studies determining the degree of heterosynapsis along entire chromosome arms in  
362 *Mcm5<sup>A7</sup>* mutants may provide more insight into how synapsis and homology interact in  
363 flies.

364 The heterosynapsis observed in this study also negates the long-standing  
365 assumption in *Drosophila* that stable synapsis occurs only between homologs, *i.e.*, if  
366 synapsis occurs in a mutant, then the mutant is proficient in pairing. Thus, mutants in  
367 *Drosophila* (and perhaps in other organisms) that have been previously believed to be  
368 competent in pairing due to the presence of stable SC should be revisited and tested for  
369 pairing deficiencies. Doing so could result in novel pairing mutants and aid in further  
370 understanding of how a meiotic chromosome pairs and synapses with its unique homolog.

371

## 372 MATERIALS and METHODS

373

### 374 *Experimental model details*

375

376 In all experiments, *Drosophila melanogaster* adult females 3-10 days old were used. Flies  
377 were maintained on standard medium at 25°C. *Drosophila* nomenclature used in this  
378 study was generalized for readership. Nomenclature and specific genotypes are listed  
379 below.

380

Manuscript Nomenclature	<i>Drosophila</i> genotype(s), if heteroallelic	Figure(s)
<i>Mcm5<sup>A7</sup></i>	<i>Mcm5<sup>A7</sup></i> (Lake et al. 2007)	1, 2, 4, 5, 6,
	<i>Df(3R)Exel7305</i> (Parks et al. 2004)	S1, S2, S3, S4
<i>WT</i>	<i>yw<sup>1118</sup></i>	1, 2, 4, 5, 6, S1, S2, S3, S4
<i>ord<sup>10</sup></i>	<i>ord<sup>10</sup></i> (Webber, Howard, and Bickel 2004)	2
<i>WT CID::RFP</i>	<i>w</i> ; <i>P{nanos::GAL4}</i> ; <i>CID::RFP</i> (Christophorou et al. 2015)	3
	<i>w</i> ; <i>P{UAS::Par1-GFP}</i> (Christophorou et al. 2015); +	
<i>Mcm5<sup>A7</sup> CID::RFP</i>	<i>w</i> ; <i>P{nanos::GAL4}</i> ; <i>Mcm5<sup>A7</sup></i> , <i>CID::RFP</i>	3
	<i>w</i> ; <i>P{UAS::Par1-GFP}</i> ; <i>Df(3R)Exel7305</i>	
<i>nos&gt;Smc1; Mcm5<sup>A7</sup></i>	<i>w</i> ; <i>P{nanos::GAL4}</i> ; <i>Df(3R)Exel7305</i>	6, S4
	<i>w</i> ; <i>P{UAS::Smc1-HA}</i> ; <i>Mcm5<sup>A7</sup></i>	
<i>nos&gt;Smc1; Mcm5<sup>D/+f</sup></i>	<i>w</i> ; <i>P{nanos::GAL4}</i> ; <i>Df(3R)Exel7305</i>	S4
	<i>yw</i> , <i>P{UAS::Smc1-HA}</i> (Gyuricza et al. 2016); +	
<i>Mcm5&gt;Mcm5<sup>WT</sup>; Mcm5<sup>A7</sup></i>	<i>w</i> ; <i>P{Mcm5::Mcm5}</i> (Lake et al. 2007); <i>Mcm5<sup>A7</sup></i>	S4
	+; <i>Df(3R)Exel7305</i>	
<i>rec<sup>1/2</sup></i>	<i>rec<sup>1</sup></i> (Grell 1978)	S4

	<i>rec</i> <sup>2</sup> (Matsubayashi and Yamamoto 2003)	
<i>nos&gt;Smc1; rec</i> <sup>1/2</sup>	<i>w; P{nanos::Gal4}; rec</i> <sup>1</sup>	S4
	<i>yw, P{UAS::Smc1-HA}; rec</i> <sup>2</sup>	

381

## 382 *Experimental details*

383

### 384 Genetic assays

385 X chromosome NDJ was evaluated by scoring the progeny from virgin females of desired  
 386 genotype crossed with *y cv v f / T(1:Y)B<sup>S</sup>* males. Viable exceptional XXY females have  
 387 *Bar* eyes, and viable exceptional X0 males have *Bar*<sup>+</sup> eyes and are *y cv v f*. To adjust for  
 388 inviable exceptional males and females, viable exceptional class was multiplied by 2. %  
 389 X-NDJ = 100\* ([2\*viable exceptional females] + [2\*viable exceptional males])/total  
 390 progeny. Statistical comparisons were performed as in Zeng *et al.*, 2010.

391 Crossovers on chromosome 2L were measured by crossing virgin *net dpp<sup>ho</sup> dp b*  
 392 *pr cn / +* females of desired genotype to *net dpp<sup>ho</sup> dp b pr cn* males. Vials of flies were  
 393 flipped after three days of mating. Resulting progeny were scored for all phenotypic  
 394 markers. Similarly, crossovers on chromosome X were measured by crossing virgin *y sc*  
 395 *cv v g f y<sup>+</sup> / +* females to *y sc cv v g f* males. Progeny were assessed for all phenotypic  
 396 markers.

397 To calculate intersister recombination, *R(1)2, y<sup>1</sup> w<sup>hd80k17</sup> f<sup>1</sup> / y<sup>1</sup>* females with desired  
 398 genotype were crossed to *y<sup>1</sup> w<sup>1118</sup>* and progeny was scored for phenotypic markers.  
 399 Exceptional progeny were able to be distinguished through phenotypes and rates were  
 400 adjusted to reflect only normal progeny, as in Webber, Howard and Bickel, 2004.

401

402 Dissection and immunofluorescence (IF) of whole mount germaria

403 Ten three- to five-day old virgin females of desired genotype were fattened overnight with  
404 yeast paste in vials with ~5 males of any genotype. Ovaries were dissected in fresh 1x  
405 PBS and incubated in fixative buffer for 20 minutes. Fixative buffer: 165  $\mu$ L of fresh 1x  
406 PBS, 10  $\mu$ L of N-P40, 600  $\mu$ L of heptane, and 25  $\mu$ L of 16% formaldehyde. After being  
407 washed 3 times in 1x PBS + 0.1% Tween-20 (PBST), ovaries were incubated for 1 hour  
408 in 1 mL PBST + 1% BSA (10 mL of PBST + 0.1 g BSA). Ovaries were incubated overnight  
409 in 500  $\mu$ L primary antibody diluted in 1 mL PBST + 1% BSA at 4° C on rocking nutator.  
410 After being washed 3x in PBST, ovaries were incubated in 500  $\mu$ L secondary antibody  
411 diluted at 1:500 in PBST + 1% BSA for 2 hours under foil. Ovaries were mounted in 35  
412  $\mu$ L of ProLong Gold + DAPI on microscope slide using fine-tip forceps to spread ovaries.

