1. Supplementary Discussion

2. Supplementary Table 2

## Supplementary Discussion

***Possible mechanistic explanations for defect conferred by top2 pathway mutants.***

As described in the text, very accurate BF best-fit simulations of all mutant data can be achieved by altering a single parameter of the beam-film model, (L), which describes the distance over which stress (relief) redistributes to either side of a CO-designation site. Thus, in the context of the BF model, the simplest mechanistic interpretation of the mutant phenotype is that the identified pathway is required for efficient redistribution of stress.

However, reasonably satisfactory BF best-fit simulations to mutant data can be obtained by varying certain other parameters.

- An increase in the maximum stress level (Smax) can give effects qualitatively similar to those conferred by a decrease in (L). In principel, changes in these two parameters have distinguishable effects: CoC relationships are more sensitive to changes in (L) while the average number and distribution of events is more sensitive to changes in (Smax) (discussion in ref. 8). However, in the present case, the mutant phenotype is sufficiently subtle that we cannot exclude an increase in Smax as the relevant effect. Nonetheless, we do not favor this possibility, for two reasons. First, Smax represents the "CO-designation driving force" which, presumably, is some type of biochemical effect; however, the mutations identified appear to involve chromosome structure and thus to act in *cis* rather than in *trans*. Second, an increase in Smax could potentially correspond to an increase in the time window available for CO-designation, e.g. as in the Drosophila inter-chromosomal effect[68].However, some mutations in the identified TopoII pathway confer no discernible alteration in the kinetics of progression through prophase (Extended Data Fig. 7 legend).

- A change in the sensitivity of precursors to stress (parameter (s) in the BF model) can change CO number and distribution with a concomitant change of $L_{CoC}$. Moreover, different effects can occur according to the response relationship between sensitivity and the level of stress (parameter (A) in the BF model). In the present case, a

two-fold reduction in (s) gives a reasonable fit to the CoC relationships and CO/bivalent distributions observed in *top2* pathway mutants.

***Reduced Gamma distribution shape parameter (ν) in top2 CO interference pathway mutants.***

Many people apply the gamma distribution to inter-focus (COs) data to evaluate the evenness of the foci distribution. A value of the gamma shape parameter (ν) >1 corresponds to even spacing (and thus positive CO interference) with higher ν values corresponding to more even spacing (and thus ‘‘stronger’’ CO interference). The gamma distribution analysis is also useful in the context of BF simulations: variations in patterning parameters (e.g. L or Smax) will coordinately change $L_{CoC}$ and ν (ref. 8).

We note however that a change in the gamma distribution shape parameter can be misleading although it is independent of models (more discussion in ref. 8). For example: (1) a CO maturation defect that occurs after CO designation has no effect on interference and thus does not affect CoC relationships but can significantly reduce the value of ν; (2) a moderate change in the precursor number does not affect the interference distance or CoC relationships but can change the value of ν. This is observed in *tel1Δ* with ~50% more DSBs than WT and no change in CoC relationships (Extended Data Fig. 7), and ν is 5.5 in *tel1Δ* vs 5.0 in WT. Furthermore these effects can be demonstrated in BF simulations (more details in ref. 8).

We have also applied the gamma distribution to the array of inter-CO (Zip3-focus) distances in WT and mutant data sets. If the array of mutant phenotypes described here does indeed reflect an alteration in patterning, the value of the gamma distribution shape parameter, ν, should be reduced (above)[8]. For the entire subset of strains described in text Fig. 4b that exhibit a WT Zip3 focus pattern (black, grey, green), ν = 5.05±0.13 whereas the subset of strains exhibiting the mutant Zip3 focus pattern (other colors) have ν = 4.65±0.09 as determined from experimental data. Analogously, in corresponding best-fit BF simulations, ν = 5.6±0.12 and ν = 5.0±0.08, respectively. These are the differences expected from an underlying alteration in patterning of COs (Zip3 foci).

***CO patterning in C.elegans.***

Recent findings by Villeneuve and colleagues in *C. elegans*[42] provide interested data regarding CO-related events that can be further considered in relation to the findings presented here.

