

Genotype sex (Col/Ler F1)	Number of cells	Total number of MLH1 foci	Total number of HEI10 foci	Total number of MLH1/HEI10 cofoci	% MLH1 colocalization with HEI10	% HEI10 colocalization with MLH1	Co-foci/cell
<i>wild type female</i>	12	86	83	82	95,3	98,8	6,8
<i>wild type male</i>	28	300	290	290	96,7	100,0	10,4
<i>zyp1 female</i>	12	150	147	147	98,0	100,0	12,3
<i>zyp1 male</i>	27	344	342	342	99,4	100,0	12,7
<i>HEI10oe female</i>	17	204	202	202	99,0	100,0	11,9
<i>HEI10oe male</i>	34	627	626	624	99,5	99,7	18,4
<i>recq4 female</i>	21	147	147	147	100,0	100,0	7,0
<i>recq4 male</i>	19	186	186	186	100,0	100,0	9,8
<i>zyp1 HEI10oe female</i>	26	817	789	774	94,7	98,1	29,8
<i>zyp1 HEI10oe male</i>	26	774	762	761	98,3	99,9	29,3
<i>recq4 HEI10oe female</i>	13	156	156	156	100,0	100,0	12,0
<i>recq4 HEI10oe male</i>	27	508	516	505	99,4	97,9	18,7
<i>zyp1 recq4 female</i>	15	197	188	187	94,9	99,5	12,5
<i>zyp1 recq4 male</i>	23	291	291	291	100,0	100,0	12,7
<i>zyp1 recq4 HEI10oe female</i>	22	426	414	412	96,7	99,5	18,7
<i>zyp1 recq4 HEI10oe male</i>	67	1264	1230	1195	94,5	97,2	17,8

Table S1. Analysis of MLH1-HEI10 foci co-localization at diplotene-diakinesis

Raw data can be found in Source_data_Figure2_HEI10 MLH1 counting - coller.xlsx

lab stock	Mutants	PCR WT primer	PCR mutant primer	size wt band	size mutant band
K#288	<i>zyp1-1 (col)</i>	288A+288B Digestion Mbol		182bp+128bp	204bp+128bp
K#293	<i>zyp1-6 (ler)</i>	288A+288B	292A+292B	503bp	1273bp
K#231	<i>recq4a-4 (col)</i>	158A+158B	158B+K5GABI_o8409	900bp	600bp
	<i>recq4b-2 (col)</i>	230A+230B	230C+K3_LB-Salk2	1000bp	800bp
K#249	<i>recq4a-W387X (ler)</i>	249A+249B Digestion ScrF1		150bp	180bp
K#133	<i>mus81-2 (col)</i>	133A+133B	133B+K3_LB-Salk2	1000bp	750bp
K#166	<i>figl1-19 (col)</i>	166C+166D	166C+K3_LB-Salk2	675bp	800bp
K#198	<i>figl1-12 (Ler)</i>	198A+198B Digestion Pfo1		253bp+71bp+45bp	298bp+71bp
K#244	HEI10oe (C2) (Col)	244E+F	244A+B	496pb	1000pb

	identification primer	PCR product size
Col/ler polymorphism	NGA151F+R	Col=160bp Ler =120bp

Primer name	Sequence(5'-3')
288A	ATAGATCGATTTTCGTCATCT
288B	GAGGTGAAATATGAATCTGCT
292A	TCCAGTTATTACCCTGTTTCTGT
292B	AGTGAGGATGATCGGCCATT
158A	ATCAGAGCCCACTCATTGTTG
158B	GTCCTGATCGTGTGGACAG
K5_GABI_o8409	ATATTGACCATCATACTCATTGC
249A	GACCAAGGCAGATATGCCTGTGATACCTG
249B	GTCCAGGGAAATTCACGACTGCTC
230A	TCAGAAAGTTGCTCTGCGTC
230B	ACCAAGACCCTGCATATTGC
230C	ACTAGAGATACTTCAGGAGCTGAGC
K3_LB-Salk2	GCTTTCTTCCCTTCCTTTCTC
133A	CATGCTGACAGTTGAAGGTC
133B	CCTCAAACGTTTCTCCAAAT
244A	TATTTCTGTGCTTGAGCTTAGTGC
244B	CAGTCACGACGTTGTAAACGACG
244E	TAATGTAATAAAATAAGAACC
244F	ACTCTTTCATCTAATTTCTTC
NGA151F	GTTTTGGGAAGTTTGTCTGG
NGA151R	CAGTCTAAAAGCGAGAGTATGATG
198A	GCATCTTTCACCAAGTTCTTC
198B	TGGTAAAGAATGATAACAAA
166C	AAGGGCAGAGACAGGCATAG
166D	CCGTGCCTCTGATGAATGTG