413 Antibodies for C(3)G(Anderson et al. 2005), SMC1(Khetani and Bickel 2007), and  
414 CID (Active Motif) were used. For Figures 4a, 4b, 4c, S2a, S2b, S2c: Images of whole  
415 mount germaria were taken Zeiss LSM880 confocal laser scanning microscope using  
416 63x/0.65 NA oil immersion objective, with a 2x zoom using ZEN software. Images were  
417 saved as .czi files and processed using FIJI(Schindelin et al. 2012). For Figures S1a,  
418 S1d, S3a, S4a: Images were taken on Nikon A1R point-scanning confocal microscope  
419 using 60x 1.49 NA oil immersion objective. Images were saved as .nd2 files and  
420 quantified as described below.

421

422 Dissection and IF of chromosome spreads

423 Before dissection, 25 mL of fixative, 5 mL of hypo-extraction buffer, and 500  $\mu$ L of 100  
424 mM sucrose were prepared. Fixative (25 mL): 23.0875 mL water, 1.5625 mL 16%  
425 formaldehyde, at 350  $\mu$ L of 10% Triton-X (1 mL of Triton-X + 9 mL water). Hypo-extraction  
426 buffer (5 mL): 3.685 mL water, 250  $\mu$ L 600 mM Tris (pH 8.2), 500  $\mu$ L 170 mM Trisodium  
427 Citrate Dihydrate, 50  $\mu$ L 500 mM EDTA, 2.5  $\mu$ L 1.0 M DTT, 12.5  $\mu$ L 200 mM Pefabloc  
428 (hypo-extraction buffer is good for only 2 hours). 100 mM Sucrose (500  $\mu$ L): 100  $\mu$ L 500  
429 mM sucrose + 400  $\mu$ L water).

430 Ovaries were dissected in 1x PBS and rinsed once in hypo-extraction buffer.  
431 Ovaries were incubated for 20 minutes in hypoextraction buffer and transferred to sucrose  
432 and minced. A super-frost slide was dipped into the fixative for 15 seconds. 10  $\mu$ L of  
433 minced ovary tips were transferred onto the middle edge of the long side of the slide and  
434 rolled to allow spreading. Slides were dried very slowly overnight in a closed humidified  
435 chamber. Once dried, slides were incubated with 500  $\mu$ L of blocking (5% normal goat  
436 serum (NGS), 2% BSA, 0.1% Triton-X in 1x PBS). Slides were rinsed 3 times in B-PBSTx  
437 (0.1% BSA, 0.1% Triton-X in 1x PBS). 250  $\mu$ L of primary antibodies diluted in B-PBSTx  
438 were incubated under parafilm overnight in humidifying chamber. Slides were washed 3  
439 times with PBSTx (0.1% Triton-X in 1x PBS). Secondary antibodies were diluted at 1:400  
440 in B-PBSTx. 100  $\mu$ L of diluted secondary were added onto slide under parafilm and  
441 incubated for an hour. Slides were rinsed 3 times in PBSTx and washed three time for  
442 10 minutes in PBSTx in Coplin jar. Slides were incubated swith 400  $\mu$ L DAPI (1 ug/ml) in  
443 1x PBS for 10 minutes in dark and washed in 1x PBS. Coverslips were mounted with  
444 ProLong Gold.

445 Antibodies for C(3)G(Anderson et al. 2005), Corolla(Collins et al. 2014),  
446 SMC1(Khetani and Bickel 2007), and CID (Active Motif) were used. Figure 5a: Images  
447 were taken on Zeiss LSM880 confocal laser scanning microscope using 63x/0.65 NA oil  
448 immersion objective with a 2x zoom using ZEN software. Images were saved as .czi files  
449 and processed using FIJI (Schindelin et al. 2012). Figures 2c and 2d: Images were taken  
450 on Nikon N-SIM using Elements software. Images were saved as .nd2 files and  
451 processed using FIJI(Schindelin et al. 2012).

452

#### 453 Generation of fluorescence in situ hybridization (FISH) probes

454 DNA from desired BAC clones (BAC PAC RPCI-98 Library) was extracted from MIDI-  
455 prep culture. For X probe (Figure 1b), six BAC clones were used, spanning cytological  
456 bands 6E-7B. Clones: 17 C09, 06 J12, 35 J16, 20 K01, 35 A18, 26 L11. For 3R probe  
457 (Figure 1c), six BAC clones were used, spanning cytological bands 93A-93E. Clones: 19  
458 P12, 05 I01, 20 N14, 10 M16, 06 L13, 34 E13. The BAC-derived template DNA was used  
459 in a nick-translation reaction to generate euchromatic biotinylated DNA probes, as  
460 described below.

461 For one BAC clone DNA template, the following was added into a 0.5 mL tube: 5  
462  $\mu$ L 10X DNA Pol I buffer, 2.5  $\mu$ L dNTP mix (1 mM each of dCTP, dATP, dGTP), 2.5  $\mu$ L  
463 biotin-11-dUTP (1 mM), 5.0  $\mu$ L 100 mM BME, 10  $\mu$ L of freshly diluted dDNase I, 1  $\mu$ L  
464 DNA Pol I, 1 ug of template DNA, water up to 50  $\mu$ L. Reaction was incubated at 15° C in  
465 thermocycler for 4 hours and eluted in 20  $\mu$ L TE. Concentration was determined using  
466 Qubit kit and diluted to a final concentration at 2 ng/ $\mu$ L in hybridization buffer.

467 Hybridization buffer: 2x Saline-Sodium Citrate (SSC) buffer, 50% formamide, 10% w/v  
468 dextran sulfate, 0.8 mg/mL salmon sperm DNA.

469 The 359-bp probe (Figure 4d) was ordered from Integrative DNA Technologies  
470 (IDT, [www.idtdna.com](http://www.idtdna.com)) with 5' Cy5, resuspended in 1x TE at 100  $\mu$ M. Sequence for 359-  
471 bp probe (5' to 3'): Cy5- GGGATCGTTAGCACTGGTAATTAGCTGC.

