Meiotic chromosome structures constrain and respond to designation of crossover sites

Diana E. Libuda¹, Satoru Uzawa², Barbara J. Meyer² & Anne M. Villeneuve¹,3

Crossover recombination events between homologous chromosomes are required to form chiasmata, temporary connections between homologues that ensure their proper segregation at meiosis¹. Despite this requirement for crossovers and an excess of double-strand DNA breaks that are the initiating events for meiotic recombination, most organisms make very few crossovers per chromosome pair². Moreover, crossovers tend to inhibit the formation of other crossovers nearby on the same chromosome pair, a poorly understood phenomenon known as crossover interference³—⁴. Here we show that the synaptonemal complex, a meiosis-specific structure that assembles between aligned homologous chromosomes, both constrains and is altered by crossover recombination events. Using a cytological marker of crossover sites in Caenorhabditis elegans⁵, we show that partial depletion of the synaptonemal complex central region proteins attenuates crossover interference, increasing crossovers and reducing the effective distance over which interference operates, indicating that synaptonemal complex proteins limit crossovers. Moreover, we show that crossovers are associated with a local 0.4–0.5-micrometre increase in chromosome axis length. We propose that meiotic crossover regulation operates as a self-limiting system in which meiotic chromosome structures establish an environment that promotes crossover formation, which in turn alters chromosome structure to inhibit other crossovers at additional sites.

Although crossovers mature in the context of assembled synaptonemal complexes (SCs), whether the SC functions in crossover interference has long been debated⁶—⁸. Largely on the basis of analysis in budding yeast, it has been argued for the last decade that the SC is irrelevant for interference⁹. Consequently, suggestive evidence that the SC might be involved in interference in other organisms has gained little traction⁹—¹⁰. The debate persists in part because of inherent limitations of some assays used to score crossovers (see Supplementary Discussion). We overcome this problem by taking advantage of the fact that the C. elegans crossover-promoting protein COSA-1, which forms foci at nascent crossover sites in vivo, serves as a robust cytological marker of the crossovers that elicit and respond to crossover interference⁶.

C. elegans exhibits robust crossover interference, with chromosome pairs normally undergoing only a single crossover. Using COSA-1 foci, we assessed whether the SC central region proteins (the SYP proteins) function in limiting the number of crossovers per chromosome pair. SYPs are essential for crossover formation¹¹—¹³, precluding the use of syp null mutants for this analysis. To circumvent this issue, we used RNA interference (RNAi) to partially deplete SYP-1 protein levels by ~60–70% (Extended Data Fig. 1). In contrast to a previous study⁶, the residual SYP-1 levels achieved using our experimental conditions were sufficient to permit assembly of SCs (albeit with reduced ratios of SYPs to axis subunits) and formation of chiasmata for all six chromosome pairs in most gonads (see Supplementary Discussion and Methods). Although SYPs are required to form crossovers, we found that partial depletion of SYP-1, SYP-2 or SYP-3 increases COSA-1 foci (Fig. 1a and Extended Data Fig. 2), indicating that a sufficient pool of SYPs is required to limit COSA-1-marked crossovers to one per chromosome pair.

In principle, an increase in COSA-1-marked crossover sites could reflect an increase in the number of double-strand breaks (DSBs) formed and/or an increase in the fraction of DSBs repaired as crossovers. Therefore, we controlled DSB number by using γ-irradiation to induce DSBs in spo-11 mutant worms, which are proficient for pairing and SC assembly but lack endogenous meiotic DSBs (Fig. 1b)¹⁴. Controls recapitulated the previous finding that increasing DSBs beyond the level needed to ensure at least one per chromosome pair (1,000 Rad, 23.5 DSBs per nucleus) did not increase the average number of COSA-1 foci per nucleus beyond six. Further, COSA-1 foci exhibited very low standard deviations (±0.14–0.27), reflecting operation of the robust crossover control system. By contrast, upon syp-1 RNAi, most nuclei

