1   Shugoshin is essential for meiotic prophase checkpoints in *C. elegans*

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21   **Abstract**

22

23   The conserved factor Shugoshin is dispensable in *C. elegans* for the two-step loss of sister

24   chromatid cohesion that directs the proper segregation of meiotic chromosomes. We show that

25   the *C. elegans* ortholog of Shugoshin, SGO-1, is required for checkpoint activity in meiotic

26   prophase. This role in checkpoint function is similar to that of the meiotic chromosomal protein,

27   HTP-3. Null *sgo-1* mutants exhibit additional phenotypes similar to that of a partial loss of

28   function allele of HTP-3: premature synaptonemal complex disassembly, the activation of

29   alternate DNA repair pathways and an inability to recruit a conserved effector of the DNA

30   damage pathway, HUS-1. SGO-1 localizes to pre-meiotic nuclei, when HTP-3 is present but not

31   yet loaded onto chromosome axes, suggesting an early role in regulating meiotic chromosome

32   metabolism. We propose that SGO-1 acts during pre-meiotic replication to ensure fully

33   functional meiotic chromosome architecture, rendering these chromosomes competent for

34   checkpoint activity and normal progression of meiotic recombination. Given that most research

35   on Shugoshin has been focused on its regulation of sister chromatid cohesion in meiosis, this

36   novel role may be conserved but previously uncharacterized in other organisms. Further, our

37   findings expand the repertoire of Shugoshin's functions beyond coordinating regulatory activities

38   at the centromere.

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40

41   **Introduction**

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43   Sexually reproducing organisms rely on the specialized cell division, meiosis, to generate

44   haploid gametes, such as sperm and eggs, so that diploidy is restored upon fertilization. To

45   promote proper disjunction of meiotic chromosomes, homologs undergo a series of

46   progressively intimate interactions during meiotic prophase. Chromosomes identify their unique

47   homolog, pair, and stabilize pairing via the assembly of the synaptonemal complex (SC) in a

48   process called synapsis. Interhomolog crossover recombination occurs in the context of

49   synapsis to produce linkages, or chiasmata, that direct meiotic chromosome segregation

50   (reviewed in [1]). Defects in pairing, synapsis or recombination can produce errors in meiotic

51   chromosome segregation and gametes with too few or too many chromosomes, also referred to

52   as aneuploidy. Fertilization of these defective gametes generates aneuploid embryos, which are

53   often inviable. It is estimated that ~30% of miscarriages are the result of aneuploidy [2] and

54   many developmental disorders, such as Down or Klinefelter's syndromes, are the product of

55   aneuploidy.

56

57   Meiotic chromosomes are structured by a variety of proteins so that they are competent for

58   pairing, synapsis and interhomolog recombination. These include the cohesin complex, which

59   mediates sister chromatid cohesion, and axis component proteins that assemble the linear axes

60   of the SC (reviewed in [3]). In addition, cohesin and axis components are involved in meiotic

61   prophase checkpoints that respond to errors by either stalling meiotic prophase progression or

62   activating apoptosis to remove defective meiocytes [4-10]. A subset of these proteins, identified

63   by a conserved domain called the HORMA domain, adopt structures reminiscent of the spindle

64   checkpoint effector, Mad2, suggesting that meiotic HORMA domain containing proteins

65   (HORMADs) might also control meiotic checkpoint signaling through the adoption of multiple

66   conformations [11, 12]. In budding yeast there is a single meiotic HORMAD (Hop1) [13], in mice

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67   there are two (HORMAD1 and 2) [14] and in *C. elegans* there are four (HTP-3, HIM-3, HTP-1

68   and HTP-2) [15-18]. Why this family has expanded so dramatically in *C. elegans* is unknown.

69

70   To halve the chromosome complement, meiosis is composed of two rounds of chromosome

71   segregation: meiosis I, in which homologous chromosomes segregate, and meiosis II, in which

72   sister chromatids segregate. This segregation scheme necessitates a two-step loss of sister

73   chromatid cohesion. Cohesin is removed distal to chiasmata to allow homologs to segregate

74   during meiosis I while being partially maintained to enable sister chromatids to partition correctly

75   during meiosis II. In organisms with point centromeres, this sequential loss of cohesion is

76   regulated by Shugoshin [19]. Shugoshin protects cohesin at the centromere until meiosis II by

77   recruiting the conserved phosphatase, PP2A, to antagonize the phosphorylation and removal of

78   the cohesin complex [20, 21]. Some organisms, such as *C. elegans*, do not have a localized

79   centromere. In this model organism, the two-step loss of cohesin is accomplished through an

80   alternate regulatory mechanism that involves the ordered, asymmetric disassembly of SC

81   components around a single crossover site in late meiotic prophase [22-24]. Further, attempts to

82   attribute a meiotic role to the worm ortholog of Shugoshin, SGO-1, have been unsuccessful [22].

83

84   We report here that SGO-1 is essential for checkpoint function in meiotic prophase in *C.*

85   *elegans.* A hypomorphic mutant allele of *sgo-1* abrogates the synapsis checkpoint that monitors

86   whether homologous chromosomes have synapsed, while a null mutation abrogates both the

87   synapsis checkpoint and the DNA damage response (DDR). However, unlike other

88   characterized synapsis checkpoint components, SGO-1 does not inhibit synapsis, indicating it

89   acts in an alternate pathway. Instead, SC disassembly is accelerated in null *sgo-1* mutants (*sgo-*

90   *1[0])*). Both this phenotype and the requirement for SGO-1 in both meiotic checkpoints are

91   reminiscent of phenotypes displayed by a partial loss of function mutation in the conserved

92   chromosome axis component and meiotic HORMA domain containing protein, HTP-3. Indeed,

93   similar to HTP-3, SGO-1 is also required for preventing the activation of alternate DNA repair

94   pathways during meiosis and the ability to recruit conserved effectors of the DDR, such as HUS-

95   1, suggesting a role in meiotic axis morphogenesis. Consistent with an early role in this process,

96   SGO-1 localizes to pre-meiotic nuclei that express HTP-3 but have not yet assembled

97   chromosome axes. We propose that SGO-1 contributes to checkpoint functionality and the

98   proper progression of meiotic recombination through its regulation of meiotic chromosome

99   architecture, potentially through its role as a regulator of cohesins, and suggest that this role

100   may be conserved.

101

102   **Results**

103

104   **SGO-1 is required for both the synapsis checkpoint and the DNA damage response**

105

106   Previous experiments showed that a hypomorphic allele of *sgo-1*, *sgo-1(tm2443)*, resulted in

107   low levels of chromosome segregation defects during meiosis [22], suggesting that SGO-1

108   might play a role in meiotic checkpoint function. The *sgo-1(tm2443)* allele results in a frameshift

109   at amino acid 157 to generate a stop codon after 30 out-of-frame codons (Figure 1A) [25]. This

110   results in a truncated protein product that is expressed at levels similar to that of full-length

111   SGO-1 in wildtype animals (Figure 1B). We tested whether SGO-1 is required for meiotic

112   prophase checkpoints by introducing this allele into *syp-1* mutants (Figure 1A). SYP-1 is

113   required for SC assembly, and in its absence, homologous chromosomes fail to synapse and

114   undergo meiotic recombination [26]. As a result, *syp-1* mutants activate two meiotic

115   checkpoints, the synapsis checkpoint and the DDR, which produces high germline apoptosis

116   (Figure 1C and D) [27]. *syp-1;sgo-1(tm2443)* double mutants had reduced germline apoptosis,

117   suggesting inactivation of either the synapsis checkpoint or the DDR (Figure 1D). Since CEP-1

118   is required for DDR-induced germline apoptosis [28, 29], elevated apoptosis in *syp-1;cep-1*

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119   double mutants is strictly due to synapsis checkpoint activity. To test whether *sgo-1* was a

120   synapsis checkpoint component, we generated *syp-1;cep-1;sgo-1(tm2443)* triple mutants. *syp-*

121   *1;cep-1;sgo-1(tm2443)* triple mutants had wildtype levels of germline apoptosis when compared

122   to *syp-1;cep-1* double mutants (Figure 1D). This indicates that SGO-1 acts in the synapsis

123   checkpoint and more specifically, the region lost in *sgo-1(tm2443)* mutants is required for its

124   function in the synapsis checkpoint (Figure 1A).

125

126   To verify this, we introduced the *sgo-1(tm2443)* mutant allele into *meDf2* mutants. *meDf2* is a

127   deletion of the X chromosome Pairing Center (PC) [30], which is required for pairing, synapsis

128   and the synapsis checkpoint [27, 31]. Although *meDf2* homozygotes fail to synapse X

129   chromosomes due to the absence of PCs, they also cannot signal to the synapsis checkpoint,

130   instead activating apoptosis via the DDR [27]. In contrast, the presence of an active PC on

131   unsynapsed X chromosomes in *meDf2* heterozygotes (*meDf2/+*) produces elevated apoptosis

132   via the synapsis checkpoint but not the DDR (Figure S1A) [27]. Consistent with *sgo-1(tm2443)*

133   mutants specifically abolishing the synapsis checkpoint and not the DDR, *meDf2;sgo-1(tm2443)*

134   double mutants had similar levels of apoptosis as *meDf2* single mutants while apoptosis was

135   reduced in *meDf2/+;sgo-1(tm2443)* double mutants in comparison to *meDf2/+* single mutants

136   (Figure S1B).

137

138   We wondered if null mutations in *sgo-1* would produce similar results. Therefore, we introduced

139   a stop codon by CRISPR/Cas9 genome editing technology 63 base pairs after the start of the

140   *sgo-1* gene (Figure 1A) and verified that these mutants did not produce SGO-1 protein (Figure

141   1B). We introduced this null mutation, *sgo-1(0)*, into *syp-1* mutants and were surprised to find

142   that *syp-1;sgo-1(0)* double mutants exhibited wildtype levels of apoptosis (Figure 1E), indicating

143   that SGO-1 function is required for both meiotic checkpoints. Consistent with this analysis, the

144   *sgo-1(0)* mutant allele also reduced apoptosis in both *meDf2* homozygotes and heterozygotes

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145   (Figure S1C). Thus, when SGO-1 function is completely abrogated, both the synapsis

146   checkpoint and the DDR are inactive.

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148   **SGO-1 regulates meiotic checkpoint function independent of spindle checkpoint**

149   **components and PCH-2**

150

151   We previously identified additional genes that are required for the synapsis checkpoint and

152   showed that they inhibit synapsis in two independent pathways [32]. One pathway involves the

153   microtubule motor, dynein, which is essential for synapsis in *C. elegans* [33]. We previously

154   demonstrated that spindle checkpoint genes, Mad1, Mad2 and Bub3, enforce this requirement

155   for dynein: loss of function mutations in these spindle checkpoint genes restore synapsis when

156   dynein function is knocked down, potentially implicating these factors in a tension-sensing

157   mechanism at PCs [4]. Shugoshin has been shown to respond to changes in tension at

158   centromeres, specifically during biorientation of chromosomes on mitotic or meiotic spindles [34-

159   38]. Further, in humans, mice and Xenopus, Shugoshin interacts directly with Mad2 [39, 40],

160   suggesting that SGO-1 may act with Mad1, Mad2 and Bub3 to regulate the synapsis

161   checkpoint. We tested whether SGO-1 may also be involved in tension-sensing during synapsis

162   by performing RNA interference against the gene that encodes dynein light chain (*dlc-1*) in

163   wildtype, *sgo-1(tm2443)*, *sgo-1(0)* and *mad-1 (mdf-1* in *C. elegans*) null mutants *(mad-1[0])*. To

164   visualize synapsis in these mutants, we performed immunofluorescence against the SC

165   components HTP-3 and SYP-1 (Figure 2A). 76% of germlines from *dlc-1^{RNAi}* animals exhibited

166   asynapsis (Figure 2B), visible as meiotic chromosomes with HTP-3 but devoid of SYP-1 (see

167   dashed line in Figure 2A). *mad-1(0);dlc-1^{RNAi}* worms suppressed asynapsis in *dlc-1^{RNAi}* animals

168   and significantly reduced the percentage of germlines with asynapsis to 24% (Figure 2B) [4]. By

169   contrast and similar to *dlc-1^{RNAi}* animals, both *sgo-1(tm2443);dlc-1^{RNAi}* and *sgo-1(0);dlc-1^{RNAi}*

170   worms (Figure 2A and data not shown) had 75% and 74%, respectively, of germlines with

171   unsynapsed chromosomes (Figure 2B), indicating that SGO-1 does not monitor or regulate

172   meiotic synapsis in the same pathway as Mad1, Mad2 or Bub3.