472

#### 473 FISH/IF of whole mount germaria

474 Ovaries were dissected in fresh 1x PBS and incubated in fixative buffer for 4 minutes.  
475 Fixative buffer: 100 mM sodium cacodylate (pH 7.2), 100 mM sucrose, 40 mM potassium  
476 acetate, 10 mM sodium acetate, 10 mM EGTA, 5% formaldehyde. Ovaries were  
477 transferred to 0.5 ml tube filled with 2x SSCT (5 ml 20x SSC, 50  $\mu$ L Tween, 45 mL water)  
478 and washed four times in 2x SSCT, 3 minutes each. Ovaries were washed 10 minutes  
479 in 2x SSCT + 20% formamide, 10 minutes 2x SSCT + 40% formamide, and then two  
480 times for 10 minutes each in 2x SSCT + 50% formamide. Ovaries were incubated at 37°  
481 C for 4 hours, at 92° C for 3 minutes and then 60° C for 20 minutes. Ovaries were  
482 transferred to tube with 36  $\mu$ L of BAC-generated probe (diluted in hybridization buffer) or  
483 with 35  $\mu$ L of hybridization buffer and 1  $\mu$ L of IDT-generated probe. Ovaries were  
484 incubated in the thermocycler for 3 minutes at 91° C then at 37° C overnight and then  
485 washed with 2x SSCT + 50% formamide for 1 hour at 37° C. Ovaries were washed in 2x  
486 SSCT + 20% formamide for 10 minutes at room temperature and rinsed in 2x SSCT four  
487 times quickly. Ovaries were incubated for 4 hours in blocking solution (6 mg/mL NGS in  
488 2x SSCT) and then washed three times quickly in 2x SSCT. Ovaries were incubated  
489 overnight in primary antibody diluted in 2x SSCT at room temperature. Ovaries were

490 washed three times quickly in 2x SSCT and incubated for two hours in secondary antibody  
491 diluted in 2x SSCT. Biotinylated probes: sample was incubated in 1.5  $\mu$ L of 488-  
492 conjugated streptavidin diluted in 98.5  $\mu$ L detection solution (0.5 mL 1M Tris, 400 mg  
493 BSA, water to 10mL) for 1 hour, washed two times quickly in 2x SSCT, washed for 1 hour  
494 in 2x SSCT, and then washed 3 hours in 2x SSCT. (If using IDT-generated probes, these  
495 steps were not performed.) Ovarioles were mounted on a slide in 35  $\mu$ L of DAPI +  
496 fluoromount.

497       Antibody for C(3)G(Anderson et al. 2005) was used. Figures 1b, 1c, 4d: Images of  
498 whole mount germaria were taken Zeiss LSM880 confocal laser scanning microscope  
499 using 63x/0.65 NA oil immersion objective with a 2x zoom using ZEN software. Figure  
500 2b: Images were obtain using AIRY-Scan on Zeiss LSM880 confocal laser scanning  
501 microscope using 40x oil immersion objective. Images were saved as .czi files and  
502 processed using FIJI(Schindelin et al. 2012).

503

#### 504 Live cell imaging

505 Ovaries were dissected in 10S Voltalef oil. The muscular sheath around each ovariole  
506 was removed and ovarioles were manually separated. Individual ovarioles were  
507 transferred to a drop of oil on coverslip. Videos were collected with an on an inverted  
508 Zeiss Axioobserver Z1 with motorized XYZ spinning-disc confocal microscope operated  
509 by Metamorph coupled to a sCMOS (Hamamatsuorca) camera and a temperature control  
510 chamber. All images were acquired with the Plan-Apochromat 100x/1.4 oil objective lens.  
511 Single-position videos in the germarium were acquired for 8 minutes at  $25 \pm 1^\circ$  C, with a  
512 10 second temporal resolution (12-slice Z-stack, 0.5  $\mu$ m per slice).



513

## 514 *Quantification and statistical analysis*

515

### 516 Recombination calculations

517 Genetic distances are expressed in centiMorgans (cM), calculated by  $100 * (R / n)$ , where  
518  $R$  is the number of recombinant progeny in a given interval (including single, double, and  
519 triple crossovers), and  $n$  is the total number of progeny scored. 95% confident intervals  
520 were calculated from variance, as in Stevens (Stevens 1936). Molecular distances (in  
521 Mb) are from the positions of genetic markers on the *Drosophila melanogaster* reference  
522 genome, release 6.12 (Thurmond et al. 2018). Crossover density (or frequency), as  
523 calculated by cM/Mb, exclude transposable elements (see Miller *et al.*, 2016; Hatkevich  
524 and Sekelsky, 2017).

525

### 526 Quantitative microscopy analysis of IF in whole mounts

527 For fixed germaria, DAPI and anti-CID with either anti-C(3)G(Anderson et al. 2005) or  
528 anti-SMC1(Khetani and Bickel 2007) stains were used. Individual nuclei were first  
529 selected and eight 0.5  $\mu$ m z-slices were used for analysis. First, fluorescence intensities  
530 were measured separately by subtracting cytoplasmic background in individual slices  
531 using an automated approach. For centromeric fluorescence intensities, centromeres  
532 were first segmented based on anti-CID (Active Motif) fluorescence using a probabilistic  
533 segmentation approach. Using segmented centromere masks, centromeric CID, C(3)G  
534 and SMC1 were quantified. For nuclear fluorescence intensities, nuclei were segmented  
535 using anti-C(3)G fluorescence. For chromosome arm fluorescence intensities,

536 centromeric fluorescence intensities were subtracted from nuclear fluorescence  
537 intensities. To account for potential staining heterogeneity, fluorescence intensities were  
538 normalized to total nuclear CID fluorescence intensity, which was assumed to be  
539 unperturbed. Fluorescence intensities represent raw integrated densities of maximum  
540 intensity projected z-stacks. Nucleus selection, background subtraction and fluorescence  
541 intensity measurements were performed semi-automatically using custom FIJI-based  
542 plugins (available upon request) (Schindelin et al. 2012).

543

#### 544 Analysis of pairing and centromere clustering

545 To determine the meiotic stage in fixed whole mount germaria, nuclear C(3)G staining  
546 patterning was used. Spots of C(3)G in early Region 2A was considered zygotene, full-  
547 length C(3)G in Region 2A was considered early pachytene, and full-length C(3)G in  
548 Region 3 was considered mid-pachytene. Two foci were considered unpaired if distances  
549 between the center of the foci were equal to or greater than 0.7  $\mu\text{m}$  (Gong, McKim, and  
550 Scott Hawley 2005). For centromere counting, any distinguishable single CID focus was  
551 counted as one, and distance between CID foci was not considered.