Figure 1 | SYP-1 partial depletion increases numbers of COSA-1 foci and chiasmata. a, Immunofluorescence images of late pachytene nuclei from control or syp-1 partial RNAi worms. DAPI, 4′,6-diamidino-2-phenylindole. Scale bar, 5 µm. b, Dose–response graph depicting mean numbers of green fluorescent protein (GFP):COSA-1 foci per nucleus in response to DSBs generated by increasing doses of γ-irradiation (IR, rad). See Methods for numbers of nuclei used; error bars indicate ± s.d. At >1,000 rad, both the mean numbers of foci and s.d. were increased in syp-1 RNAi relative to control. c, Three-dimensionally rendered images of individual diakinesis bivalents comprising the mn112 (XIV) fusion chromosome pair. Dashed lines (white) indicate traced HTP-3 axes, with crossing of axes indicating chiasmata. Scale bar, 1 µm.
exposed to >1,000 rad of γ-irradiation had >6 COSA-1 foci and standard deviations were much higher (±1.2–1.6), indicating impairment of crossover control. This experiment shows that increased DSBs alone cannot account for the increase in COSA-1 foci following SYP-1 partial depletion, and that for any given level of DSBs, SYP-1 has a role in determining the fraction that will mature into cytologically differentiated crossovers.

Our data suggest that SYP-1 partial depletion impairs crossover interference. To investigate this further, we used worms homozygous for the two-chromosome fusion mnT12 (X chromosome fused with chromosome IV)10,15. Previous work showed that although crossover interference limits the mnT12 chromosome pair to a single COSA-1 focus in the majority of meioses, mnT12 chromosome pairs with two COSA-1 foci also occur5. Under our control conditions, 51% of mnT12 pairs had a single COSA-1 focus, whereas 49% had two foci (Fig. 2a, b and Methods). This occurrence of two COSA-1 foci along mnT12 allowed us to assess interference strength in the context of wild-type SYP-1 levels and compare it to crossover/chiasma interference in the context of SYP-1 partial depletion (Figs 1c and 2–4).

Increased COSA-1 foci following syp-1 RNAi correlated with an increased number of cytologically resolvable chiasmata on mnT12 bivalents at diakinesis, the last stage of prophase (Fig. 1c and Extended Data Fig. 3). Consistent with mnT12 having only one or two COSA-1 foci at the late pachytene stage in controls (average of 1.49 COSA-1 foci per mnT12 pair), control mnT12 diakinesis bivalents had only one or two chiasmata. Upon syp-1 RNAi, an average of 2.57 COSA-1 foci per mnT12 pair were observed at late pachytene (49% having ≥3 foci; Fig. 2b), and 47% of diakinesis bivalents had ≥3 chiasmata. These and other data (Extended Data Fig. 3) indicate that the extra COSA-1 foci in syp-1 RNAi worms represent bona fide cytologically differentiated inter-homologue crossovers.

We conducted quantitative analyses evaluating the positions, distributions and distances between COSA-1-marked sites on computationally straightened mnT12 chromosomes (with HTP-3 immunofluorescence marking chromosome axes16 and with immunofluorescence for the HIM-8 protein, which localizes near the left end of the X chromosome17, serving as an orientation marker; Fig. 2a and Methods). The average distance between COSA-1 foci on mnT12 with ≥2 COSA-1 foci in syp-1 RNAi was 4.5 μm, substantially shorter than the average distance of 8.5 μm for control mnT12 with two foci (P < 0.0001). Further, even when only syp-1 RNAi mnT12 with two foci were considered, the average distance (6.5 μm) was still significantly shorter than in controls (P < 0.0001; Fig. 2c). These data show that syp-1 RNAi decreases the effective distance over which interference operates. The average distance between COSA-1 foci on control mnT12 (8.5 μm) exceeds the average axis length of unfused autosomes (6.4 μm, P < 0.0001), reinforcing the indirect inference from ref. 10 that interference in C. elegans operates over distances longer than the length of a normal chromosome.