173

174   The second pathway that inhibits synapsis involves the conserved ATPase, PCH-2 [32]. Similar

175   to mutation of SGO-1, loss of PCH-2 does not suppress the defect in synapsis observed when

176   dynein activity is knocked down [4]. However, loss of PCH-2 rescues the defect in synapsis

177   observed in *meDf2* heterozygotes (*meDf2/+*), suggesting that PCH-2 inhibits synapsis from non-

178   PC sites [32]. We evaluated synapsis in *meDf2/+, meDf2/+;sgo-1(tm2443)* and *meDf2/+;sgo-*

179   *1(0)* mutants (Figure 2C and data not shown). We took advantage of the spatio-temporal

180   organization of meiotic nuclei in the germline, dividing the germline into six equivalently sized

181   zones (see cartoon in Figure 2D), and quantified the percentage of nuclei that had competed

182   synapsis. We could not detect any effect on the progression of synapsis in the double mutants

183   (Figure 2D, zones 2 and 3). Instead, we observed what appeared to be more rapid SC

184   disassembly in *meDf2/+;sgo-1(0)* mutants (Figure 2D, zones 5 and 6).

185

186   In addition to its effect on synapsis, loss of PCH-2 stabilizes pairing intermediates [32]. We

187   hypothesize that this stabilization of pairing, particularly at PCs, satisfies the synapsis

188   checkpoint in *pch-2;syp-1* double mutants [32]. To assay pairing, we localized the X

189   chromosome PC protein, HIM-8, in *syp-1* mutants (Figure S2A), which allows us to visualize

190   pairing intermediates in the absence of synapsis [26, 41]. We then quantified the percentage of

191   meiotic nuclei that had a single HIM-8 focus, indicating that X chromosomes had paired, as a

192   function of meiotic progression in *syp-1* single mutants as well as *syp-1;sgo-1(tm2443)* and *syp-*

193   *1;sgo-1(0)* double mutants (Figure S2B). Unlike what we observe in *pch-2;syp1* mutants [32],

194   the progression of pairing in *syp-1;sgo-1(tm2443)* and *syp-1;sgo-1(0)* double mutants was

195   indistinguishable from *syp-1* single mutants (Figure S2B). Altogether, these data suggest that

196   SGO-1 also does not act in the same pathway as PCH-2. Therefore, SGO-1 identifies a third,

197   alternate pathway that regulates synapsis checkpoint function.

198

199   **A null mutation in *sgo-1* resembles a partial loss of function allele in the meiotic**

200   **HORMAD, HTP-3**

201

202   To determine whether loss of SGO-1 had any effect on synapsis, we monitored synapsis in

203   wildtype worms and *sgo-1* single mutants (Figure 3A) as a function of meiotic progression

204   (Figure 3B), similar to our experiment in Figure 2D. Unlike *mad-1*, *bub-3* or *pch-2* mutants [4,

205   32], we did not detect an acceleration of SC assembly (Figure 3B, zones 2 and 3). Instead,

206   similar to what we observed in *meDf2/+;sgo-1(0)* double mutants (Figure 2D), we observed that

207   SC disassembly was slightly more rapid in *sgo-1(tm2443)* and significantly more rapid in *sgo-*

208   *1(0)* mutants than wildtype (see unsynapsed chromosomes in *sgo-1(tm2443)* and *sgo-1(0)* in

209   Figure 3A and Figure 3B, zones 5 and 6).

210

211   This phenotype reminded us of the reported phenotype of a partial loss of function mutant allele

212   of the meiotic HORMAD, *htp-3*, *htp-3*^{H96Y} (Figures 3A and B) [43]. This mutation converts a

213   histidine at position 96 of the HTP-3 protein to a tyrosine. This amino acid lies in the HORMA

214   domain and is not conserved but resides next to two invariant residues shared between the four

215   meiotic HORMA domain containing proteins in *C. elegans* (HTP-3, HIM-3, HTP-1, and HTP-2),

216   suggesting it might affect HORMA domain function. Given that *sgo-1* mutants resemble *htp-*

217   *3*^{H96Y} mutants in the context of SC disassembly (Figure 3B), and we showed that a subset of

218   meiotic HORMADs are required for checkpoint-induced germline apoptosis [42], we tested what

219   effect this allele had on meiotic checkpoint activation by introducing it into *syp-1* mutants. We

220   found that mutation of the HORMA domain abolished both the synapsis checkpoint and the

221   DDR (Figure 3C), similar to null mutations in *htp-3*, *him-3* [42] and *sgo-1* (Figure 1D). Thus, both

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222   *htp-3*^{H96Y} and *sgo-1(0)* mutants abrogate meiotic checkpoint function and prematurely

223   disassemble the SC, suggesting they act in the same pathway.

224

225   **SGO-1 limits non-homologous DNA repair and promotes crossover assurance**

226

227   HTP-3^{H96Y} also affects the progression of DNA repair [43] by inappropriately activating non-

228   homologous DNA repair mechanisms. We tested the role of SGO-1 in meiotic recombination.

229   We focused these experiments on the null mutation of *sgo-1*, since this allele also affected the

230   DDR and exhibited additional phenotypes that most closely resembled *htp-3*^{H96Y} mutants

231   (Figures 1E and 3).

232

233   First, we monitored the progression of DNA repair. For this experiment, we performed

234   immunofluorescence against the DNA repair factor RAD-51 (Figure 4A). RAD-51's appearance

235   on meiotic chromosomes indicates the formation of double strand breaks and its disappearance

236   shows entry into a DNA repair pathway [44]. When we follow the dynamics of RAD-51

237   appearance and disappearance in wildtype and *sgo-1(0)* single mutants, they are exceedingly

238   similar (Figure 4B). However, when we did this experiment in the *syp-1* mutant background, in

239   which the inability to synapse prevents DNA repair from using a homologous chromosome as a

240   template [44], we saw that *syp-1;sgo-1(0)* double mutants had sharply reduced average number

241   of RAD-51 foci, particularly in zone 5, suggesting these double mutants repair double strand

242   breaks more rapidly than *syp-1* single mutants (Figure 4B).

243

244   DSB-1 and DSB-2 localize to chromosomes and are required for the formation of double strand

245   breaks. When meiosis is defective, DSB-1 and DSB-2 remain on chromosomes [45, 46] and

246   their persistence depends on the recruitment of a subset of meiotic HORMADs to chromosomes

247   [5]. To determine whether the reduction in RAD-51 foci in *syp-1;sgo-1(0)* double mutants is

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248   because *sgo-1(0)* mutants fail to activate this meiotic feedback mechanism, we visualized DSB-

249   1 and DSB-2 in *syp-1* and *syp-1;sgo-1(0)* double mutants and could detect no difference in their

250   staining pattern (data not shown). From these data, we conclude that SGO-1 typically prevents

251   the activation of alternate DNA repair mechanisms, such as using the sister chromatid as a

252   repair template, to promote homologous DNA repair.

253

254   We reasoned that this effect on DNA repair might have consequences on crossover formation.

255   We monitored crossover formation by evaluating both GFP::COSA-1 localization and bivalent

256   formation. COSA-1 localizes to presumptive crossovers in late meiotic prophase (Figure 4C).

257   The six pairs of chromosomes in *C. elegans* exhibit crossover assurance, in which every pair of

258   chromosomes has at least one crossover, and strict crossover control, in which every pair of

259   chromosomes enjoys only a single crossover. As a result, we observe 6 GFP::COSA-1 foci in

260   greater than 98% of meiotic nuclei in wildtype animals (Figure 4B). *sgo-1(0)* mutants show a

261   significant increase (p value < 0.01, Fisher's exact test) in nuclei with five GFP::COSA-1 foci,

262   indicating a subtle loss of crossover assurance (Figure 4D). Interestingly, we could not detect a

263   loss of crossover assurance in *htp-3*^{H96Y} mutants (Figure 4D), suggesting that either the

264   requirement for SGO-1 in regulating meiotic DNA repair might be stronger than that of a single

265   meiotic HORMAD or HTP-3^{H96Y} might still retain some activity.

266

267   Next, we assessed bivalent formation. In wildtype nuclei and *htp-3*^{H96Y} mutants, all chromosome

268   pairs are linked by chiasmata in late meiotic prophase and we always see 6 DAPI stained

269   bodies (Figure 4E, top image). We observed non-recombinant chromosome pairs, or univalents

270   (Figure 4E, bottom image), in *sgo-1(0)* mutants in 3% of meiotic nuclei in late prophase (p value

271   < 0.05, Fisher's exact test), verifying the subtle loss of crossover assurance in this mutant

272   background (Figures 4D and E).

273

274 **SGO-1 promotes the recruitment of HUS-1::GFP to sites of DNA damage**

275

276 Given the effect that loss of SGO-1 has on meiotic DNA repair and recombination, we wondered

277 if SGO-1's role in the DDR could be involved in recruiting early DDR components. An early

278 event in DDR is the recruitment of the conserved 9-1-1 complex, which includes the factors

279 MRT-2 (the *C. elegans* Rad1 ortholog), HPR-9 (the *C. elegans* Rad9 ortholog) and HUS-1, to

280 sites of damage [47, 48]. To visualize recruitment of the 9-1-1 complex, we localized HUS-

281 1::GFP in wildtype, *sgo-1(0)*, *htp-3^{H96Y}*, *syp-1*, *syp-1;sgo-1(0)* and *syp-1;htp-3^{H96Y}* mutants

282 (Figure 5A). Wildtype meiotic nuclei had very few HUS-1::GFP foci (Figure 5B). Both *sgo-1(0)*

283 and *htp-3^{H96Y}* single mutants exhibited slightly more HUS-1::GFP foci (Figure 5B), indicating that

284 the DDR is weakly active in these backgrounds despite normal levels of apoptosis (Figures 1E

285 and 3C). This may reflect the inappropriate activation of non-homologous DNA repair in these

286 mutants, despite the apparent normal progression of DNA repair ([43] and Figure 4E). Meiotic

287 nuclei in *syp-1* single mutants displayed many more HUS-1::GFP foci (Figure 5B). By contrast,

288 we observed a sharp reduction in the average number of HUS-1::GFP foci in *syp-1;sgo-1(0)* and

289 *syp-1;htp-3^{H96Y}* double mutants (Figure 5B), albeit not to the average numbers we observed in

290 the single mutant backgrounds. This defect in the ability to recruit HUS-1::GFP is entirely

291 consistent with the reduction in DDR-induced apoptosis we also detected in these double

292 mutants (Figures 1E, S1C and 3C). Thus, SGO-1 is required to robustly recruit components of

293 the 9-1-1 complex, acting early in the meiotic DDR.