552

#### 553 Live cell imaging tracking

554 The use of PAR1::GFP on live germaria allowed the identification of the different cyst  
555 stages. For live germaria, images shown are the projection of all Z-series of a single (t)  
556 projection. Three-dimensional tracking of spinning-disc data was performed using Imaris  
557 software (Bitplane). The CID::RFP signal was tracked using the 'spots' function with an  
558 expected diameter of 0.3  $\mu\text{m}$ . Automatically generated tracks were then edited manually

559 to eliminate inappropriate connections, including connections between foci in different  
560 nuclei or between foci of different sizes or intensity when more likely assignments were  
561 apparent or multiple spots assigned to the same focus.

562 To remove global movements of the germarium, each nucleus containing a  
563 CID::RFP focus was assigned to the nearest fusome foci. Then, the position of the  
564 reference fusome was subtracted from each CID::RFP focus for each time point of the  
565 tracking to get the relative tracks. These relative tracks were then compiled using a  
566 custom MATLAB (MathWorks) routine that computes the minimum volume of the ellipsoid  
567 that encloses all of the three-dimensional points of the trajectory.

568 To analyze centromere trajectories: Positions of individual centromeres were  
569 tracked every 10 seconds during 8 minutes to quantify the volume covered by each  
570 centromere. This raw volume was then corrected both for overall movements of the tissue  
571 and for variations in total nuclear volume. First, we subtracted the motion of the  
572 germarium using the position of the fusome as a reference within each cyst. Second, to  
573 take into account the nuclear volume at 8cc, we computed the relative volume, which is  
574 the raw volume divided by the mean value of the nuclear volume at 8cc stage. Finally, we  
575 normalized durations of each track by calculating the relative covered volume per second  
576 (as shown in Figure 3e).

577

### 578 Transparent Reporting

579 Each microscopy experiment performed in this study was repeated independently at least  
580 two times. We did not use explicit power analysis; rather, in each experiment, at least 8  
581 independent germaria were imaged, and meiotic cells within the germaria were quantified,

582 giving the final sample size per experiment. The total number of samples ( $n$ ) is the sum  
583 of the final sample sizes per experiment.

584

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600

## 601 AUTHOR CONTRIBUTIONS

602

603 Conceptualization, TH and JS; Methodology, TH, JS, VB, TR, and JRH; Investigation,  
604 TH, VB, and TR; Resources, JS, PSM, and JRH; Writing – Original, TH and JS; Writing –  
605 Editing, TH, JS, VB, TR, and JRH; Visualization, TH, VB, and TR; Supervision, JS, PSM,  
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## 607 COMPETING INTERESTS

608

609 The authors declare no competing interests.

610

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753

## 754 FIGURE LEGENDS

755

756 **Figure 1. Meiotic pairing is perturbed in *Mcm5<sup>A7</sup>* mutants.** a. Schematic depiction of  
757 the *Drosophila* germarium. At the anterior portion (the pre-meiotic region, Region 1), the  
758 germline stem cell (brown cell) divides to yield a cytoblast, which undergoes four  
759 subsequent rounds of division to yield a 16-cell cyst. In the pre-meiotic region, meiotic  
760 proteins, such as SMC1 and C(3)G, are enriched at the centromeres, and within the 8-  
761 cell cyst, chromosomes exhibit centromere-direct rapid movements. Within the first 16-  
762 cell cyst (zygotene; Region 2A), homologous chromosomes pair, centromeres cluster into

763 1 or 2 groups, and up to four cells initiate meiosis, expressing patches of SC (red dots).  
764 As the 16-cell cyst enters early pachytene (EP) (Region 2A), only two continue as pro-  
765 oocytes to form full length synaptonemal complex (red). Meiotic double-strand breaks  
766 (DSBs) are formed and repaired via homologous recombination (HR) throughout the  
767 germarium's posterior (Regions 2A, 2B) to yield noncrossover and crossover products.  
768 At the most posterior tip, signifying mid-pachytene (MP), only one cell within the cyst has  
769 been selected to become the oocyte, and all DSBs are repaired. b. Top: schematic of *X*  
770 chromosome and relative location of *X*-probe, not drawn to scale. Left: Representative  
771 images paired (*WT*) and unpaired (*Mcm5<sup>A7</sup>*) *X*-probes (green) in meiotic cells, indicated  
772 by C(3)G expression (magenta). Images are of meiotic nuclei in Region 2A. Scale bar =  
773 1  $\mu$ m. Right: Quantification of percent paired and unpaired cell in *WT* and *Mcm5<sup>A7</sup>* in Z  
774 (*WT*  $n = 33$ , *Mcm5<sup>A7</sup>* = 32), EP (*WT*  $n = 130$ , *Mcm5<sup>A7</sup>* = 118; \*\*\* $p < 0.0001$ , chi-square),  
775 and MP (*WT*  $n = 10$ , *Mcm5<sup>A7</sup>* = 11; \* $p = 0.01$ , chi-square). c. Top: schematic of the right  
776 arm of chromosome 3 (3R) and relative location of 3R-probe, not drawn to scale. Left:  
777 Representative images paired (*WT*) and unpaired (*Mcm5<sup>A7</sup>*) 3R-probes (green) in meiotic  
778 cells, represented by C(3)G expression (magenta). *WT* image is of 2A nucleus, *Mcm5<sup>A7</sup>*  
779 is of Region 3 nucleus. Right: Quantification of percent paired and unpaired cell in *WT*  
780 and *Mcm5<sup>A7</sup>* in Z (*WT*  $n = 37$ , *Mcm5<sup>A7</sup>* = 33), EP (*WT*  $n = 104$ , *Mcm5<sup>A7</sup>* = 97; \*\*\* $p < 0.0001$ ,  
781 chi-square), and MP (*WT*  $n = 10$ , *Mcm5<sup>A7</sup>* = 9; \*\* $p = 0.0066$ , chi-square). Brightness,  
782 contrast, and texture (smoothed) of images have been adjusted for clarity.