We assessed crossover interference further by dividing the mnT12 axis into eight evenly spaced intervals and binning the positions of COSA-1 foci into these intervals (Fig. 2d, e and Extended Data Fig. 4). First, we compared the distributions of COSA-1 foci among these intervals for chromosomes with different numbers of foci. In controls, COSA-1 foci exhibited a specific non-random distribution indicative of interference: for the subset of chromosomes with only one COSA-1 focus, the single focus usually occurred near the middle of mnT12, whereas for the subset of chromosomes with two COSA-1 foci, those foci predominantly occurred near the chromosome ends (Fig. 2d). By contrast, syp-1 RNAi resulted in a fairly even distribution of foci along mnT12, regardless of the number of COSA-1 foci, consistent with attenuation of crossover interference. Second, plotting the separation between adjacent pairs of COSA-1 foci (Fig. 2e) revealed wide spacing (≥4 interval boundaries) separating adjacent COSA-1 foci in controls, and reduced separation between adjacent foci in syp-1 RNAi worms, both when all pairs of foci or only chromosomes with two foci were considered.

We also used two quantitative methods to calculate interference strength. First, we performed a coefficient of coincidence analysis, with mnT12 divided into four intervals (Fig. 3a, Extended Data Fig. 4, Extended Data Table 1 and Methods). Controls exhibit a robust signature of interference (I): complete interference (I = 1) for adjacent interval pairs; strong but reduced interference for pairs at intermediate spacing between adjacent intervals (0.5 < I < 1). Partial depletion of SYP-1 impairs interference, with a lower number of chromosomes carrying ≥2 COSA-1 foci (20% having ≥2 COSA-1 foci; Fig. 3b). Inside the interference region, the number of chromosomes with ≥2 COSA-1 foci further decreases, consistent with a partial depletion of SYP-1 during spermatogenesis (Fig. 3b, asterisks).
distance; and high ‘negative’ interference ($I < 0$) for the pair of intervals including opposite ends of the chromosome, indicating that a chromosome with a focus in one end-interval has an increased likelihood of a second focus in the opposite end-interval (Fig. 2d, top). By contrast, the $syp-1$ RNAi data show reduced interference for adjacent inter-vals, and no interference for interval pairs separated by $\geq 1$ interval, indicating attenuated interference. Second, we used a gamma distribution to model inter-focus distances (expressed as a percentage of total axis length), and generated best-fit probability density curves where the shape parameter ($\gamma$) is a relative indicator of interference strength, with $\gamma = 1$ indicating no interference and higher values signifying stronger interference ($\gamma = 37$). This fit was compared with a gamma distribution where the control displayed very strong interference ($\gamma = 37$) and was also observed in controls (Fig. 4a). Moreover, comparison of $mnT12$ axis lengths in a $spo-11$ mutant (which lacks crossovers) and in $spo-11$/+ heterozygous controls yielded a similar linear relationship ($R^2 = 0.952$). Analysis of unfused autosomes from $spo-11$, controls and $syp-1$ RNAi worms likewise showed each COSA-1 focus associating with a 0.4-μm increase in axis length (Fig. 4c; $R^2 = 0.999$), demonstrating that the relationship between crossovers and axis length is generalizable to other chromosomes. Together, these data indicate that extension of chromosome axes occurs in response to crossover designation. Finally, assessment of the distance between the left end of the chromosome and the position of the HIM-8 focus on control $mnT12$ (Fig. 4d and Extended Data Fig. 6) revealed that the mean length of this specific short axis segment was increased by 0.4–0.5 μm when a COSA-1 focus was present in the segment. As this value is comparable to that inferred from the linear regression analyses, we conclude that the increase in axis length associated with each crossover is predominantly a local effect.

Our demonstration that partial depletion of SYPs attenuates the robust crossover interference in $C. elegans$ indicates a role for SC central region proteins in crossover control. This effect of SYP depletion on interference could reflect a decreased ability to propagate an inhibitory signal, a reduced sensitivity of recombination precursors to inhibition, and/or a prolonged state of competence for crossover designation. Our finding that SC central region subunits have a role in achieving the high level of interference characteristic of $C. elegans$ meiosis can be reconciled with studies concluding that the SC is dispensable for interference in budding yeast $^{19-21}$, on the basis of several considerations. There is a growing body of evidence that, at least in some organisms, non-random distribution of prospective crossover intermediates may occur in (at least) two different steps $^{22-24}$. This suggests that multiple layers of regulation can contribute to the final interference distribution of crossovers, raising the possibility that the relative contributions of different crossover control mechanisms may differ between organisms. Thus, given that the attenuated interference observed following $SYP-1$ partial depletion in $C. elegans$ ($\gamma = 4.9$) appears at least as strong or stronger than the interference observed in wild-type budding yeast ($\gamma = 1.9$) $^{25}$, we suggest that $C. elegans$ may use a layer of crossover control that does not make an appreciable contribution in budding yeast.