294

295 **SGO-1 localizes to premeiotic and late meiotic prophase nuclei**

296

297 We localized the SGO-1 protein in the hermaphrodite germline. To our surprise its staining was

298 limited to nuclei just prior to entry into meiotic prophase, which are often defined as pre-meiotic,

299 and in late meiotic prophase (Figure 6A). HTP-3 was also present in these pre-meiotic nuclei

12

300   but was not yet visibly assembled into chromosome axes, suggesting that SGO-1 may be

301   regulating early events in axis morphogenesis. Upon the appearance of discrete HTP-3 axes in

302   early prophase nuclei, SGO-1 protein was conspicuously absent (Figure 6B). When the SC

303   undergoes ordered disassembly in diplotene of meiotic prophase, SGO-1 reappears in meiotic

304   nuclei (Figure 6C). This localization pattern was unchanged in *sgo-1(tm2443)* mutants and

305   absent in *sgo-1(0)* mutants (data not shown).

306

307   **Discussion**

308

309   The phenotypes we have characterized when Shugoshin function is compromised or abolished

310   in *C. elegans* are highly reminiscent of mutations in cohesin or other meiotic axis components,

311   namely the inability to activate meiotic checkpoints [4-10], the loss of crossover assurance, and

312   the activation of homolog-independent, presumably sister chromatid-dependent, DNA repair

313   mechanisms (reviewed in [3]). Indeed, we show that *sgo-1* null mutants resemble a loss of

314   function mutation in the meiotic axis component and HORMAD protein, HTP-3 [43]. Despite

315   being dispensable for normal pairing and synapsis in *C. elegans* (Figures S2 and 3B), we

316   propose that SGO-1 is required to generate meiotic chromosome architecture competent for

317   checkpoint activation and the normal progression of meiotic recombination (Figure 7). Further,

318   we hypothesize that this role is conserved but unappreciated given the focus on Shugoshin's

319   role in regulating two-step loss of sister chromatid cohesion during meiotic chromosome

320   segregation. The requirement for Shugoshin in maintaining meiotic synapsis in rice, a

321   phenotype startlingly similar to the premature SC disassembly we detect in *sgo-1* mutants,

322   strongly supports this possibility [49]. More importantly, our findings expand the repertoire of

323   Shugoshin's functions in controlling chromosome segregation beyond being a platform or

324   adapter protein at centromeric regions.

325

13

326   Given our proposal that SGO-1 acts in the same pathway as meiotic HORMADs for meiotic

327   checkpoint function, we were surprised to see that *sgo-1* mutants did not resemble *pch-2*

328   mutants (Figures 2C, 2D, S2 and 3B). In budding yeast and mice, PCH-2, and its mammalian

329   ortholog TRIP13, regulate meiotic HORMADs in a feedback mechanism that signals proper

330   meiotic progression [7, 10, 14, 50]. This discrepancy may be because meiotic HORMADs

331   regulate meiotic checkpoint function through multiple mechanisms, one involving PCH-2 and

332   one involving SGO-1. Further, our identification of at least three separate pathways that trigger

333   germline apoptosis in response to defects in synapsis [4, 42, this study] demonstrates the

334   stringency of the synapsis checkpoint in *C. elegans*, presenting a marked contrast to oogenesis

335   in mammalian cells [51]. This stringency may reflect the importance of synapsis to the

336   generation of chiasma [44] or the regulation of germline apoptosis in *C. elegans*.

337

338   The localization of SGO-1 in the pre-meiotic zone corresponds to when the meiosis specific

339   cohesin complex defined by the kleisin, REC-8, is loaded onto meiotic chromosomes [52] and

340   pre-meiotic replication occurs. However, it precedes when the meiosis specific cohesin complex

341   defined by the kleisins, COH-3/COH-4, is loaded onto meiotic chromosomes [53]. Therefore, we

342   suggest that SGO-1's role in promoting fully functional chromosome axes is likely associated

343   with pre-meiotic replication and involves cohesin, specifically cohesin complexes defined by

344   REC-8. In addition to SGO-1's localization, this is based on its characterized role regulating

345   sister chromatid cohesion during meiotic chromosome segregation [19-21], the reported

346   biochemical interaction between meiotic HORMADs and cohesin [11, 54] and the observation

347   that complete loss of cohesin function also affects the ability to recruit HUS-1::GFP [6].

348   Unfortunately, we could not detect any obvious defects in the localization of REC-8, COH-3 or

349   COH-4 in *sgo-1(0)* mutants (data not shown). Shugoshin also regulates additional factors

350   required for chromosome structure and function, such as condensin [55, 56], raising the

351   possibility that Shugoshin's effect on meiotic prophase events occurs through factors in addition

352   to or independent of cohesin.

353

354   SGO-1's localization is similar to that of the cohesin regulator WAPL-1, whose early localization

355   during pre-meiotic replication also affects meiotic axis structure [57]. Based on this

356   colocalization and reports that Shugoshin and Wapl may antagonize each other [58], we tested

357   whether we could detect a genetic interaction between mutations in *wapl-1* and *sgo-1*,

358   specifically whether loss of *sgo-1* would suppress the reduction in meiotic axis length observed

359   in *wapl-1* mutants. However, meiotic chromosomes in *wapl-1;sgo-1* double mutants resembled

360   those in *wapl-1* single mutants (data not shown), suggesting that these two factors appear not to

361   antagonize each other when regulating meiotic axis structure.

362

363   The *tm2443* mutant allele acts as a separation of function allele (Figures 1 and S1), indicating

364   that SGO-1's role in the synapsis checkpoint and the DDR are separable. Two functional

365   portions of SGO-1 are absent in the protein produced by the *tm2443* allele and are potentially

366   essential for the synapsis checkpoint but dispensable for the DDR. These include the highly

367   conserved basic "SGO motif," which mediates binding to histone H2A phosphorylated by the

368   conserved cell cycle kinase and spindle checkpoint component Bub1 [59]. Given our interest in

369   roles for spindle checkpoint components in regulating and monitoring meiotic synapsis [4],

370   testing whether Bub1 and its kinase activity are required for the synapsis checkpoint is a current

371   focus. The middle section of the protein that is highly divergent in both length and sequence is

372   also missing in the protein generated by the *tm2443* mutant allele. This region appears to

373   mediate interactions with a wide array of proteins, including cohesin, Mad2 and the microtubule

374   motor, MCAK [39, 40, 58, 60]. Considering that loss of *sgo-1* fails to suppress the synapsis

375   defect when the microtubule motor dynein is knocked down (Figures 2A and B), unlike loss of

376   Mad2 [4], and that SGO-1 is enriched in the nucleus (Figure 6), where microtubules are not

15

377   present [33], we think it unlikely that either interaction with Mad2 or MCAK explains SGO-1's

378   function in the synapsis checkpoint. This region is also subject to phospho-regulation by

379   important cell cycle kinases in some organisms (reviewed in [61]), raising the possibility that

380   regulation of this portion of Shugoshin contributes to its synapsis checkpoint role. The N-

381   terminal coiled-coil region, which has been shown to promote dimerization [62] as well as

382   interact with both PP2A phosphatase [63] and the chromosome passenger complex (CPC) [64],

383   may be required for Shugoshin's role in the DDR as well as in regulating meiotic recombination.

384   However, the conserved kinase Aurora B, a component of the CPC, is prevented from

385   interacting with meiotic chromosomes in *C. elegans* to promote sister chromatid cohesion [65]

386   and is not required for either the synapsis checkpoint or the DDR (data not shown), ruling out

387   that an interaction with this complex explains SGO-1's contribution to meiotic axis function. No

388   role for PP2A in meiotic prophase has been reported but it's possible a role early in meiosis has

389   been overlooked, similar to Shugoshin's. Additional domain analysis and identification of

390   Shugoshin's meiotic interactors in *C. elegans* will determine how Shugoshin manages its

391   multiple roles during meiotic prophase.

392

393   The premature disassembly of the SC in both *sgo-1* null mutants and *htp-3*^{H96Y} mutants provides

394   a potential opportunity to reconcile what previously appeared to be disparate observations in

395   multiple meiotic systems. The stability of axis and SC components on meiotic chromosomes is

396   tightly controlled and linked to the progression of meiotic recombination. In budding yeast and

397   mice, this includes the Pch2/Trip13-dependent redistribution or removal of meiotic HORMADs

398   from chromosomes as chromosomes synapse [14, 50, 66]. Given the multiple roles meiotic

399   HORMADs play during prophase, this redistribution or removal likely accomplishes three things:

400   1) it prevents additional double strand breaks [7, 67, 68]; 2) it allows any remaining double

401   strand breaks to be repaired using the sister chromatid as a template or by mitotic-like

402   mechanisms [69-72]; and 3) it signals the proper progression of meiotic prophase [7-10]. In

16

403   budding yeast, central element components of the SC also undergo turnover, but it is limited to

404   regions associated with meiotic recombination [73].

405

406   In *C. elegans*, relocalization or redistribution of meiotic HORMADs does not occur until SC

407   disassembly. However, several reports have highlighted how the SC becomes more stable later

408   in meiotic prophase before undergoing ordered disassembly (see Figure 7) [74-77]. This stability

409   relies on the presence of a crossover-specific intermediate in cis [74, 76]: chromosomes that fail

410   to undergo crossover recombination disassemble their SCs prematurely, similar to *sgo-1* null

411   and *htp-3^{H96Y}* mutants (Figures 3A, B and [43]). Since this portion of meiotic prophase coincides

412   with a loss of homolog access during DNA repair [78] and a release from meiosis-specific DNA

413   repair mechanisms [79], it seems likely that some modification of axis components also occurs

414   during this period of meiotic prophase. We suggest that this modification may contribute to SC

415   stabilization, and its eventual ordered disassembly, potentially analogous to the remodeling of

416   meiotic HORMADs in budding yeast and mice. SC disassembly is accelerated in *sgo-1* null and

417   *htp-3^{H96Y}* mutants despite the presence of crossover-specific recombination intermediates

418   (Figures 3A, B and [43]), suggesting that a fully functional meiotic axis is important for this

419   stabilization (Figure 7). SC disassembly is delayed in *C. elegans pch-2* mutants, implicating this

420   factor in the process, analogous to yeast and mammals [32]. We speculate that this remodeling

421   manifests itself differently in *C. elegans* than in yeast or mice because *C. elegans* relies on

422   synapsis for early events in meiotic recombination, such as ZHP-3 recruitment [80], and uses

423   axis components, including meiotic HORMADs, to direct the two step loss of sister chromatid

424   cohesion [22, 23].

425

426   Materials and Methods

427

428   Genetics and worm strains

429   The wildtype *C. elegans* strain background was Bristol N2 [81]. All experiments were performed

430   on adult hermaphrodites at 20°C under standard conditions unless otherwise stated. Mutations

431   and rearrangements used were as follows:

432   LG I: *mnDp66*, *cep-1(gk138), htp-3(vc75), hus-1(op241)*

433   LG II: *meIs8 [Ppie-1::GFP::cosa-1 + unc-119(+)]*

434   LG IV: *sgo-1(tm2443), sgo-1(blt2), nT1[unc-?(n754) let-?(m435)] (IV, V), nTI [qIs51]*

435   LG V: *syp-1(me17)*, *mad-1(gk2), dpy-11(e224), bcIs39 [lim-7p::ced-1::GFP + lin-15(+)]*

436   LG X: *meDf2*

437   *opIs34 [Phus-1::hus-1::GFP + unc-119(+)]*

438

439   *meDf2* is a terminal deficiency of the left end of the X chromosome that removes the X

440   chromosome PC as well as numerous essential genes [30]. For this reason, homo- and

441   hemizygous *meDf2* animals also carry a duplication (*mnDp66*) that includes these essential

442   genes but does not interfere with normal X chromosome segregation [82] or synapsis

443   checkpoint signaling [27]. For clarity, it has been omitted from the text.