1. It was demonstrated that depletion of an SC component (Syp-1) results in an increase in the number of COs and thus a decrease in inter-CO distance. Such an effect is analogous to the effects described for the TopoII pathway in the present work and may reflect a decrease in the distance over which the interference signal spreads (but see also above Supplementary Discussion).

2. It was emphasized that involvement of the SC in *C. elegans* contrasts with absence of SC involvement in budding yeast. The difference between the two cases could be only a consequence of biology: in budding yeast, CO patterning arises before the SC forms, and thus does not use the SC, whereas, in *C. elegans*, CO patterning arises after the SC forms and the SC is correspondingly involved.

More specifically: we have proposed that interference involves redistribution of mechanical stress along chromosome axes. Documentation of a role for the SC in *C. elegans* CO interference is fully consistent with this proposal. The important feature in our model is the mechanical robustness of the axes, i.e. the ability of the axes to transmit mechanical stress over long distances regardless of whether homolog axes are linked periodically by inter-axis bridges before SC formation (as in yeast) or via the SC (as in *C.elegans*).

3. The *C. elegans* study also shows that chromosome axes become extended at/around the sites of COs. We have suggested previously (refs 4 and 14) and again in the present study (above) that, immediately prior to CO-designation, chromosome axes are under mechanical stress due to chromatin expansion that is constrained by the axis meshwork (giving "chromatin meshwork stress"). In this model, CO-designation would be promoted by that constrained expansion. Furthermore, a stress-promoted event results

in alleviation of stress. Local extension of the chromosome axes as observed in *C. elegans* would be one way to alleviate such stress.

We also note that Villeneuve and colleagues[42] suggest that CO-designation requires local axis expansion but that CO-designation is not a stress-promoted event. However, a stress-promoted event can only occur if stress can be locally alleviated to give a lower energy state. Thus, a stress and stress relief model in which local axis expansion alleviates stress would automatically also imply that CO-designation will require local axis expansion.

4. Villeneuve and colleagues[42] propose that local chromatin expansion causes "crowding of axis/SC subunits in flanking regions....thereby reducing the capacity for axis expansion at [affected] sites". Therefore, their proposed model seems to envision expansion-mediated pushing of subunits from the expanded region outward. If so, this model implicitly involves interference by redistribution of mechanical (pushing) stress outward from the position of local expansion, dissipating with distance as is characteristic of stress redistribution in complex elastic systems.

The proposed model further suggests that the density of SC components determines whether axes can or cannot expand (and thus whether a CO-designation event occurs or not). However, the presented results also show that depletion of Syp-1 does not result in any increase in the extent of axis expansion per CO-designation. This finding appears to contradict the motivating idea that the density of SC components (Syp-1) determines the probability of expansion-driven CO-designation.

In summary: the results of Villeneuve and colleagues are consistent with a fully mechanical stress and stress relief model.

**Supplementary References:**

68. Joyce, E. F. & McKim, K. S. Meiotic checkpoints and the interchromosomal effect on crossing over in Drosophila females. *Fly (Austin)* **5**, 134-140 (2011).

Supplementary Table 2. Genetic crossover interference on three chromosomes in WT, *sir2RK* and *slx5Δ*.