Integrating our findings with previous data showing that crossover designation in $C. elegans$ occurs in the context of assembled SCs $^{5,26}$, we suggest a model in which crossover designation requires a capacity for local expansion of previously assembled meiotic chromosome structures. We suggest that local expansion in turn alters the thickness, density and/or rigidity of structures beyond the local area, thereby reducing the capacity for axis expansion at other sites (see Supplementary Discussion). Overall, our work supports the idea that meiotic crossover regulation operates as a self-limiting system in which meiotic chromosome structures create an environment that promotes crossovers, which in turn modify chromosome structures to inhibit crossover formation at additional neighbouring sites.

**METHODS SUMMARY**

For assessing the effects of partial depletion of $SYP-1$, worms were exposed to RNAi treatment at 25 °C from the L1 stage until 16–22 h post-L4 stage, when their gonads were then dissected and processed for immunofluorescence. For Fig. 1,
chromosomes in three-dimensionally preserved meiotic prophase nuclei were imaged using wide-field deconvolution microscopy. For analyses involving measurements of chromosome axis lengths and positions of GFP–COSA-1 foci (Figs 2–4), three-dimensionally preserved nuclei were imaged using confocal microscopy, followed by three-dimensional tracing and subsequent computational straightening of chromosomes before recording of position coordinates. All P values reported in the main text are two-tailed and calculated from Mann–Whitney tests.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 25 April; accepted 16 August 2013.
Published online 9 October 2013.

1. Page, S. L. & Hawley, R. S. Chromosome choreography: the meiotic ballet. Science 301, 785–789 (2003).
2. Martínez-Perez, E. & Colaiácovo, M. P. Distribution of meiotic recombination events: talking to your neighbors. Curr. Opin. Genet. Dev. 19, 105–112 (2009).
3. Muller, H. J. The mechanism of crossing-over. Am. Nat. 50, 193–221 (1916).
4. Sturtevant, A. H. Synaptonemal complex and crossing-over: structural support or interference? Heredity 41, 233–237 (1978).
5. Maguire, M. P. Can abortive early homologous associations promote increased crossing-over in an adjacent rearranged segment? Genome 30, 469–472 (1988).
6. Hayashi, M., Miyazaki, Y. & Villeneuve, A. M. The synaptonemal complex shapes the crossover landscape through cooperative assembly, crossover promotion and crossover inhibition during Caenorhabditis elegans meiosis. Genetics 159, 2428–2442 (2002).
7. MacQueen, A. J., Colaiácovo, M. P., McDonald, K. & Villeneuve, A. M. Synaptonemal complex assembly in C. elegans is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev. Cell 5, 463–474 (2003).
8. MacQueen, A. J., Colaiácovo, M. P., McDonald, K. & Villeneuve, A. M. Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in C. elegans. Genes Dev. 16, 2428–2442 (2002).
9. Smolikov, S. et al. SYP-3 restricts synaptonemal complex assembly to bridge paired chromosome axes during meiosis in Caenorhabditis elegans. Genetics 176, 2015–2025 (2007).
10. Hillers, K. J. & Villeneuve, A. M. Chromosome-wide control of meiotic crossing over during C. elegans meiosis. Dev. Cell 14, 263–274 (2008).
11. Phillips, C. M. et al. HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell 123, 1051–1063 (2005).
12. McPeek, M. S. & Speed, T. P. Modeling interference in genetic recombination. Genetics 139, 1031–1044 (1995).
13. Fung, J. C., Rockmill, B., Odell, M. & Roeder, G. S. Imposition of crossover interference through the nonrandom distribution of synopsis initiation complexes. Cell 116, 795–802 (2004).
14. Börner, G. V., Kleckner, N. & Hunter, N. Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117, 29–45 (2004).
15. Storlazzi, A., Xu, L., Schwacha, A. & Kleckner, N. Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. Proc. Natl Acad. Sci. USA 93, 9043–9048 (1996).
16. Cole, F. et al. Homeostatic control of recombination is implemented progressively in mouse meiosis. Nature Cell Biol. 14, 424–430 (2012).
17. de Boer, E., Stam, P., Dietrich, A. J., Pastink, A. & Heyting, C. Two levels of interference in mouse meiotic recombination. Proc. Natl Acad. Sci. USA 103, 9607–9612 (2006).
18. Reynolds, A. et al. RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. Nature Genet. 45, 269–278 (2013).
19. Chen, S. Y. et al. Global analysis of the meiotic crossover landscape. Dev. Cell 15, 401–415 (2008).
20. Rosu, S., Libuda, D. E. & Villeneuve, A. M. Robust crossover assurance and regulated interhomolog access maintain meiotic crossover number. Science 334, 1286–1289 (2011).
21. Merts, D. G. & Meyer, B. J. Condensins regulate meiotic DNA break distribution, thus crossover frequency, by controlling chromosome structure. Cell 139, 73–86 (2009).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank A. Derbina and M. Zetka for antibodies and the CGC (funded by National Institutes of Health (NIH) P40 OD010440) for strains. We thank K. Hillers and K. Zawadzki for comments on the manuscript. This work was supported by Helen Hay Whitney Foundation Postdoctoral Fellowship, a Katharine McCormick Advanced Postdoctoral Fellowship, and NIH K99 HD076165 to D.E.L. and by NIH R01 GM067268 to A.M.V. B.J.M. is an investigator of the Howard Hughes Medical Institute.