783

784 **Figure 2. Synaptonemal complex exhibits no observable defects in *Mcm5<sup>A7</sup>***  
785 **mutants.** a. Schematic depiction of SC between two homologous chromosomes. The

786 SC is composed of two lateral elements (LEs) and one central region (CR). The LEs are  
787 predecessors of the axial element, which is formed between sister chromatids and are  
788 composed of two cohesion complexes (blue and pink ovals). The CR consists, in part, of  
789 a C(3)G (green) dimer spanning the LEs, with pillar proteins such as Corolla (yellow)  
790 embedded within the CR. Enrichment of proteins at the centromere is not depicted. b.  
791 Super-resolution images of C(3)G and X-probe in *WT* (paired) and *Mcm5<sup>A7</sup>* (unpaired) in  
792 whole-mount germaria. The images of *Mcm5<sup>A7</sup>* is of the same nucleus but of different *Z*  
793 slices to capture both X-probes. Brightness and contrast have been adjusted for clarity.  
794 Scale bar = 1  $\mu\text{m}$ . Refer to Supplemental Movies 1 and 2. c. Top: Representative image  
795 of C(3)G (magenta) and Corolla (green) in a *WT* meiotic chromosome spread. Brightness  
796 and contrast have been adjusted for clarity. Yellow arrowhead indicates area magnified  
797 in lower panel (middle). Scale bar = 2  $\mu\text{m}$ . Middle: Magnification to detail the localization  
798 of C(3)G and Corolla. Scale bar = 2  $\mu\text{m}$ . Yellow line indicates the area that was quantified  
799 for normalized intensity. Bottom: Normalized intensity of C(3)G and Corolla to  
800 demonstrate localization. d. Top: Representative image of C(3)G (magenta) and Corolla  
801 (green) in *Mcm5<sup>A7</sup>* meiotic chromosome spread. Yellow arrowhead indicates area  
802 magnified in lower panel (middle). Scale bar = 2  $\mu\text{m}$ . Middle: Magnification to detail the  
803 localization of C(3)G and Corolla. Scale bar = 2  $\mu\text{m}$ . Yellow line indicates the area that  
804 was quantified for normalized intensity. Bottom: Normalized intensity of C(3)G and  
805 Corolla to demonstrate localization. e. Left panel: Quantification of nuclear C(3)G signal  
806 at early pachytene in *WT* ( $n = 52$ ) and *Mcm5<sup>A7</sup>* ( $n = 41$ ) meiotic nuclei.  $p = 0.5601$ ,  
807 unpaired T-test. Data are represented as mean  $\pm$  SD. Right panel: Quantification of  
808 nuclear C(3)G signal in mid-pachytene in *WT* ( $n = 12$ ) and *Mcm5<sup>A7</sup>* ( $n = 11$ ) meiotic nuclei.

809  $p = 0.3993$ , unpaired T-test. Data are represented as mean  $\pm$  SD. Refer to Supplemental  
810 Figure 1 for images and further analysis. f. *Mcm5<sup>A7</sup>* ( $n = 1194$ ) and *ord<sup>10</sup>* ( $n = 250$ ) mutants  
811 examined for inter-sister recombination through the ratio of Ring chromosome to Rod  
812 chromosome transmission. *WT* ( $n = 2574$  for *Mcm5<sup>A7</sup>* experiment,  $n = 1204$  for *Ord*  
813 experiment) was normalized to 1. Ratios above 1 suggest less inter-sister recombination;  
814 ratios below 1 suggest more inter-sister recombination. Refer to Table S1 for complete  
815 dataset.

816

817 **Figure 3. Centromeres in *Mcm5<sup>A7</sup>* mutants exhibit dynamic, rapid movements.** a.  
818 Projection of Z-sections of live *WT* (left) and *Mcm5<sup>A7</sup>* 8-cell cysts expressing CID::RFP  
819 (magenta) and Par-1::GFP (fusome, green). Circles represent individual nuclei within the  
820 8-cell cysts. Yellow arrow heads denote representative analyses shown in b. and c. and  
821 quantified by time points in d.. Scale bars = 2 $\mu$ m. For videos, refer to Supplemental Video  
822 3 and Video 4. b. Selected projections from one *WT* 8-cell cyst nucleus in (A, indicated  
823 by yellow arrow head) over a 3-minute time course. See Video S5 for full movie. c.  
824 Selected projections from one *Mcm5<sup>A7</sup>* 8-cell cyst nucleus in (A, indicated by yellow arrow  
825 head) over a 3-minute time course. See Video S6 for full movie. Time-colored tracking  
826 for CID-RFP dots indicated by yellow arrow heads are shown in right panels for b. and c..  
827 Scale bars = 2 $\mu$ m. d. 3-dimensional representations demonstrating the covered volume  
828 of a representative track for all time points in *WT* (50 time points, volume = 12.9  $\mu$ m<sup>3</sup>) and  
829 *Mcm5<sup>A7</sup>* (48 time points, volume = 15.7  $\mu$ m<sup>3</sup>). e. Distribution of the relative covered volume  
830 (raw covered volume/nuclear volume) per second for each track in *WT* ( $n = 103$

831 centromere foci) and *Mcm5<sup>A7</sup>* ( $n = 80$  centromere foci).  $p = 0.75$ , Kolmogorov-Smirnov  
832 test. Data are represented as mean  $\pm$  SD.

833

834 **Figure 4. Centromere clustering is disrupted in *Mcm5<sup>A7</sup>* mutants.** a. Left:

835 Representative images of centromere clustering, or lack thereof, in wild-type (*WT*) and  
836 *Mcm5<sup>A7</sup>* meiotic nuclei located in zygotene. Magenta: C(3)G, green: CID (centromere).

837 In these images, *WT* nucleus contains 1 CID focus, and *Mcm5<sup>A7</sup>* contains 6 CID foci.

838 Scale bar = 1  $\mu$ m. Circles represent outline of nuclei. CID foci not localized with C(3)G is

839 from adjacent, non-meiotic cells (refer to Supplemental Figure 2a). Right: Quantification

840 of CID foci in zygotene in *WT* ( $n = 24$ ) and *Mcm5<sup>A7</sup>* ( $n = 16$ ). \*\*\* $p < 0.0001$ , unpaired T-

841 test. Data are represented as mean  $\pm$  SD. b. Left: Representative images of centromere

842 clustering in *WT* and *Mcm5<sup>A7</sup>* early pachytene nuclei. Magenta: C(3)G, green: CID. In

843 these images, *WT* nucleus contains 2 CID foci, and *Mcm5<sup>A7</sup>* contains 5 CID foci. Scale

844 bar = 1  $\mu$ m. CID foci not localized with C(3)G is from adjacent, non-meiotic cells (refer to