444

445   The *sgo-1* null allele *(sgo-1[0])*, *blt2*, was created by CRISPR-mediated genomic editing as

446   described in [83, 84]. pDD162 was mutagenized using Q5 mutagenesis and oligos

447   TAAAACTGCAGCATGTGCCGTTTTAGAGCTAGAAATAGCAAGT and

448   CAAGACATCTCGCAATAGG. The resulting plasmid was sequenced and three different correct

449   clones (50ng/ul total) were mixed with pRF4 (120ng/ul) and the repair oligo

450   ATTTGTATTTTACACATAAACTTTGTAAATATAATAATACCTTCTTTAGAGCTAGCTTGGTCG

451   TTTTTTTGCTGCTACAATTCCTCCAAAAATAGATTGTGCAGTTT (30ng/ul). Wildtype worms

452   were picked as L4s, allowed to age 15-20 hours at 20°C and injected with the described mix.

453   Worms that produced rolling progeny were identified and F1 rollers, as well as their wildtype

454   siblings, were placed on plates seeded with OP50, 1-2 rollers per plate and 6-8 non-rolling

455   siblings per plate, and allowed to produce progeny. PCR and NheI digestions were performed

456   on these F1s to identify worms that contained the mutant allele and individual F2s were picked

457   to identify mutant homozygotes. Multiple homozygotes carrying the *sgo-1(blt2)* mutant allele

458   were backcrossed against wildtype worms at least three times and analyzed to determine

459   whether they produced the same mutant phenotype.

460

461   Quantification of Germline Apoptosis

462   Scoring of germline apoptosis was performed as previously descried in [27] with the following

463   exceptions. L4 hermaphrodites were allowed to age for 22 hours. They were then mounted

464   under coverslips on 1.5% agarose pads containing 0.2mM levamisole for wildtype moving

465   strains or 0.1mM levamisole for *dpy-11* strains. A minimum of twenty-five germlines were

466   analyzed for each genotype.

467

468   Antibodies, Immunostaining and Microscopy

469   DAPI staining and immunostaining was performed as in [27] 20 to 24 hours post L4 stage.

470   Primary antibodies were as follows (dilutions are indicated in parentheses): rabbit anti-SYP-1

471   (1:500) [26], chicken anti-HTP-3 (1:1000) [31], guinea pig anti-HIM-8 (1:250)[41], rabbit anti-

472   SGO-1 (1:30,000) [25], mouse anti-GFP (1:100) (Invitrogen) and rabbit anti-RAD-51 (1:5000)

473   [85]. Secondary antibodies were Cy3 anti-rabbit, anti-guinea pig and anti-chicken (Jackson

474   Immunochemicals) and Alexa-Fluor 488 anti-guinea pig and anti-rabbit (Invitrogen). All

475   secondary antibodies were used at a dilution of 1:500. DAPI staining of meiotic nuclei in late

476   meiotic prophase to visualize bivalents was performed 48 hours post-L4 stage.

477

478   Quantification of synapsis and RAD-51 foci was performed with a minimum of three whole

479   germlines per genotype as in [41] on animals 24 hours post L4 stage.

480

19

481   All images were acquired using a DeltaVision Personal DV system (Applied Precision) equipped

482   with a 100X N.A. 1.40 oil-immersion objective (Olympus), resulting in an effective XY pixel

483   spacing of 0.064 or 0.040 µm. Three-dimensional image stacks were collected at 0.2-µm Z-

484   spacing and processed by constrained, iterative deconvolution. Image scaling and analysis

485   were performed using functions in the softWoRx software package. Projections were calculated

486   by a maximum intensity algorithm. Composite images were assembled and some false coloring

487   was performed with Adobe Photoshop.

488

489   Westerns

490   For immunoblotting, samples were run on SDS-PAGE gels, transferred to nitrocellulose,

491   blocked in a PBST + 5% (w/v) non-fat milk solution, and then probed with rabbit anti-SGO-1

492   (dilution 1:30,000) and anti-GAPDH (1:5000) overnight at 4°C. Blots were washed 3x for 10

493   minutes in PBST, probed for 1 hour using an HRP-conjugated secondary antibody (rabbit or

494   mouse; GE Healthcare), washed 3x for 10 minutes in PBST, and then analyzed using a

495   chemiluminescent substrate (Thermo Scientific).

496

497   Feeding RNAi

498   For RNAi $dlc-1^{RNAi}$ and empty vector (L4440) clones from the Ahringer laboratory [86] were

499   used. Bacteria strains containing $dlc-1^{RNAi}$ and empty vector controls were cultured overnight in

500   10ml LB + 50ug/ul carbenicillin, centrifuged, and resuspended in 0.5 ml LB + 50ug/ul

501   carbenicillin. Sixty microliters of the RNAi bacteria was spotted onto NGM plates containing

502   1mM IPTG + 50ug/ul carbenicillin and allowed to grow at room temperature overnight. L4

503   hermaphrodite worms were picked into M9, transferred to these plates, allowed to incubate for

504   2-3 hours and then transferred to fresh RNAi plates to be dissected 48 hours post L4. A

505   minimum of 28 germlines were scored for each genotype.

506

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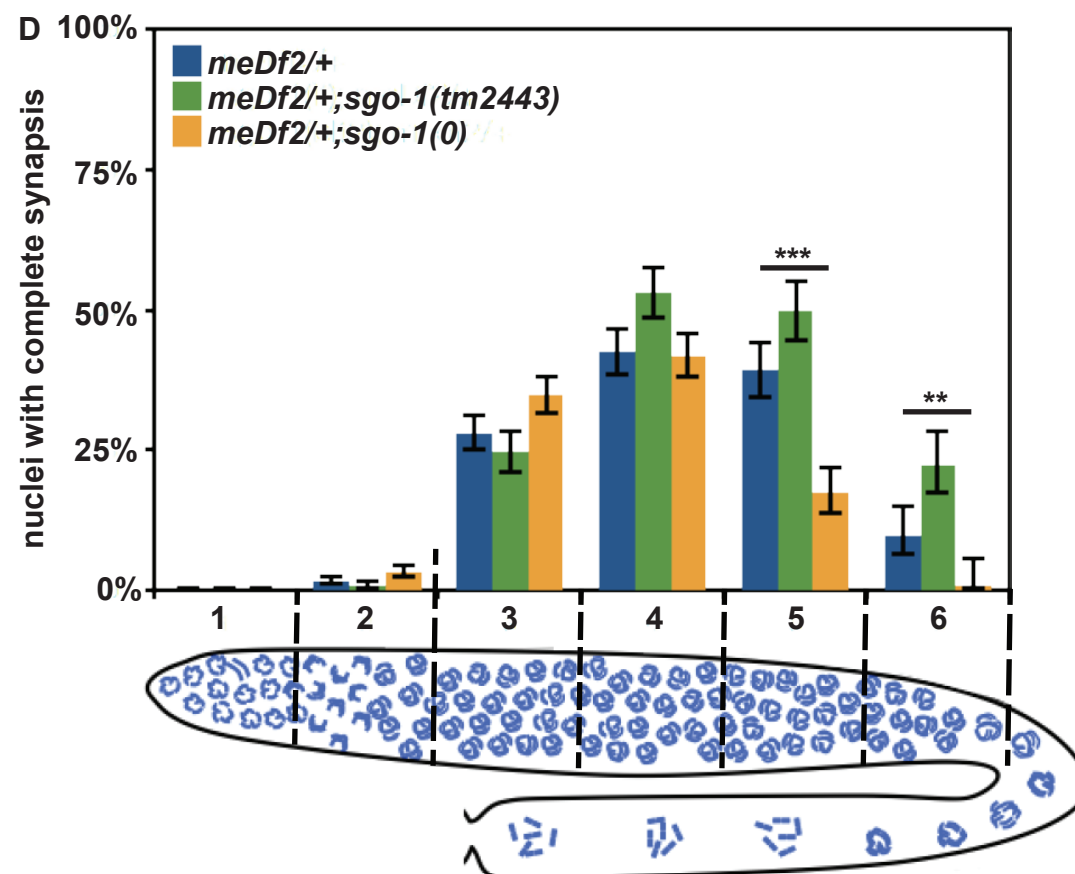755   systematic RNA interference. Nature *408*, 325-330.

756

757

Figure 1: SGO-1 is required for the synapsis checkpoint and the DNA damage response.

A. Cartoon of SGO-1 protein with relevant mutations indicated. **B.** *sgo-1(0)* mutants have no functional SGO-1 protein expression. Lysates from wildtype, *sgo-1(0)* mutants and *sgo-1(tm2443)* mutants blotted with antibodies against SGO-1 and GAPDH as a loading control. # indicates a background band present in all samples. Arrowhead indicates the truncated version of SGO-1 expressed in *sgo-1(tm2443)* mutants. **C.** Cartoon of meiotic checkpoint activation in *C. elegans*. **D.** A partial loss of function allele of *sgo-1* (*sgo-1[tm2443])* reduces apoptosis in *syp-1* single mutants and *syp-1;cep-1* double mutants. **E.** A null mutation in *sgo-1* (*sgo-1[0]*) reduces apoptosis to wildtype levels in *syp-1* mutants. Error bars indicate 2XSEM. Significance was assessed by performing t-tests. In all graphs, a * indicates a p value < 0.05, a ** indicates a p value < 0.01, and a *** indicates a p value < 0.0001.

A coiled-coil (PP2A binding) conserved basic region

STOP codon inserted to generate *sgo-1(0)*

absent in *tm2443*

B

wildtype *sgo-1(0)* *sgo-1(tm2443)*

37kDa –   SGO-1
          #
          ◄

37kDa –   GAPDH

C wildtype

synapsis checkpoint

syp-1 (no SC)

synapsis checkpoint → apoptosis

DNA damage checkpoint → apoptosis

cep-1;syp-1 (no SC)

synapsis checkpoint → apoptosis

DNA damage checkpoint

○ Pairing Center (PC)

≡ Synaptonemal Complex (SC)

★ DNA damage

D

average no. of apoptotic nuclei/germline

*

**

wildtype *sgo-1 (tm2443)* *syp-1* *syp-1; sgo-1 (tm2443)* *syp-1; cep-1* *syp-1; cep-1; sgo-1 (tm2443)*

E

average no. of apoptotic nuclei/germline

***

wildtype *sgo-1(0)* *syp-1* *syp-1; sgo-1(0)*

Figure S1. SGO-1 is required for the synapsis checkpoint and the DNA damage response.

A. Cartoon of meiotic checkpoint activation in *C. elegans*. **B.** A partial loss of function allele of

sgo-1 (*sgo-1[tm2443]*) reduces apoptosis in *meDf2* heterozygotes (*PCΔ/+*) but not *meDf2*

homozygote mutants (*PCΔ/ PCΔ*). **C.** A null mutation in *sgo-1* (*sgo-1[0]*) reduces apoptosis in

both *meDf2* homozygotes (*PCΔ/ PCΔ*) and *meDf2* heterozygotes (*PCΔ/+*). Error bars indicate

2XSEM. Significance was assessed by performing t-tests.