Author Contributions D.E.L. and A.M.V. conceived and designed the experiments, analysed the data and wrote the paper. D.E.L. performed the experiments. S.U. deconvolved confocal images. S.U. and B.J.M. provided technical expertise for computational straightening of chromosomes.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.M.V. (annve@stanford.edu).
METHODS
C. elegans strains, genetics and culture conditions. All strains are from the Bristol N2 background and were maintained and crossed at 20 °C under standard conditions. Temperatures used for specific experiments are indicated in Figures and below.

The following strains were used in this study: (1) AV307: syg-1(me17) V/nt1 [unc-80[ng64-1]; tr18; dpy-11]; meIs18; spo-11/me44 IV; dpy-5(e1166) mnT12 (IV; X) (that is, SYP-1 was not detected on all chromosomes) was excluded from this analysis, as gonads in this category would have been excluded from our experiments evaluating numbers and distribution COSA-1 foci on the basis of presence of asynapsed chromosomes and/or inability to trace continuous chromosome axes (as a result of asynapsis). For each gonad, background fluorescence in the SYP-1 and HTTP-3 channels was assessed by measuring fluorescence intensities for three separate areas between nuclei in the scored region and using these to calculate the average background fluorescence per pixel. For each individual nucleus, SYP-1 and HTTP-3 fluorescence measurements were calculated by dividing the total intensity by the total area of the projected nucleus, and then subtracting the average background fluorescence for the gonad. To obtain an average SYP-1:HTTP-3 ratio for each gonad, the SYP-1:HTTP-3 ratios calculated for each nucleus within a given gonad were averaged. Once average SYP-1:HTTP-3 ratios were measured for all gonads, two different approaches were used to compare SYP-1:HTTP-3 ratios for syg-1 RNAi gonads and control gonads: (1) ‘mean of experiments’: for each experiment, the average SYP-1:HTTP-3 ratios across all experiments. Note that this latter approach makes it possible to convey the variability in the control measurements in the corresponding graph.