845 Supplemental Figure 2b). Right: Quantification of early pachytene CID foci in *WT* ( $n = 65$ )

846 and *Mcm5<sup>A7</sup>* ( $n = 94$ ). \*\*\* $p < 0.0001$ , unpaired T-test. Data are represented as mean  $\pm$

847 SD. c. Left: Representative images of centromere clustering, or lack thereof, in mid-

848 pachytene *WT* and *Mcm5<sup>A7</sup>* nuclei. Magenta: C(3)G, Green: CID. In these images, *WT*

849 nucleus contains 1 CID focus, and *Mcm5<sup>A7</sup>* contains 4 CID foci. Scale bar = 1  $\mu$ m. CID

850 foci not localized with C(3)G is from adjacent, non-meiotic cells (refer to Supplemental

851 Figure 2c). Right: Quantification of mid-pachytene CID foci in *WT* ( $n = 16$ ) and *Mcm5<sup>A7</sup>*

852 ( $n = 19$ ). \*\*\* $p < 0.0001$ , unpaired T-test. Data are represented as mean  $\pm$  SD. d.

853 Schematic representing relative location of 359-bp locus on Chromosome X (not drawn

854 to scale). Top panel: Representative image of meiotic nucleus with 1 359-bp (green) focus  
855 (*WT*, Region 2A). Bottom panel: Representative image of meiotic nucleus with 2 359-  
856 bp (green) foci *Mcm5<sup>A7</sup>*, Region 2A). Right: Percentage of nuclei with paired 359-bp loci  
857 (one focus) or unpaired (two loci) in *WT* ( $n = 88$ ) and *Mcm5<sup>A7</sup>* ( $n = 63$ ) meiotic nuclei. \*\*\* $p$   
858  $< 0.0001$ , as determined by two-tailed Fisher's exact test. Contrast and brightness of all  
859 images were adjusted for clarity.

860

861 **Figure 5. Centromeric SMC1 is significantly reduced in *Mcm5<sup>A7</sup>* mutants.** a.  
862 Representative images of chromosome spreads in *WT* and *Mcm5<sup>A7</sup>* meiotic nuclei  
863 examining localization of SMC1 (magenta) and CID (green). Green arrow: SMC1  
864 enrichment at the centromere, yellow arrow and tract: SMC1 along the chromosome arm.  
865 Scale bar = 2  $\mu\text{m}$ . Contrast and brightness of images were adjusted for clarity. b.  
866 Quantification of SMC1 at CID foci in meiotic nuclei at meiotic onset (zygotene + early  
867 pachytene, Region 2A) at *WT* ( $n = 225$ ) and *Mcm5<sup>A7</sup>* ( $n = 398$ ) meiotic centromeres. \*\*\* $p$   
868  $< 0.0001$ , unpaired T-test. Data are represented as mean  $\pm$  SD. c. Quantification of SMC1  
869 at chromosome arm in meiotic nuclei at meiotic onset (zygotene + early pachytene,  
870 Region 2A) in *WT* ( $n = 81$ ) and *Mcm5<sup>A7</sup>* ( $n = 93$ ) meiotic nuclei. *n.s.* = 0.0548, unpaired  
871 T-test. Data are represented as mean  $\pm$  SD. Refer to Supplemental Figure 3 for  
872 representative images.

873

874 **Figure 6. Overexpression of SMC1 in *Mcm5<sup>A7</sup>* mutants rescue clustering, pairing,**  
875 **crossover formation, and NDJ.** a. Quantification of SMC1 signal at the centromeres at  
876 *WT*, *Mcm5<sup>A7</sup>*, and *nos>Smc1; Mcm5<sup>A7</sup>* ( $n = 427$ ) meiotic centromeres at meiotic onset.



877 See Supplemental Figure 4 for representative images of *nos>Smc1; Mcm5<sup>A7</sup>* nuclei. *WT*  
878 and *Mcm5<sup>A7</sup>* data are repeated from Figure 5. \*\*\* $p < 0.0001$ , unpaired T-test. Data are  
879 represented as mean  $\pm$  SD. b. Number of centromeres (CID foci) in *WT*, *Mcm5<sup>A7</sup>*, and  
880 *nos>Smc1; Mcm5<sup>A7</sup>* ( $n = 94$ ) meiotic nuclei at early pachytene. *WT* and *Mcm5<sup>A7</sup>* data are  
881 repeated from Figure 2. \*\*\* $p < 0.0001$ , unpaired T-test. Data are represented as mean  
882  $\pm$  SD. c. Percent of total paired and unpaired in *WT*, *Mcm5<sup>A7</sup>*, and *nos>Smc1; Mcm5<sup>A7</sup>*  
883 (total  $n = 169$ ) nuclei at early pachytene, combining X-probe and 3R-probe data. *WT* and  
884 *Mcm5<sup>A7</sup>* data are repeated from Figure 4 and are represented as X-probe plus 3R-probe  
885 early pachytene data. Significance comparing *Mcm5<sup>A7</sup>* and *nos>Smc1; Mcm5<sup>A7</sup>*: \*\* $p =$   
886  $0.0002$ , chi-square d. Crossover levels on chromosome 2L as shown in cM in *WT* ( $n =$   
887  $4222$ ) (Hatkevich et al. 2017), *Mcm5<sup>A7</sup>* ( $n = 2070$ ), and *nos>Smc1; Mcm5<sup>A7</sup>* ( $n = 933$ ). \*\*\* $p$   
888  $< 0.001$ , chi-square. Data are represented as mean  $\pm$  95% CI. Refer to Table S2 for full  
889 2L crossover dataset. e. NDJ of the X chromosome in *WT* (0.07%,  $n = 3034$ ), *Mcm5<sup>A7</sup>*  
890 (26.5%,  $n = 1979$ ), *nos>Smc1; Mcm5<sup>A7</sup>* (11.5%,  $n = 2282$ ). \*\*\* $p < 0.0001$  (Zeng et al.  
891 2010). Data are represented as mean  $\pm$  95% CI. Refer to Table S3 for full NDJ dataset.  
892

893 **Figure 7. Centromere clustering-dependent pairing model.** *WT*: In pre-meiotic cysts,  
894 homologous chromosomes (pink = homolog pair 1, black = homolog pair 2) enter the  
895 germline unpaired. During pre-meiotic cell cycles, chromosome arms and centromeres  
896 pair, with centromeres anchored at the nuclear envelope. Prior to meiotic onset, SMC1  
897 is enriched at the centromeres (yellow) and centromere-directed chromosome movement  
898 (double-headed arrows) occurs. These events yield centromere clustering at meiotic  
899 initiation. As synapsis nucleates along arms (green bars), paired chromosomes are able