Table S2. Genotyping primers

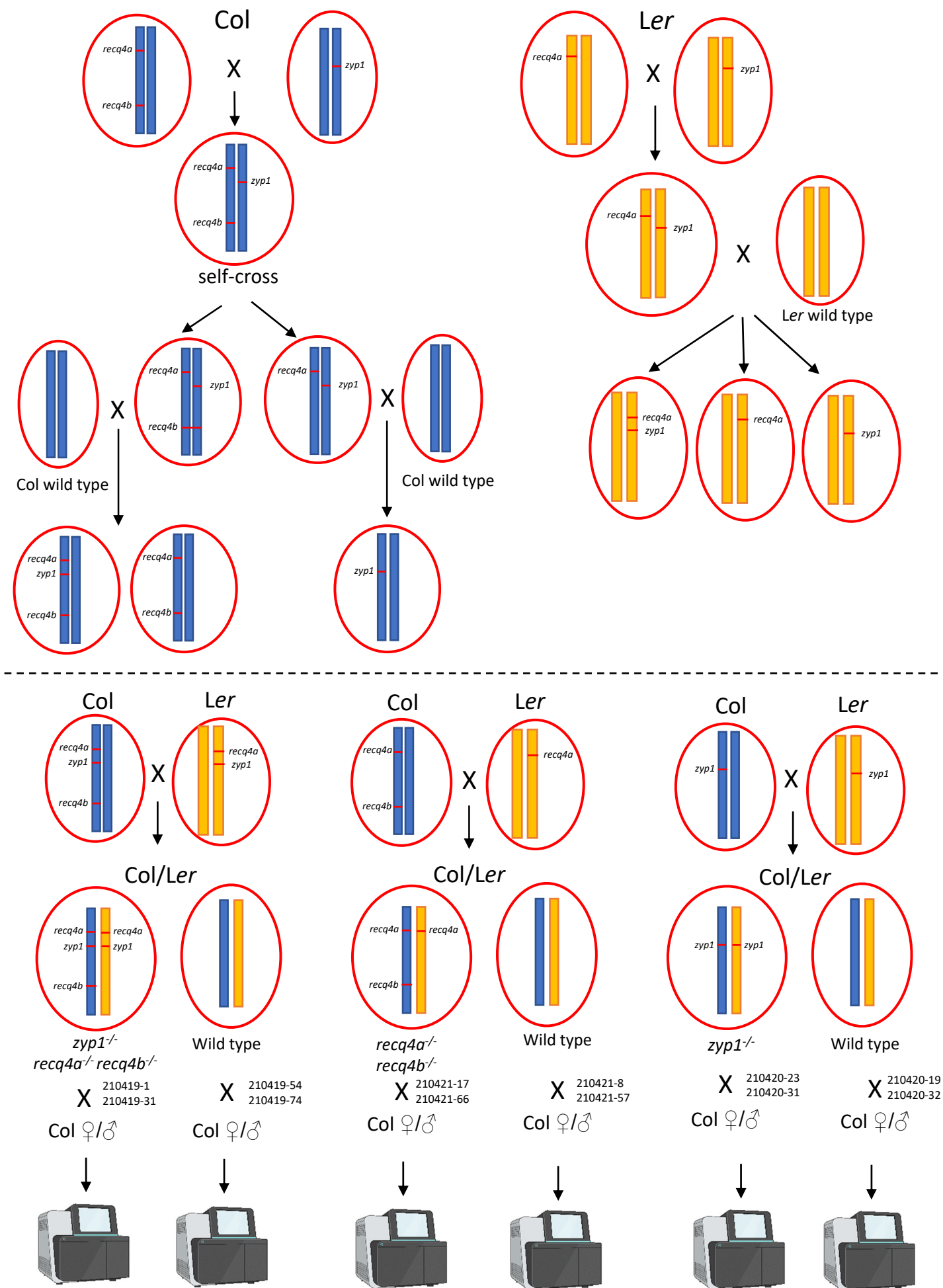


Fig S1.