Numbers of gonads assessed: experiment no. 1: control = 7 gonads, syg-1 RNAi = 7 gonads; experiment no. 2: control = 15 gonads, syg-1 RNAi = 6 gonads; experiment no. 3: control = 10 gonads, syg-1 RNAi = 8 gonads. Of the two approaches, the western blot analysis provides a somewhat lower estimate of residual SYP-1 levels, in part because the subset of worms with more severe SYP-1 depletion (which would have been excluded from our experiments) are included in the protein lysates. Thus, the immunofluorescence approach probably provides a better estimate of the residual SYP-1 levels present in the nuclei analysed in our experiments.

Because our data indicate that a 60–70% reduction in SYP-1 levels can lead to increased COSA-1-marked crossover sites and impaired interference, we also tested whether the syg-1 locus might be haploinsufficient. As we observed 6.02 ± 0.13 (mean ± s.d., n = 53) and 6.00 ± 0.13 (mean ± s.d., n = 114) GFP::COISA-1 foci per late pachytene nucleus of syg-1(me17)/+ and syg-1(ok7583)/+ worms, respectively, we conclude that reducing syg-1 gene dose by half is not sufficient to impair the robust crossover control system.

Immunofluorescence. Immunofluorescence experiments were performed as described previously29, with modifications. Gonads from adult worms at 18–24 h post-L4 stage were dissected. Slides were mounted with Vectashield (except for confocal images, which were mounted with Invitrogen ProLong Gold) and a
The document contains detailed experimental procedures and results related to the study of meiotic recombination in Caenorhabditis elegans. It describes the use of confocal microscopy and immunofluorescence techniques to analyze meiotic events such as synapsis and chiasma formation. The text includes methodologies for scoring chiasmata and the quantification of axis lengths and positions. It also mentions the use of statistical analyses, including linear regression, to interpret the data. The document references several experimental conditions and controls to validate the findings. Additionally, it discusses the use of specific markers, such as HIM-8 and COSA-1, for the analysis of meiotic events.

Key findings include the observation of chiasmata distribution and the examination of their frequency and distribution across different genetic backgrounds. The study also highlights the role of different markers and their interactions with each other in the regulation of meiotic processes. The text is rich with technical detail, including the use of specific software tools for data analysis and visualization. It is a comprehensive resource for understanding the molecular and cellular bases of meiotic behavior in C. elegans.
31. Zetka, M. C., Kawasaki, I., Strome, S. & Muller, F. Synapsis and chiasma formation in Caenorhabditis elegans require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes Dev. 13, 2258–2270 (1999).

32. Chen, H., Hughes, D. D., Chan, T. A., Sedat, J. W. & Agard, D. A. IVE (Image Visualization Environment): a software platform for all three-dimensional microscopy applications. J. Struct. Biol. 116, 56–60 (1996).
Extended Data Figure 1 | Quantification of SYP-1 partial depletion by attenuated RNAi. 

**a**, Representative western blot analysis of protein lysates from control, syp-1 partial RNAi and syp-1 null worms. A dilution series of control samples was used to estimate that the level of SYP-1 protein was reduced to approximately 25–30% of the control SYP-1 level following syp-1 partial RNAi under our experimental conditions. All panels shown are from the same membrane probed with indicated antibodies. Similar results were obtained for three independent experiments. 

**b**, Representative immunofluorescence images of late pachytene nuclei co-stained for SYP-1 (green), chromosome axis marker HTP-3 (red) and GFP::COSA-1 (blue), showing reduction of SYP-1 fluorescence relative to HTP-3 fluorescence and increase in GFP::COSA-1 foci in syp-1 partial RNAi nucleus compared to control nucleus. Except for right-most panels, images shown are sum projections through three-dimensional data stacks encompassing whole nuclei. For the first four pairs of control and syp-1 partial RNAi panels, identical exposure times and dynamic range settings for image display were used to highlight the reduction in the SYP-1:HTP-3 ratio. In the last two panels, SYP-1 signal levels were adjusted for syp-1 partial RNAi images to facilitate visualization of the SYP-1 tracts. Because some SCs from the top and bottom halves of the nuclei are superimposed in the full projections encompassing whole nuclei, partial projections showing half nuclei are also provided (right-most images). Scale bar, 2 μm. 