A

wildtype

PCΔ/PCΔ

PCΔ/+

B

C
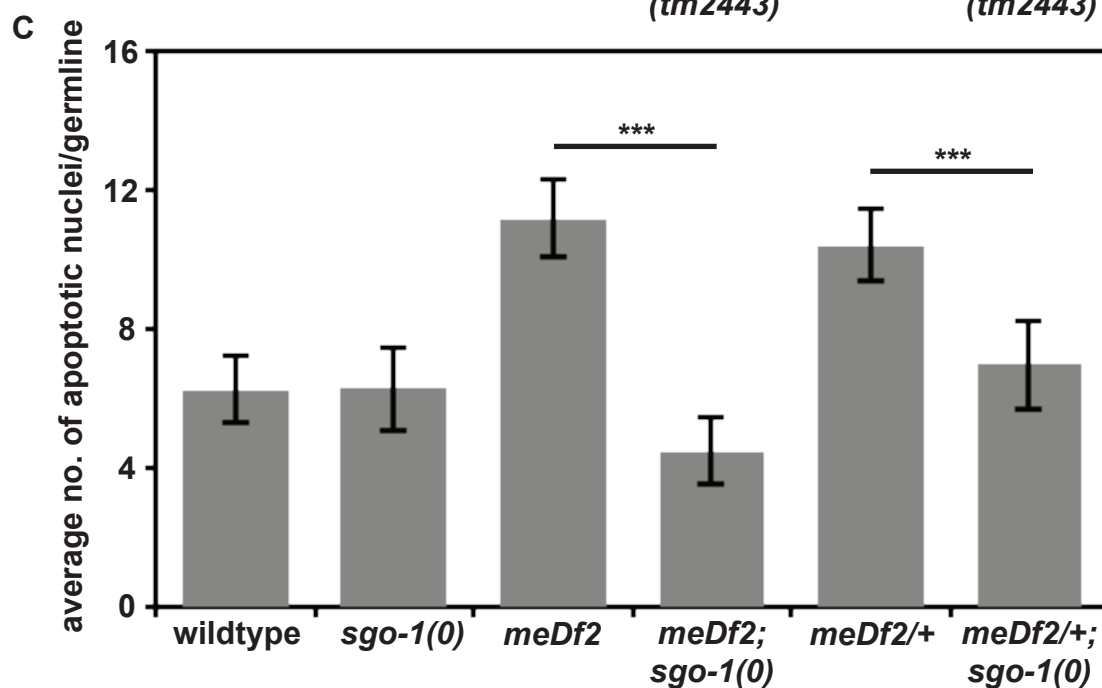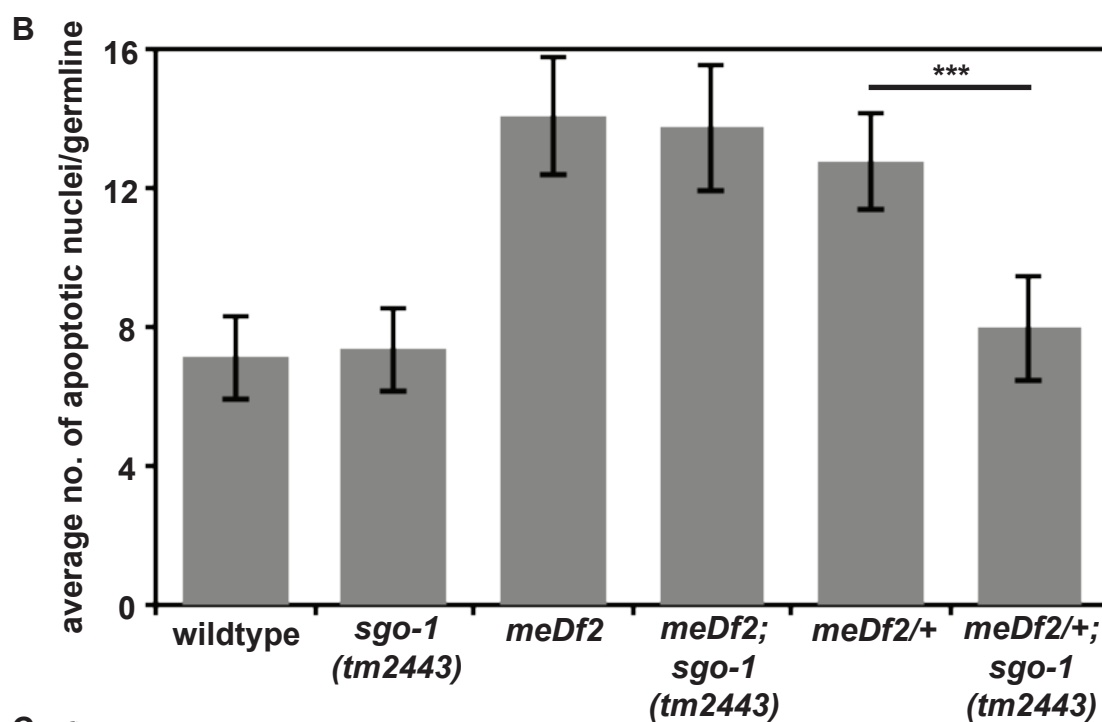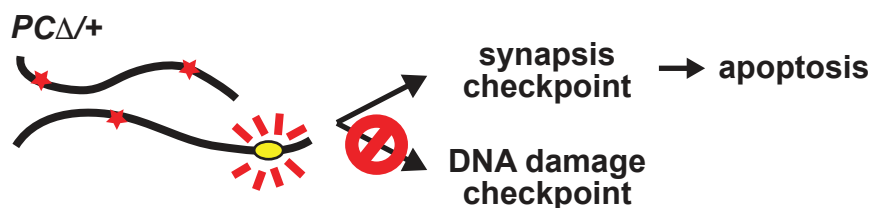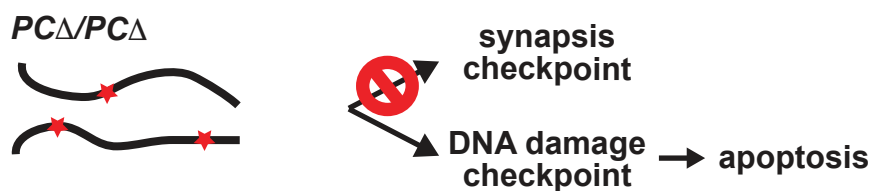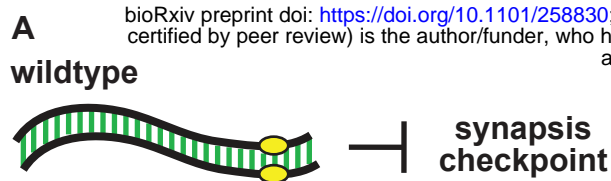
Figure 2: SGO-1 acts in a pathway independent of spindle checkpoint components and PCH-2. A. Germlines stained with antibodies against HTP-3 and SYP-1. Dashed white lines highlight regions of asynapsis and scale indicates 20 micrometers. **B.** Mutations in *sgo-1*, unlike loss of *mad-1*, do not suppress asynapsis in *dlc-1^RNAi* mutants. Error bars indicate 95% confidence intervals. **C.** Meiotic nuclei stained with antibodies against HTP-3 and SYP-1. Unsynapsed chromosomes identified by arrows and scale bar indicates 4 micrometers. **D.** Mutations in *sgo-1* do not suppress asynapsis in *meDf2/+* mutants. In all graphs that include a cartoon depiction of the *C. elegans* germline, meiotic progression is from left to right. Significance was assessed by performing Fisher's exact test.

A *dlc-1^RNAi*

dlc-1^RNAi; mad-1(0)

dlc-1^RNAi; sgo-1(0)

HTP-3
SYP-1

B

unsynapsed germlines

100%
75%
50%
25%
0%

dlc-1^RNAi *dlc-1^RNAi; mad-1(0)* *dlc-1^RNAi; sgo-1 (tm2443)* *dlc-1^RNAi; sgo-1(0)*

C *meDf2/+*

meDf2/+;sgo-1(0)

HTP-3
SYP-1

D

nuclei with complete synapsis

100%
75%
50%
25%
0%

■ *meDf2/+*
■ *meDf2/+;sgo-1(tm2443)*
■ *meDf2/+;sgo-1(0)*

1   2   3   4   5   6
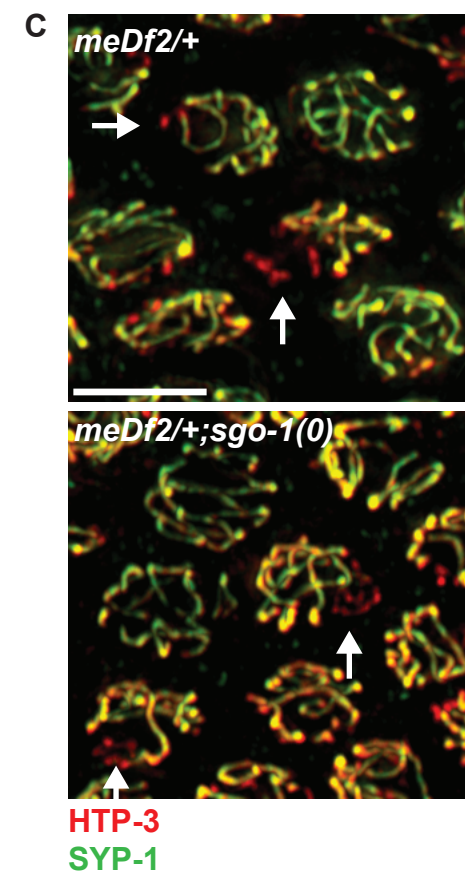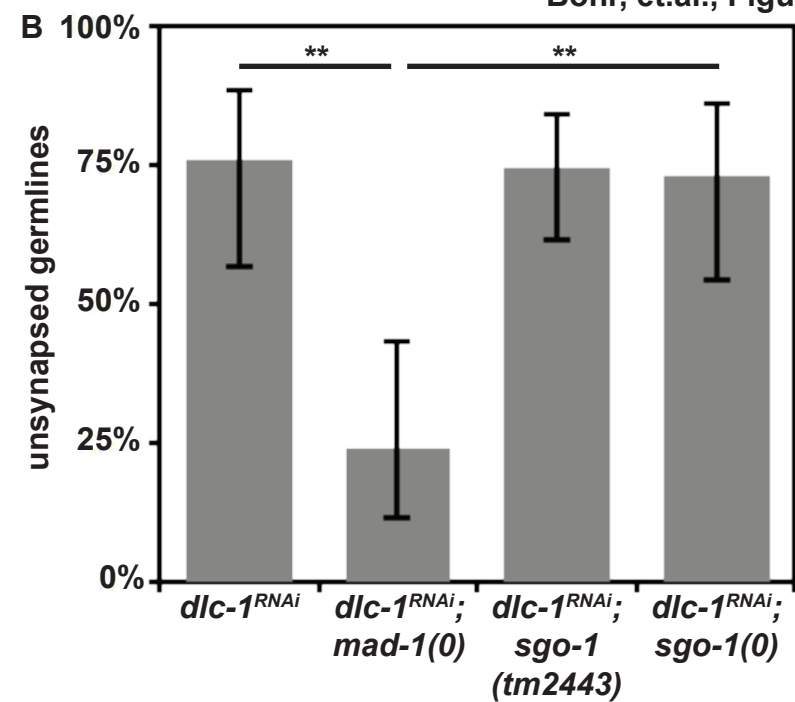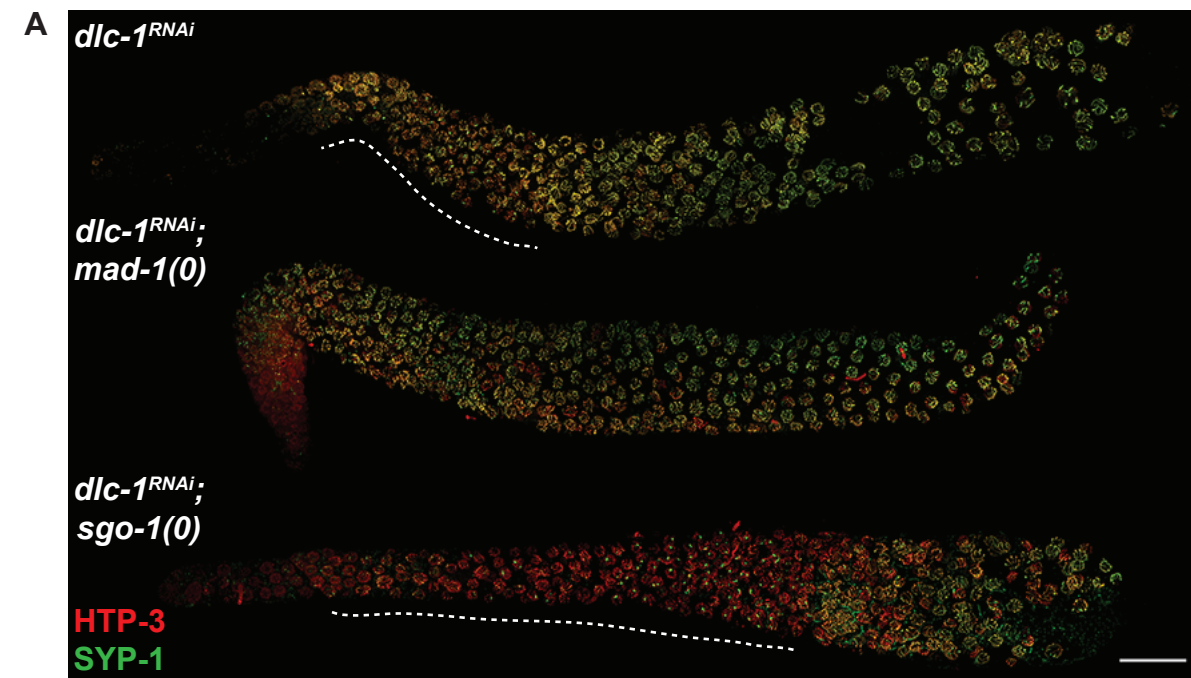
Figure S2: SGO-1 does not regulate pairing. A. Meiotic nuclei stained with DAPI and antibodies against HIM-8. Scale bar indicates 4 micrometers. **B.** Pairing in *syp-1;sgo-1* double mutants is indistinguishable from pairing in *syp-1* single mutants. Error bars indicate 95% confidence intervals.