Fig S1. Generation of *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-}*(Col/Ler), *recq4a^{-/-} recq4b^{-/-}* (Col/Ler), *zyp1^{-/-}* (Col/Ler) and their respective wild type (Col/Ler) controls.

zyp1^{+/-}(Col) plants were crossed with *recq4a^{+/-} recq4b^{+/-}*(Col) plants to get *zyp1^{+/-} recq4a^{+/-} recq4b^{+/-}*(Col), and then self-crossed to get *zyp1^{+/-} recq4a^{+/-} recq4b^{-/-}*(Col) and *recq4a^{+/-} zyp1^{+/-}*(Col) plants. Note that ZYP1, RECQ4A and RECQ4B are all located on chromosome 1, to make sure the mutation of the three genes are all on the same chromosome, *zyp1^{+/-} recq4a^{+/-} recq4b^{-/-}*(Col. *zyp1*, *recq4a*, *recq4b* mutation in trans) were back crossed with Col wild type to get *zyp1^{+/-} recq4a^{+/-} recq4b^{+/-}*(Col. *zyp1*, *recq4a* and *recq4b* in cis) and *recq4a^{+/-} recq4b^{+/-}*(Col. *recq4a*, *recq4b* in cis); *recq4a^{+/-} zyp1^{+/-}*(Col, *recq4a* and *zyp1* in trans) were crossed with Col wild type to get *zyp1^{+/-}*(Col). *zyp1^{+/-}*(Ler) were crossed with *recq4a^{+/-}*(Ler) to get *zyp1^{+/-} recq4a^{+/-}*(Ler, *zyp1* and *recq4* in trans), then *zyp1^{+/-} recq4a^{+/-}*(Ler, *zyp1* and *recq4* in trans) were backcrossed with wild type (Ler) to get *zyp1^{+/-} recq4a^{+/-}*(Ler, *zyp1* and *recq4* in cis), *recq4a^{+/-}*(Ler) and *zyp1^{+/-}*(Ler). Then *zyp1^{+/-} recq4a^{+/-} recq4b^{+/-}* (Col, *zyp1*, *recq4a* and *recq4b* in cis) were crossed with *zyp1^{+/-} recq4a^{+/-}* (Ler, *zyp1* and *recq4a* are in cis) to get *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-}*(Col/Ler) and sister wild type(Col/Ler) control; *recq4a^{+/-} recq4b^{+/-}* (Col) were crossed with *recq4a^{+/-}* (Ler) to get *recq4a^{-/-} recq4b^{-/-}* (Col/Ler) and sister wild type(Col/Ler) control; *zyp1^{+/-}*(Col) were crossed with *zyp1^{+/-}*(Ler) plants to get *zyp1^{-/-}*(Col/Ler) and sister wild type(Col/Ler) control. Created in BioRender. Lian, Q. (2025) <https://BioRender.com/m7vjhfg>