**c**, Graphs showing quantification of the reduction in SYP-1 fluorescence relative to HTP-3 fluorescence following syp-1 partial RNAi. Two different methods for analysing the data (see Methods) yield similar results, indicating that under the syp-1 partial RNAi conditions used for our experimental analysis, SYP-1 levels are reduced to approximately 30–40% of control levels. Error bars indicate s.d. Numbers of gonads assessed: experiment no. 1: control = 7 gonads, syp-1 RNAi = 7 gonads; experiment no. 2: control = 15 gonads, syp-1 RNAi = 6 gonads; experiment no. 3: control = 10 gonads, syp-1 RNAi = 8 gonads.
Extended Data Figure 2 | Partial depletion of SYPs increases numbers of COSA-1 foci. Graph depicting the mean numbers of GFP::COSA-1 foci per late pachytene nucleus detected following exposure to syp-1/F26D2.2, syp-2/C24G6.1 or syp-3/F39H2.4 RNAi or empty vector control. RNAi and control conditions were identical to those described in Methods, except that worms were dissected for immunofluorescence at 24 h post-L4 stage on RNAi or control plates at 25 °C. Error bars indicate s.d. Control nuclei had an average of six COSA-1 foci per nucleus and a very low standard deviation, indicating operation of the highly robust crossover control system. Partial RNAi treatment for any of the syp genes resulted both in a significant increase in the average number of GFP::COSA-1 foci >6 per nucleus (Mann–Whitney, two-tailed P > 0.0001 for syp-1, syp-2 and syp-3 RNAi) and in a much higher s.d., indicating impairment of crossover control. Numbers of nuclei counted were: control, n = 78; syp-1, n = 64; syp-2, n = 129; syp-3, n = 87.
Extended Data Figure 3 | GFP::COSA-1 foci in syp-1 RNAi nuclei correspond to inter-homologue crossovers. a, Quantification of chiasmata on the mnT12 bivalent in diakinesis-stage oocytes, showing that the incidence of chiasmata corresponds well with the incidence of GFP::COSA-1 foci observed at late pachytene (Fig. 2a). For bivalents with only one or two chiasmata, each individual chiasma was readily scored; bivalents with $\geq$ 3 chiasmata were pooled into a single category owing to their highly contorted structures, which in some cases made it difficult to discriminate whether 3, 4 or 5 chiasmata were present. In control oocytes, all mnT12 bivalents had one or two chiasmata. By contrast, 47% of syp-1 RNAi oocytes had mnT12 bivalents with $\geq$ 3 chiasmata, corresponding well with 49% of late pachytene syp-1 RNAi mnT12 having $\geq$ 3 GFP::COSA-1 foci. As formation of each chiasma requires an inter-homologue crossover event, the close correspondence between the numbers of GFP::COSA-1 foci at late pachytene and chiasmata at diakinesis on syp-1 RNAi mnT12 bivalents indicates that most, and probably all, GFP::COSA-1 foci in syp-1 RNAi late pachytene nuclei are marking inter-homologue crossovers.