900 to withstand opposing forces because of physical stabilization provided by centromere  
901 clustering, permitting homosynapsis. After DSB formation and repair (not depicted),  
902 crossovers between homologs are formed, promoting proper disjunction at the end of  
903 meiosis I. *Mcm5<sup>A7</sup>*: Chromosomes enter the germline unpaired, and centromeres are  
904 attached to the nuclear envelope. In pre-meiotic cycles, chromosomes initially pair, but  
905 centromeres do not. Centromere-directed chromosome movements occur, but SMC1 is  
906 not enriched at the centromere, causing a lack of centromere clustering at meiotic onset.  
907 As the SC nucleates at the arms, opposing forces push the paired chromosome arms  
908 apart. Synapsis spreads between the nearest chromosomal regions, independent of  
909 homology, yielding high frequency of heterosynapsis. During heterosynapsis, DSBs are  
910 not repaired by HR, yielding non-recombinant chromosomes that nondisjoin at the end of  
911 Meiosis I.

912

## 913 SUPPLEMENTAL INFORMATION

914

915 Supplemental Information includes four figures, four tables, and six movies.

916

## 917 SUPPLEMENTAL FIGURE LEGENDS

918

919 **Supplemental Figure 1 (related to Figure 2). Quantitative analysis of C(3)G in *WT***  
920 **and *Mcm5<sup>A7</sup>* mutants.** a. Representative images of *WT* and *Mcm5<sup>A7</sup>* meiotic nuclei in  
921 whole mount germaria that were quantified in b., c., and Figure 2e examining C(3)G

922 (magenta) and CID (green) in early pachytene. b. Quantification of C(3)G signal at the  
923 centromere (CID) in *WT* and *Mcm5<sup>A7</sup>* early pachytene nuclei.  $p = 0.4327$ , unpaired T-  
924 test. Data are represented as mean  $\pm$  SD. c. Quantification of C(3)G signal at  
925 chromosome arm in *WT* and *Mcm5<sup>A7</sup>* early pachytene nuclei.  $p = 0.6358$ , unpaired T-  
926 test. Data are represented as mean  $\pm$  SD. d. Representative images of *WT* and *Mcm5<sup>A7</sup>*  
927 meiotic nuclei of whole mount germaria that were quantified in e., f., and Figure 2f  
928 examining C(3)G (magenta) and CID (green) at mid-pachytene. e. Quantification of C(3)G  
929 signal at the centromere (CID) in *WT* and *Mcm5<sup>A7</sup>* mid-pachytene nuclei.  $p = 0.3615$ ,  
930 unpaired T-test. Data are represented as mean  $\pm$  SD. f. Quantification of C(3)G signal at  
931 chromosome arms in *WT* and *Mcm5<sup>A7</sup>* mid-pachytene nuclei.  $p = 0.5489$ , unpaired T-  
932 test. Data are represented as mean  $\pm$  SD.

933

934 **Supplemental Figure 2 (related to Figure 4). Multiple germarium nuclei are depicted**  
935 **per frame.** (A, B, C) DAPI included images of *WT* and *Mcm5<sup>A7</sup>* in Figure 4a, b, c,  
936 respectively, to demonstrate that additional CID foci are of neighboring nuclei. d. DAPI  
937 included images of meiotic nuclei with 1 359-bp focus (*WT*, top panel) and 2 359-bp foci  
938 (*Mcm5<sup>A7</sup>*, bottom panel) from Figure 4d. Scale bars = 1  $\mu$ m. Contrast and brightness of  
939 all images were adjusted for clarity.

940

941 **Supplemental Figure 3 (related to Figure 5). Images quantified in Figure 5. a.**  
942 Representative images quantified in Figure 5b and 5c. Scale bar = 1  $\mu$ m. b.  
943 Representative images quantified in Figure 5d and 5e. Scale bar = 1  $\mu$ m. Magenta:  
944 SMC1, Green: CID. Images are of whole mount germaria.

945

946 **Supplemental Figure 4 (related to Figure 6). SMC1 overexpression in *Mcm5<sup>A7</sup>***

947 **mutants.** a. Representative images of *nos>Smc1, Mcm5<sup>A7</sup>* meiotic nuclei at meiotic  
948 onset, quantified in Figure 6a. b. Crossovers levels on Chromosome X in *WT* ( $n = 2179$ ,  
949 62.8cM (Hatkevich et al. 2017) and *Mcm5<sup>A7</sup>* ( $n = 2743$ , 3.8 cM), similar to levels previously  
950 reported(Lake et al. 2007). These data show that the crossover defect severity in *Mcm5<sup>A7</sup>*  
951 mutants is chromosome-specific. Due to genetics of the SMC1 transgene, we were  
952 unable to test *nos>Smc1; Mcm5<sup>A7</sup>* crossover levels on the X. Data are represented as  
953 mean  $\pm$  95% CI. See Table S4 for complete crossover dataset. c. Left: NDJ of the X  
954 chromosome in *WT* (0.07%,  $n = 3034$ ) and controls *nos>Smc1; Mcm5<sup>Df/+</sup>* (0.16%,  $n =$   
955 1273) and *Mcm5>Mcm<sup>WT</sup>; Mcm5<sup>A7</sup>* (0.26%,  $n = 753$ ). Right: NDJ of *rec<sup>1/2</sup>* (19.1%,  $n =$   
956 1563), and *nos>Smc1, rec<sup>1/2</sup>* (24.1%,  $n = 1187$ ) to demonstrate that SMC1  
957 overexpression NDJ rescue is specific to *Mcm5<sup>A7</sup>*. Data are represented as mean  $\pm$  95%  
958 CI.

959

960 **Supplemental Table 1 (related to Figure 2). Complete inter-sister recombination**

961 **dataset.** Complete Ring:Rod dataset. \*Adjusted females: Assuming male and female  
962 NDJ are equal, we subtract the amount of male NDJ from the y Normal female progeny;  
963 in these y females, one cannot distinguish between y/y female versus a y/y/Y female.  
964 *Ord<sup>10</sup>* exceptional females were distinguishable due to an additional phenotypic marker.

965

966 **Supplemental Table 2 (related to Figure 6). Recombination dataset for**  
967 **Chromosome 2L.** Recombination events across Chromosome 2L in progeny of *WT*,  
968 *Mcm5<sup>A7</sup>*, and *nos>Smc1, Mcm5<sup>A7</sup>* mothers.