A. Chromosome III

| Genotype | Reference interval: | | *his4-leu2* | | *Leu2-CEN3* | | *CEN3-MAT* | |
|---|---|---|---|---|---|---|---|---|
| | Test interval: | | *Leu2-CEN3* | *CEN3-MAT* | *His4-leu2* | *CEN3-MAT* | *His4-leu2* | *Leu2-CEN3* |
| WT | P | P:N:T | 312:0:93 | 250:0:157 | 312:2:169 | 281:2:200 | 250:2:110 | 281:1:79 |
| | | cM | 11.5 | 19.3 | 18.7 | 21.9 | 16.9 | 11.8 |
| | T+N | P:N:T | 171:1:23 | 112:3:80 | 93:0:24 | 81:1:36 | 157:0:83 | 202:0:37 |
| | | cM | 7.4 | 25.1 | 10.3 | 17.9 | 17.3 | 7.7 |
| | sig.(p) | | 0.0012 | 0.026 | 0.0048 | 0.081 | 0.21 | 0.084 |
| | ratio | | 0.63 | 1.3 | 0.55 | 0.82 | 1.02 | 0.65 |
| *sir2RK* | P | P:N:T | 371:1:101 | 323:3:147 | 371:6:225 | 408:8:186 | 323:5:167 | 408:1:86 |
| | | cM | 11.3 | 17.4 | 21.7 | 19.4 | 19.9 | 9.3 |
| | T+N | P:N:T | 231:0:28 | 172:6:81 | 102:1:27 | 87:1:42 | 150:2:85 | 194:0:43 |
| | | cM | 5.4 | 22.6 | 12.7 | 18.5 | 20.5 | 9.1 |
| | sig.(p) | | 0.00067 | 0.16 | 0.0008 | 0.82 | 0.84 | 0.66 |
| | ratio | | 0.48 | 1.30 | 0.59 | 0.95 | 1.03 | 0.98 |
| *Slx5Δ* | P | P:N:T | 96:2:25 | 79:1:43 | 96:1:43 | 92:0:48 | 79:0:41 | 92:1:27 |
| | | cM | 15 | 19.9 | 17.5 | 17.1 | 17.1 | 13.8 |
| | T+N | P:N:T | 44:1:13 | 41:0:17 | 27:0:14 | 28:1:12 | 44:1:16 | 48:2:11 |
| | | cM | 16.4 | 14.7 | 17.1 | 22 | 18 | 18.9 |
| | sig.(p) | | 0.95 | 0.50 | 0.72 | 0.20 | 0.21 | 0.41 |
| | ratio | | 1.09 | 0.74 | 0.98 | 1.28 | 1.05 | 1.37 |

Supplementary Table 2, continued.

B. Chromosome VII

| Genotype | Reference interval: | | *lys5-met13* | | *met13-cyh2* | | *cyh2-trp5* | |
|---|---|---|---|---|---|---|---|---|
| | Test interval: | | *met13-cyh2* | *cyh2-trp5* | *lys5-met13* | *cyh2-trp5* | *lys5-met13* | *met13-cyh2* |
| WT | P | P:N:T | 275:1:107 | 135:18:230 | 274:2:181 | 304:0:76 | 135:3:84 | 157:1:64 |
| | | cM | 14.8 | 44.1 | 21.1 | 45.8 | 23 | 15.8 |
| | T+N | P:N:T | 186:0:33 | 87:7:125 | 104:2:24 | 61:3:67 | 248:3:129 | 304:0:76 |
| | | cM | 7.5 | 38.1 | 12.8 | 32.4 | 19.3 | 10 |
| | sig.(p) | | 0.0007 | 0.42 | 1.3e-5 | 1.3e-12 | 0.48 | 0.017 |
| | ratio | | 0.51 | 0.86 | 0.6 | 0.7 | 0.83 | 0.63 |
| *sir2RK* | P | P:N:T | 332:0:128 | 162:11:288 | 332:5:205 | 185:12:345 | 162:4:96 | 185:1:75 |
| | | cM | 13.9 | 38.4 | 21.7 | 38.5 | 22.9 | 15.5 |
| | T+N | P:N:T | 210:10:51 | 100:5:166 | 128:6:55 | 76:4:109 | 299:7:164 | 357:9:104 |
| | | cM | 20.5 | 36.2 | 24.1 | 35.2 | 21.9 | 16.8 |
| | sig.(p) | | 1.8e-06 | 0.81 | 0.02 | 0.33 | 0.89 | 0.03 |
| | ratio | | 1.47 | 0.94 | 1.11 | 0.91 | 0.96 | 1.08 |
| *Slx5Δ* | P | P:N:T | 59:1:32 | 26:4:62 | 59:2:60 | 33:5:83 | 26:2:22 | 33:1:16 |
| | | cM | 20.7 | 46.7 | 29.8 | 46.7 | 34 | 32 |
| | T+N | P:N:T | 62:5:22 | 24:5:60 | 33:3:24 | 17:4:39 | 66:3:62 | 88:5:38 |
| | | cM | 29.2 | 50.6 | 35 | 52.5 | 30.5 | 26 |
| | sig.(p) | | 0.09 | 0.92 | 0.27 | 0.75 | 0.79 | 0.77 |
| | ratio | | 1.41 | 1.08 | 1.17 | 1.12 | 0.9 | 1.18 |

Supplementary Table 2, continued.