Numbers of diakinesis nuclei scored: control, 86 nuclei from 35 gonads; syp-1 RNAi, 156 nuclei from 53 gonads. b, GFP::COSA-1 foci are not detected on asynapsed chromosome segments. In the regions of the mnT12 control and mnT12 syp-1 RNAi germ lines that were imaged for analyses of GFP::COSA-1 foci, we identified a subset of nuclei in which portions of the mnT12 fusion chromosome pair were asynapsed. These asynapsed segments were found at comparable frequencies among analysed nuclei from control (8%) and syp-1 RNAi (12%) germ lines and may represent early stages of desynapsis as cells transition from late pachynema to early diplonema. (Within these nuclei, the asynapsed mnT12 segments comprised approximately 18% of the total mnT12 axis length for control and 26% for syp-1 RNAi.) The mnT12 bivalents for all control (top left) and syp-1 RNAi (top right) nuclei in this category are represented in schematic form, with the chromosome axes (HTP-3, red) cartooned to depict both the approximate location and size of the asynapsed segment(s) relative to the total axis length, and the positions of GFP::COSA-1 (green) and HIM-8 (blue) foci. Notably, all GFP::COSA-1 foci on these partially asynapsed chromosome pairs were associated with synapsed segments, located either within a synapsed segment or at the boundary between a synapsed segment and an asynapsed segment; GFP::COSA-1 foci were never found on the asynapsed axis segments. Given the fraction of total axis length that was asynapsed in these syp-1 RNAi mnT12 nuclei, the observed restriction of GFP::COSA-1 foci to synapsed segments (where homologues are closely juxtaposed) represents a highly significant ($\chi^2$ test; $P = 0.0002$) departure from the distribution expected if GFP::COSA-1 foci were equally likely to occur on synapsed segments and asynapsed segments (where homologues are separated), consistent with the interpretation that these GFP::COSA-1 foci correspond to inter-homologue recombination events.
Extended Data Figure 4 | Distribution of GFP::COSA-1 foci among evenly spaced intervals along mnT12. a, Bar graph for eight-interval analysis of mnT12 (X;IV) fusion chromosomes, indicating the frequencies of GFP::COSA-1 foci in each interval for control (blue) and syp-1 partial RNAi (purple) worms. b, Table for eight-interval analysis indicating both the focus frequencies and the numbers of GFP::COSA-1 foci in each interval. c, Table for four-interval analysis indicating for each interval both the numbers and the percentages of mnT12 chromosome pairs with ≥ 1 GFP::COSA-1 focus in that interval (used for interference strength calculations in Fig. 3a and Extended Data Table 1).
Extended Data Figure 5 | Gamma probability distribution modelling of inter-COSA-1 focus distances. a, b, Histograms of the distribution of inter-focus distances (reported as percentage of total axis length) for binned control data \((n = 47)\) (a) and \textit{syp-1} RNAi data \((n = 183)\) (b). The best-fit gamma probability distribution curves generated from modelling the binned data sets (Fig. 3a) are overlaid on the histograms.
Extended Data Figure 6 | Association between local axis length and GFP::COSA-1 foci at 20 °C. Scatter plot of length measurements (μm) for the segment of *mnT12* chromosome axis from the left end of *mnT12* to the centre of the HIM-8 focus (as seen in Fig. 4d), for *spo-11/+* nuclei without (blue diamonds) or with (green diamonds) a GFP::COSA-1 focus in this chromosome segment and for the *spo-11* mutant (red triangles), which lacks meiotic DSBs and crossovers. Middle lines indicate mean and error bars indicate s.d. Mean length measurements for *spo-11* nuclei (0.31 μm, n = 92) and *spo-11/+* nuclei (0.35 μm, n = 88) lacking a focus in this chromosome segment were not significantly different from each other (Mann–Whitney, two-tailed $P = 0.062$), whereas both were significantly lower (Mann–Whitney, two-tailed $P = 0.0010$; $P = 0.0011$) than for *spo-11/+* nuclei that had a GFP::COSA-1 focus in this segment (0.83 μm, n = 4).
Extended Data Table 1 | Four-interval analysis of interference.

| control   | Interference Strength (I) | p-value (Fisher’s exact)* |
|-----------|---------------------------|---------------------------|
|           | AB | BC | CD | AC | BD | AD |
| Interference Strength (I) | 1  | 1  | 1  | 0.68 | 0.71 | -0.81 |
| p-value (Fisher’s exact)* | <0.0001 | 0.0033 | <0.0001 | 0.0115 | <0.0001 | <0.0001 |

| syv-f partial RNAi | Interference Strength (I) | p-value (Fisher’s exact)* |
|------------------|---------------------------|---------------------------|
|                  | AB | BC | CD | AC | BD | AD |
| Interference Strength (I) | 0.53 | 0.6 | 0.37 | -0.1 | -0.1 | 0.09 |
| p-value (Fisher’s exact)* | <0.0001 | <0.0001 | <0.0001 | 0.067 | 0.1448 | 0.2086 |

* P values from Fisher’s exact tests assessing the probability of obtaining the observed data set assuming independent behaviour of the two intervals.