A

DAPI
HIM-8

B
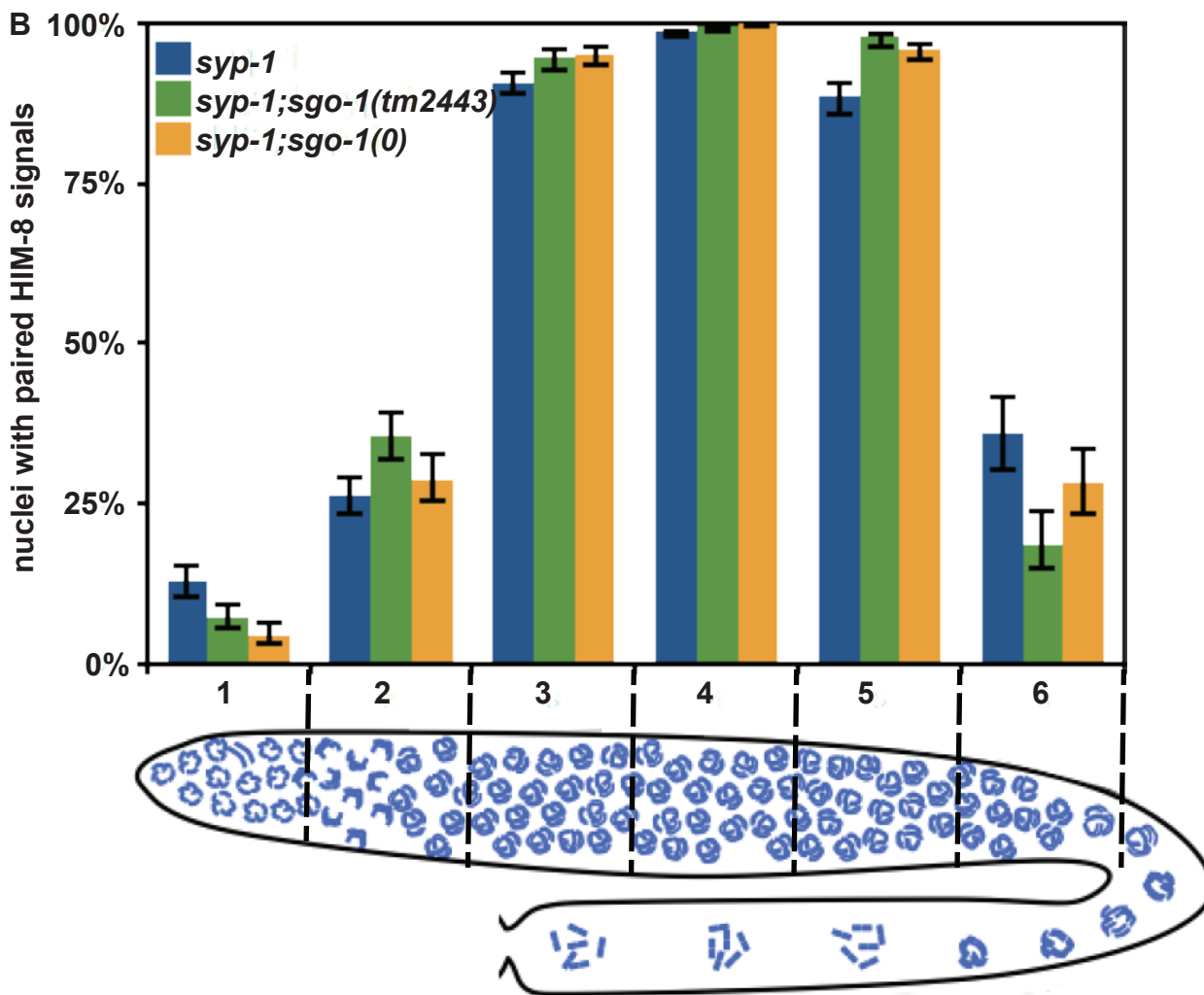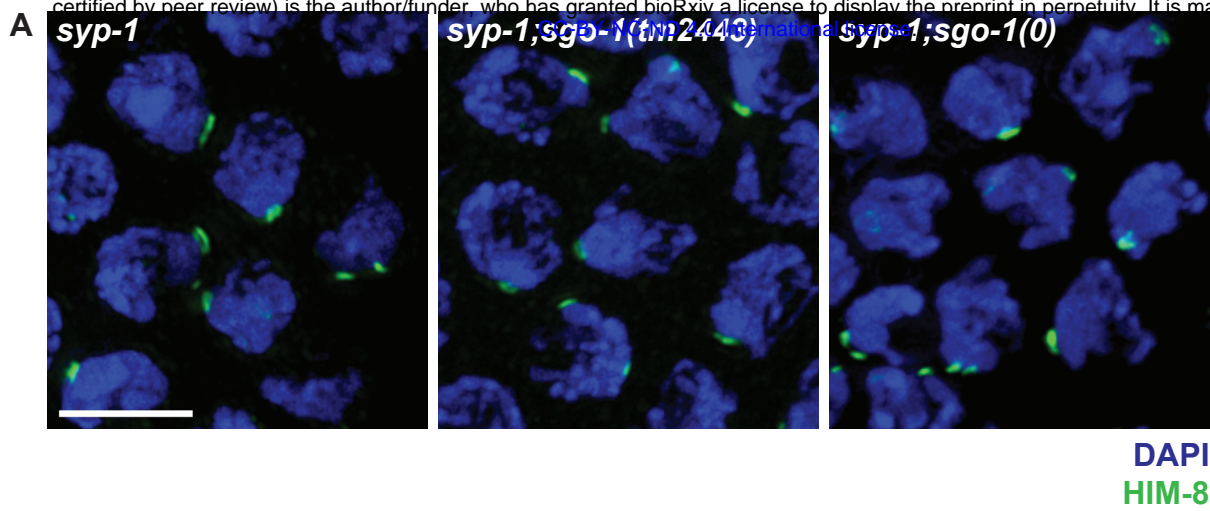
Figure 3: *sgo-1* mutants resemble a hypomorphic mutation in *htp-3*. A. Meiotic nuclei in late pachytene stained with antibodies against SC components HTP-3 and SYP-1. Arrows identify chromosomes undergoing SC disassembly. Scale bar indicates 4 micrometers. **B.** Mutations in *sgo-1* accelerate SC disassembly, similar to a partial loss of function mutation in htp-3 (*htp-3^{H96Y}*). Error bars indicate 95% confidence intervals. Significance was assessed by performing Fisher's exact test. **C.** A partial loss of function mutation in htp-3 (*htp-3^{H96Y}*) reduces apoptosis to wildtype levels in *syp-1* mutants. Error bars indicate 2XSEM. Significance was assessed by performing t- tests.