C. Chromosome VIII

| Genotype | Reference interval: | | *CEN8-arg4* | | *arg4-thr1* | | *thr1-cup1* | |
|---|---|---|---|---|---|---|---|---|
| | Test interval: | | *arg4-thr1* | *thr1-cup1* | *CEN8-arg4* | *thr1-cup1* | *CN8-arg4* | *arg4-thr1* |
| WT | P | P:N:T | 334:1:86 | 184:2:236 | 342:1:128 | 196:2:265 | 191:1:79 | 195:1:66 |
| | | cM | 10.9 | 29.4 | 14.2 | 29.9 | 15.7 | 13.7 |
| | T+N | P:N:T | 127:1:5 | 88:0:59 | 101:1:8 | 76:0:30 | 252:1:57 | 266:1:25 |
| | | cM | 4.1 | 20.1 | 6.4 | 14.2 | 10.2 | 5.3 |
| | sig.(p) | | 1.8e-06 | 0.0019 | 4.6e-07 | 1.9e-07 | 0.0092 | 6.4e-07 |
| | ratio | | 0.38 | 0.68 | 0.45 | 0.47 | 0.65 | 0.39 |
| *sir2RK* | P | P:N:T | 434:2:118 | 254:13:287 | 434:0:147 | 264:11:306 | 254:1:107 | 264:1:97 |
| | | cM | 11.7 | 32.9 | 12.7 | 32 | 15.6 | 14.2 |
| | T+N | P:N:T | 147:3:27 | 108:1:68 | 120:3:27 | 98:3:49 | 300:2:67 | 317:4:48 |
| | | cM | 12.7 | 20.9 | 15 | 22.3 | 10.7 | 9.8 |
| | sig.(p) | | 0.05 | 0.009 | 0.002 | 5.3e-05 | 0.001 | 7.5e-06 |
| | ratio | | 1.09 | 0.64 | 1.18 | 0.70 | 0.68 | 0.69 |
| *Slx5Δ* | P | P:N:T | 84:0:47 | 51:8:72 | 84:4:38 | 58:4:64 | 51:4:30 | 58:0:26 |
| | | cM | 17.9 | 45.8 | 24.6 | 34.9 | 31.8 | 15.5 |
| | T+N | P:N:T | 38:0:8 | 31:1:16 | 47:0:8 | 26:5:24 | 80:0:17 | 68:0:29 |
| | | cM | 8.7 | 23.4 | 7.3 | 49.1 | 8.8 | 14.9 |
| | sig.(p) | | 0.054 | 0.0079 | 0.012 | 0.24 | 0.00059 | 0.99 |
| | ratio | | 0.49 | 0.51 | 0.30 | 1.41 | 0.28 | 0.96 |

Supplementary Table 2, continued. Genetic crossover interference was analyzed on three chromosomes by using of the method as described in ref. 21. Each time, one interval was used as a reference interval, and tetrads were divided into two groups according there is a crossover in this reference interval or not (i.e. P or N+T). And other intervals were test intervals. Then, for each test interval, the distribution of tetrad types was compared between the two groups (P or N+T) and the difference level with statistical significance (sig.) was assessed by G test with $p<0.05$. Map distances (cM) were calculated using the web site tool (http://www.molbio.uoregon.edu/~fstahl/). And the ratio of the two map distances from N+T and P groups was calculated as a measurement for the level of the CO interference. A higher ratio means a lower interference level. P, parental ditype; N, nonparental ditype; T, tetratype.