969  
970 **Supplemental Table 3 (related to Figure 6). Complete X-NDJ dataset.** Total normal  
971 and exceptional progeny from experimental and control lines.

972  
973 **Supplemental Table 4 (related to Figure 6). Recombination dataset for X**  
974 **Chromosome.** Recombination events across X Chromosome in progeny of *WT*,  
975 *Mcm5<sup>A7</sup>*, and *nos>Smc1, Mcm5<sup>A7</sup>* mothers.

976  
977 **Supplemental Video 1 (related to Figure 2). Rotation of *WT* meiotic nucleus shown**  
978 **in Figure 2b.** Meiotic nucleus demonstrating full length tracts of C(3)G (magenta) and  
979 localization of X-homologs (X-probes, green) in *WT*.

980  
981 **Supplemental Video 2 (related to Figure 2). Rotation of *Mcm5<sup>A7</sup>* meiotic nucleus**  
982 **shown in Figure 2b.** Meiotic nucleus demonstrating full length tracts of C(3)G (magenta)  
983 and localization of X-homologs (X-probes, green) in *Mcm5<sup>A7</sup>*.

984  
985 **Supplemental Video 3 (related to Figure 3). Dynamics of centromere clusters in 8-**  
986 **cell cyst nuclei in *WT*.** Time lapse microscopy (spinning disc) expressing the  
987 centromere CID::RFP (magenta) and fusome marker Par-1::GFP (driven by the *nanos*  
988 promoter) (green). Frames were taken every 10 seconds.

989

990 **Supplemental Video 4 (related to Figure 3). Dynamics of centromere clusters in 8-**  
991 **cell cyst nuclei in *Mcm5*<sup>A7</sup>.** Time lapse microscopy (spinning disc) expressing the  
992 centromere CID::RFP (magenta) and fusome marker Par-1::GFP, (driven by the *nanos*  
993 promoter) (green). Frames were taken every 10 seconds.

994

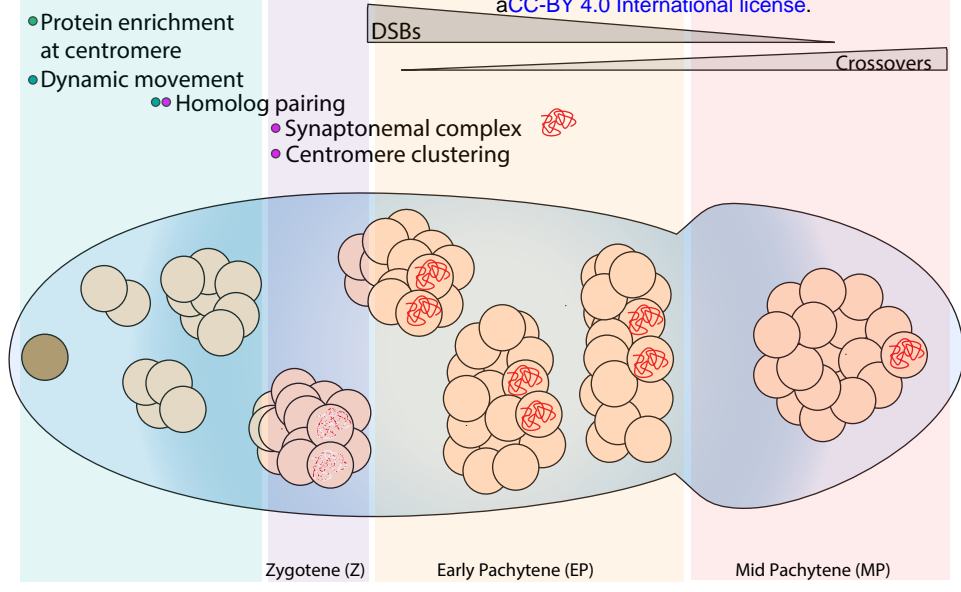
995 **Supplemental Video 5 (related to Figure 3). Dynamics of one centromere cluster in**  
996 **one 8-cell cyst nucleus in *WT*.** Time lapse microscopy (spinning disc) expressing the  
997 centromere CID::RFP (magenta) in one nucleus within an 8-cell cyst (dotted circle).  
998 Frames were taken every 10 seconds.

999

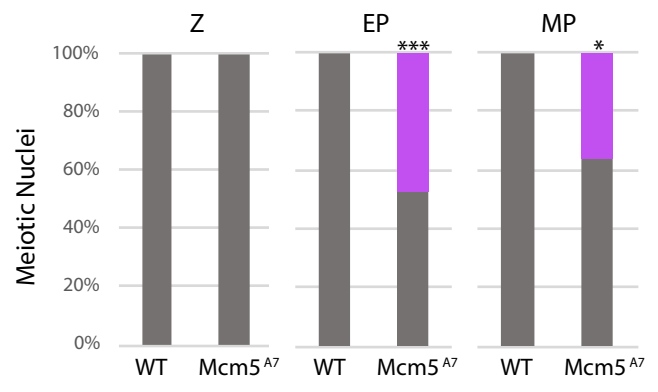
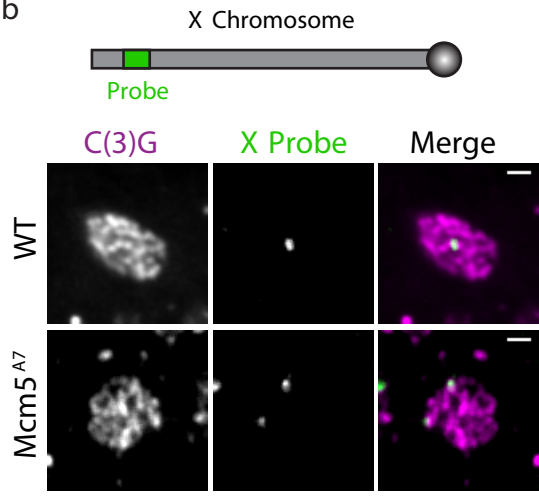
1000 **Supplemental Video 6 (related to Figure 3). Dynamics of one centromere cluster in**  
1001 **one 8-cell cyst nucleus in *Mcm5*<sup>A7</sup>.** Time lapse microscopy (spinning disc) expressing  
1002 the centromere CID::RFP (magenta) in one nucleus within an 8-cell cyst (dotted circle).  
1003 Frames were taken every 10 seconds.

1004

1005



b



c