HTP-3
SYP-1
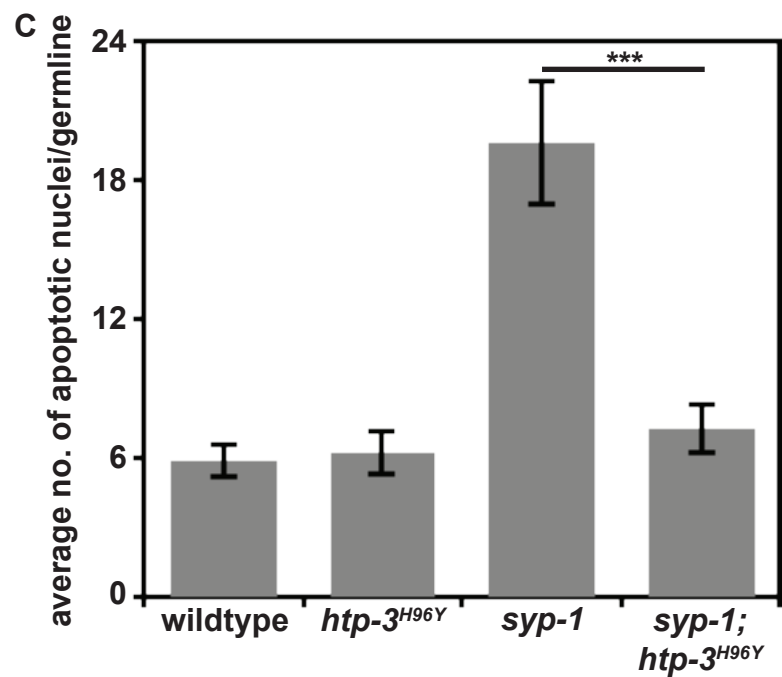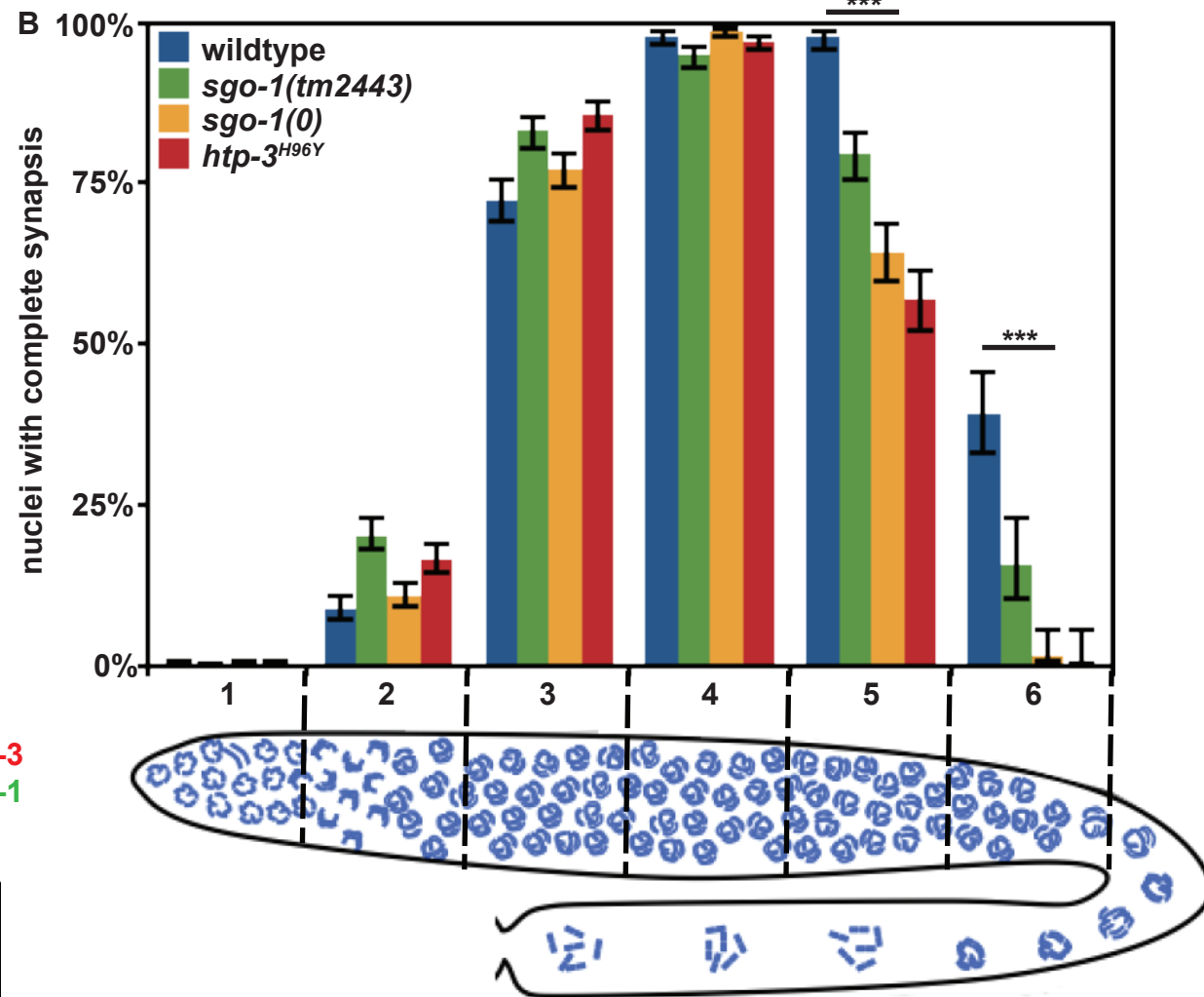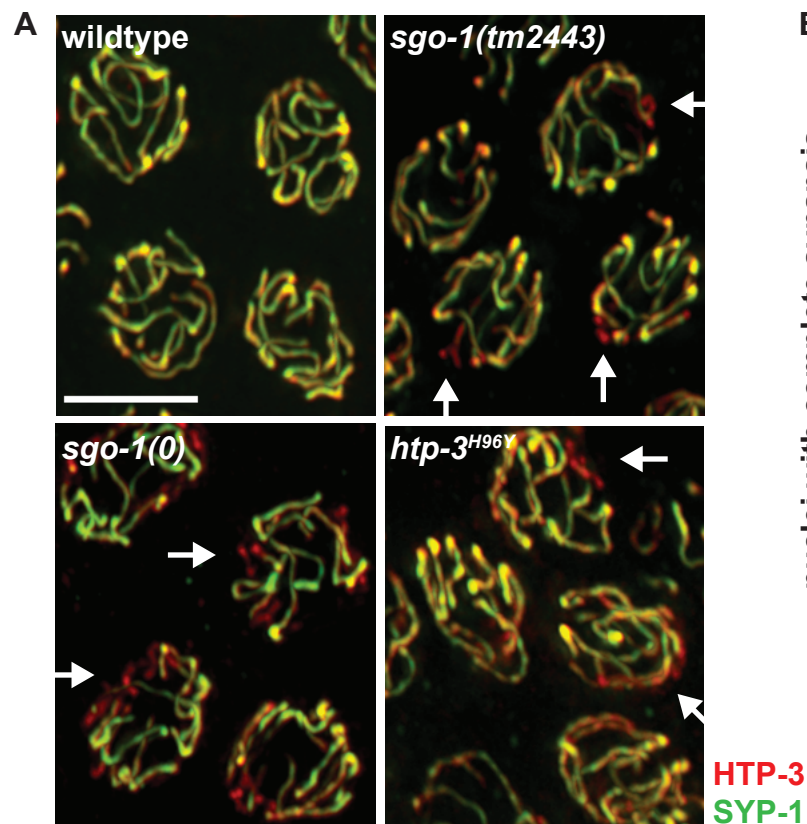
Figure 4: SGO-1 is not essential for crossover formation but is required to promote inter-homolog DNA repair and crossover assurance. A. Meiotic nuclei stained with DAPI and antibodies against RAD-51. Error bars indicate 2XSEM. **B.** Homolog-independent mechanisms of DNA repair are active in *syp-1;sgo-1(0)* double mutants. In all images, scale bar indicates 4 micrometers. Significance was assessed by performing t- tests. **C.** Meiotic nuclei stained to visualize DNA (DAPI) and GFP::COSA-1. **D.** The percentage of meiotic nuclei with 5, 6, 7 and 8 GFP::COSA-1 foci. **E.** The percentage of meiotic nuclei with 6 (top image) or 7 DAPI staining bodies (bottom image).
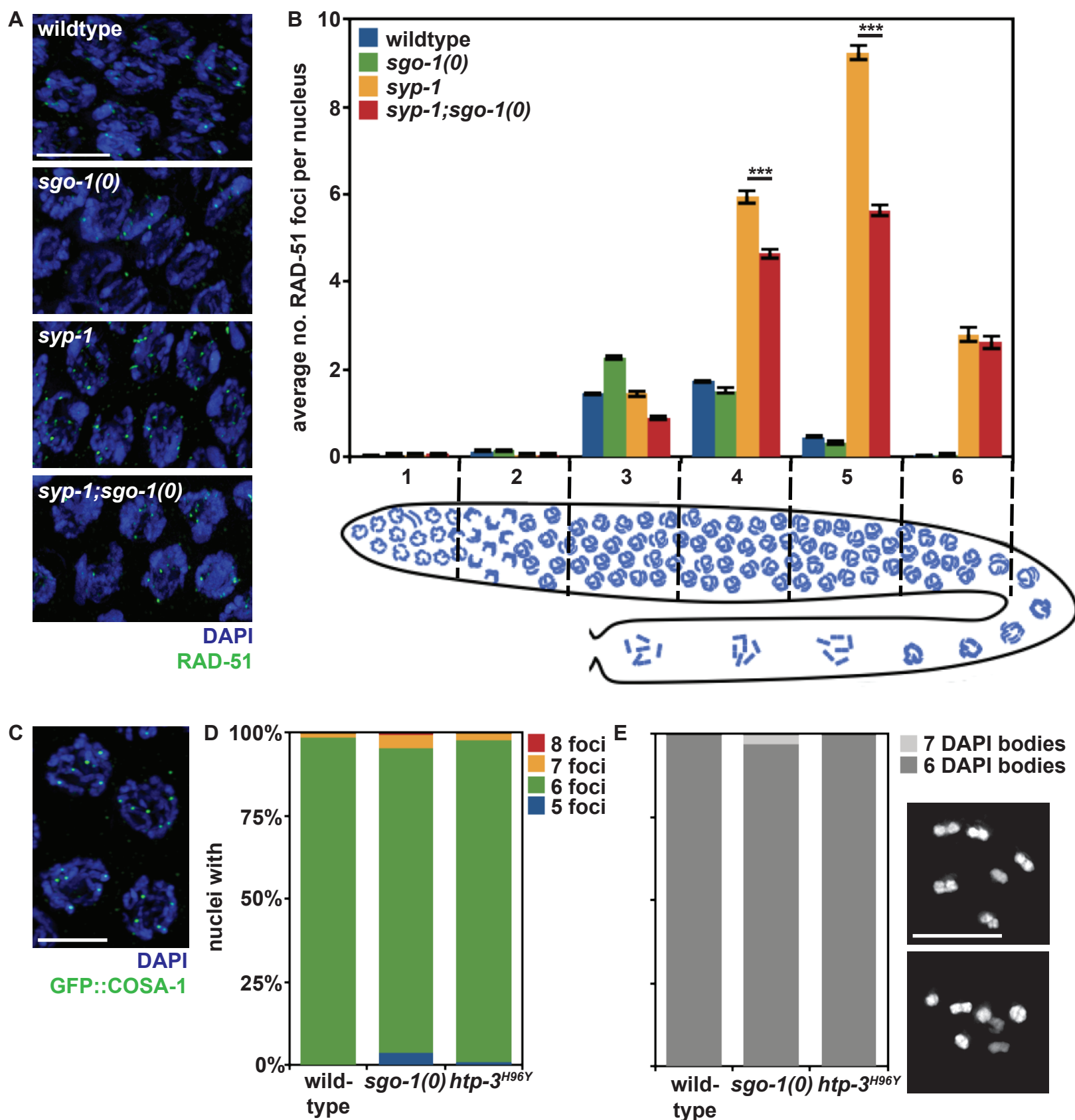
Figure 5: SGO-1 is required for robust localization of HUS-1::GFP to sites of DNA damage. A. Meiotic nuclei stained to visualize DNA (DAPI) and HUS-1::GFP. Scale bar indicates 5 micrometers. **B.** SGO-1 and HTP-3 function are required for robust recruitment of HUS-1::GFP when the DNA damage checkpoint is active. Error bars indicate 2XSEM. Significance was assessed by performing t- tests.
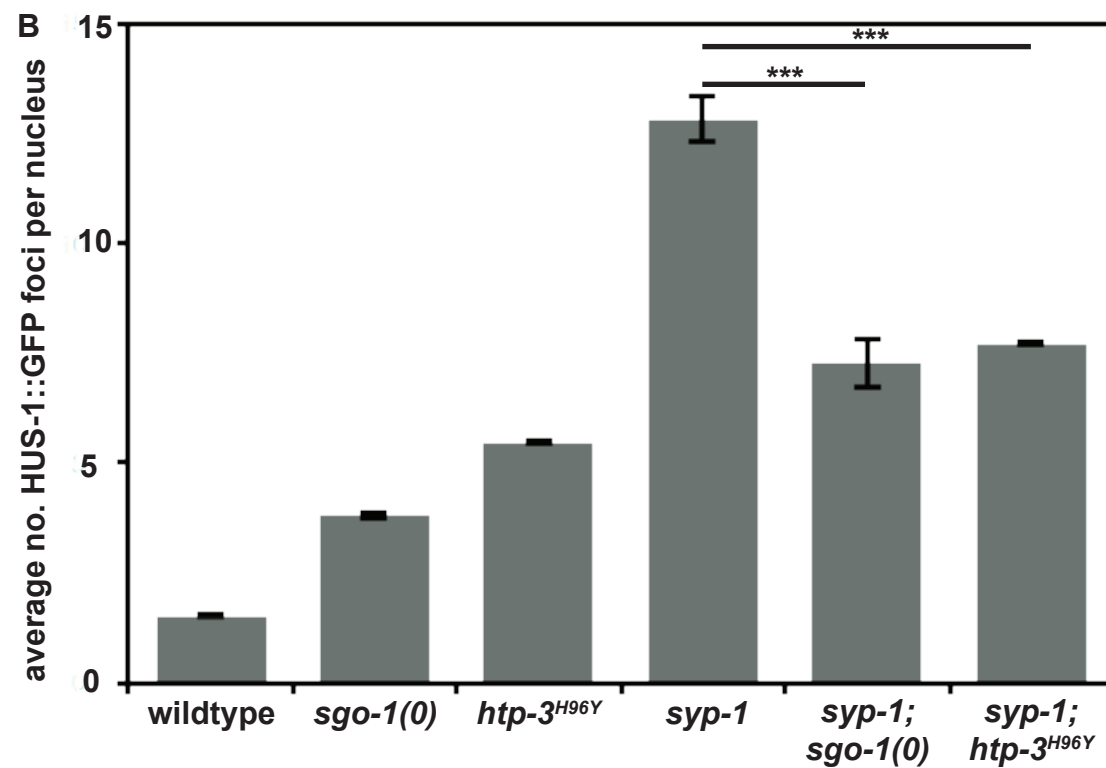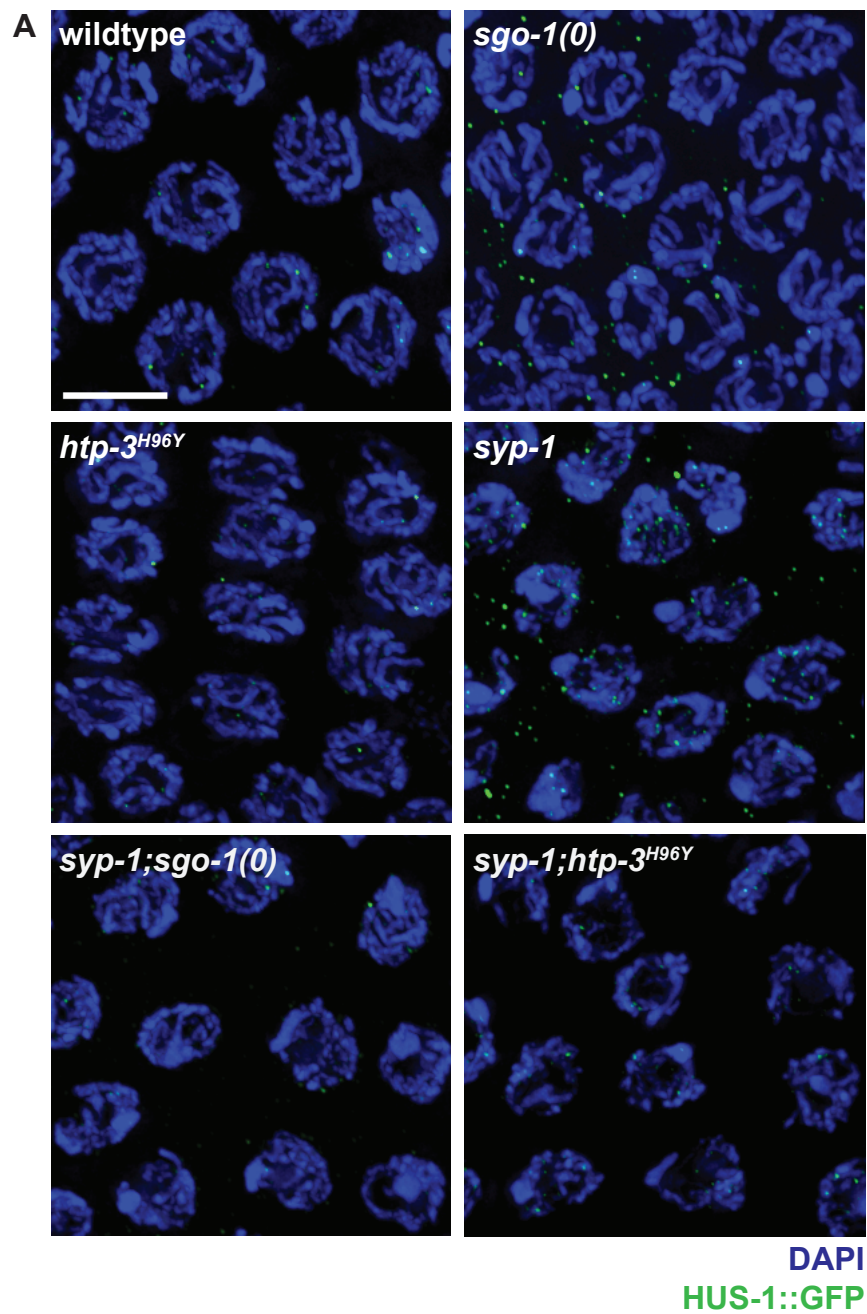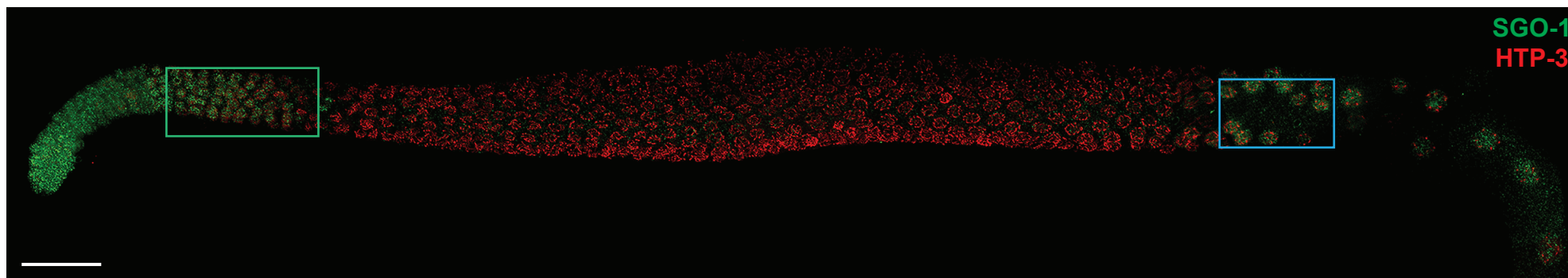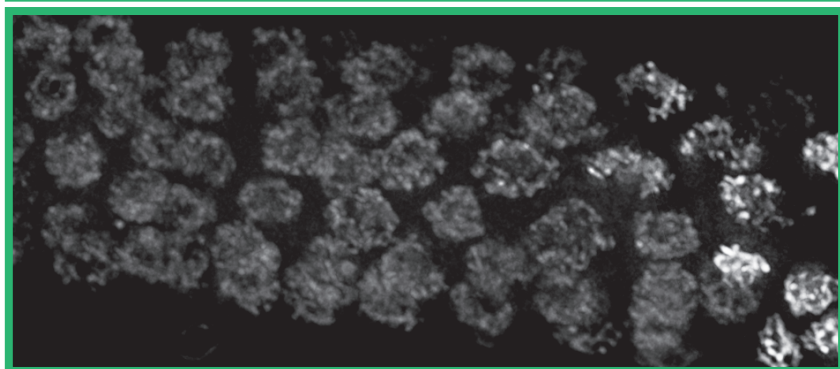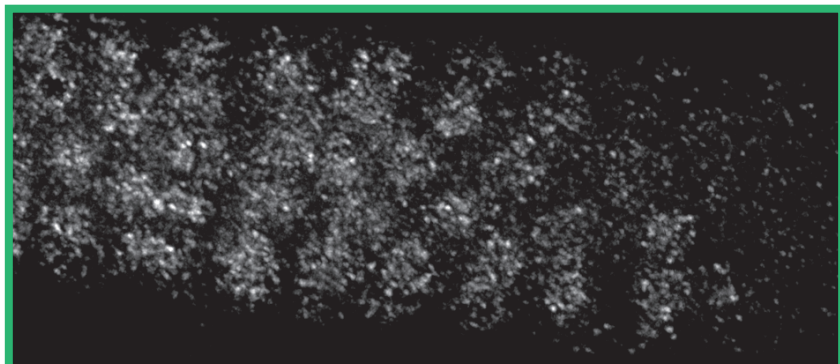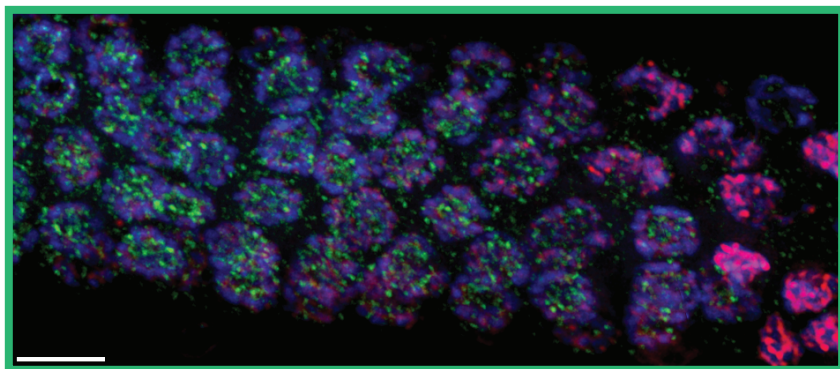
DAPI
HUS-1::GFP

Figure 6: SGO-1 localizes to pre-meiotic nuclei. A. Wildtype germline stained with antibodies against SGO-1 and HTP-3. Scale bar indicates 20 micrometers. Pre-meiotic nuclei (**B**) and nuclei in late meiotic prophase (**C**) stained with DAPI and antibodies against SGO-1 and HTP-3. Scale bars in B and C indicates 4 micrometers.
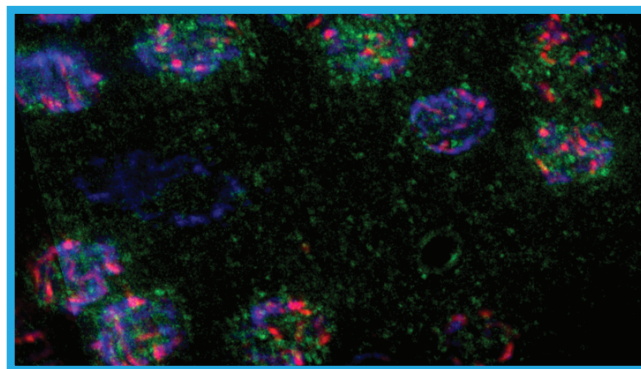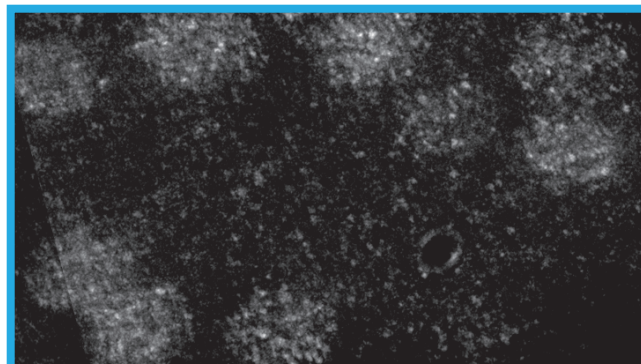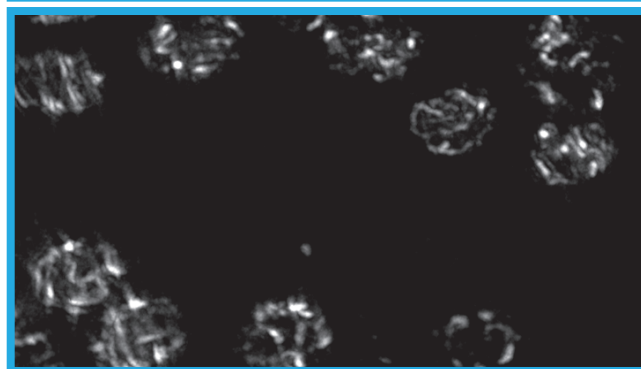
A

SGO-1
HTP-3

B

C

DNA
SGO-1
HTP-3

SGO-1

HTP-3

Figure 7: Model for SGO-1 function in meiotic prophase. Orange and blue dotted lines indicate homologous chromosomes prior to replication. During pre-meiotic replication in wildtype nuclei, meiotic axis metabolism initiates (continuous blue and orange lines), generating axes functional for pairing, synapsis (dotted green line), checkpoint signaling, limiting non-homologous recombination and eventual stabilization of the SC (solid green line). *sgo-1* mutants fail to form fully functional axes (represented by the continued presence of dotted blue and orange lines), resulting in a loss of checkpoint signaling, the activation of non-homologous recombination mechanisms and premature disassembly of the SC.

wildtype   *sgo-1(0)*

functional
meiotic axis
metabolism

pre-meiotic
replication

defective
meiotic axis
metabolism

axes capable
of checkpoint
signaling
and limiting
non-homologous
recombination

pairing,
synapsis
and
recombination

axes incapable
of checkpoint
signaling
and limiting
non-homologous
recombination

stabilization of
synaptonemal
complex

premature
disassembly of
synaptonemal
complex