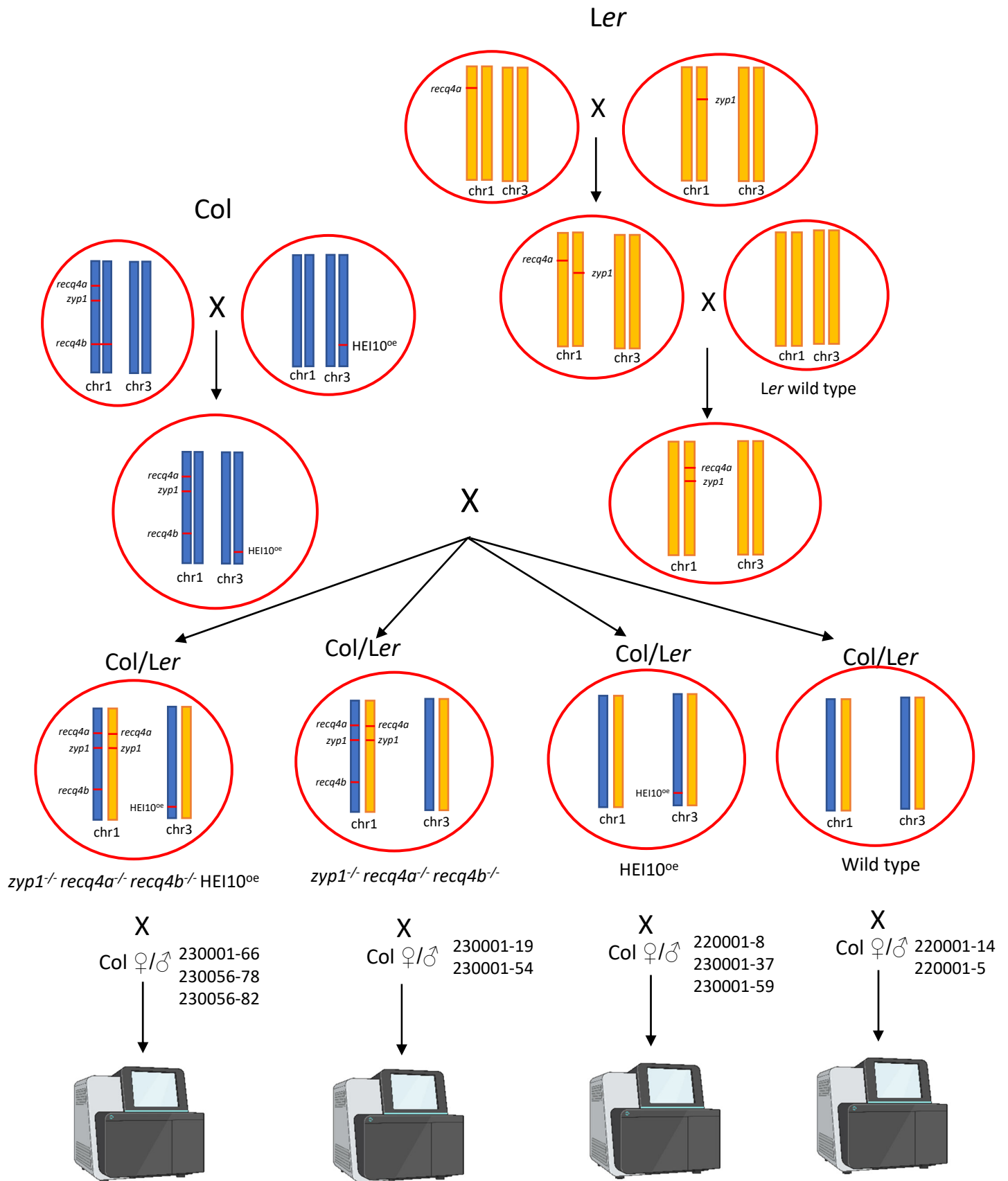


Fig S2. Generation of *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} HEI10^{oe} (Col/Ler), *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} (Col/Ler), HEI10^{oe} (Col/Ler) and wild type (Col/Ler)

zyp1^{+/-} *recq4a*^{+/-} *recq4b*^{-/-} (Col) were crossed with HEI10^{oe} (Col) to get *zyp1*^{+/-} *recq4a*^{+/-} *recq4b*^{-/-} HEI10^{oe} (Col). *zyp1*^{+/-} *recq4a*^{+/-} *recq4b*^{-/-} HEI10^{oe} (Col) were crossed with *zyp1*^{+/-} *recq4a*^{+/-} (Ler, *zyp1* and *recq4a* in cis) to get *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} HEI10^{oe} (Col/Ler), *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} (Col/Ler), HEI10^{oe} (Col/Ler) and wild type. Created in BioRender. Lian, Q. (2025) <https://BioRender.com/m7vjhfg>.

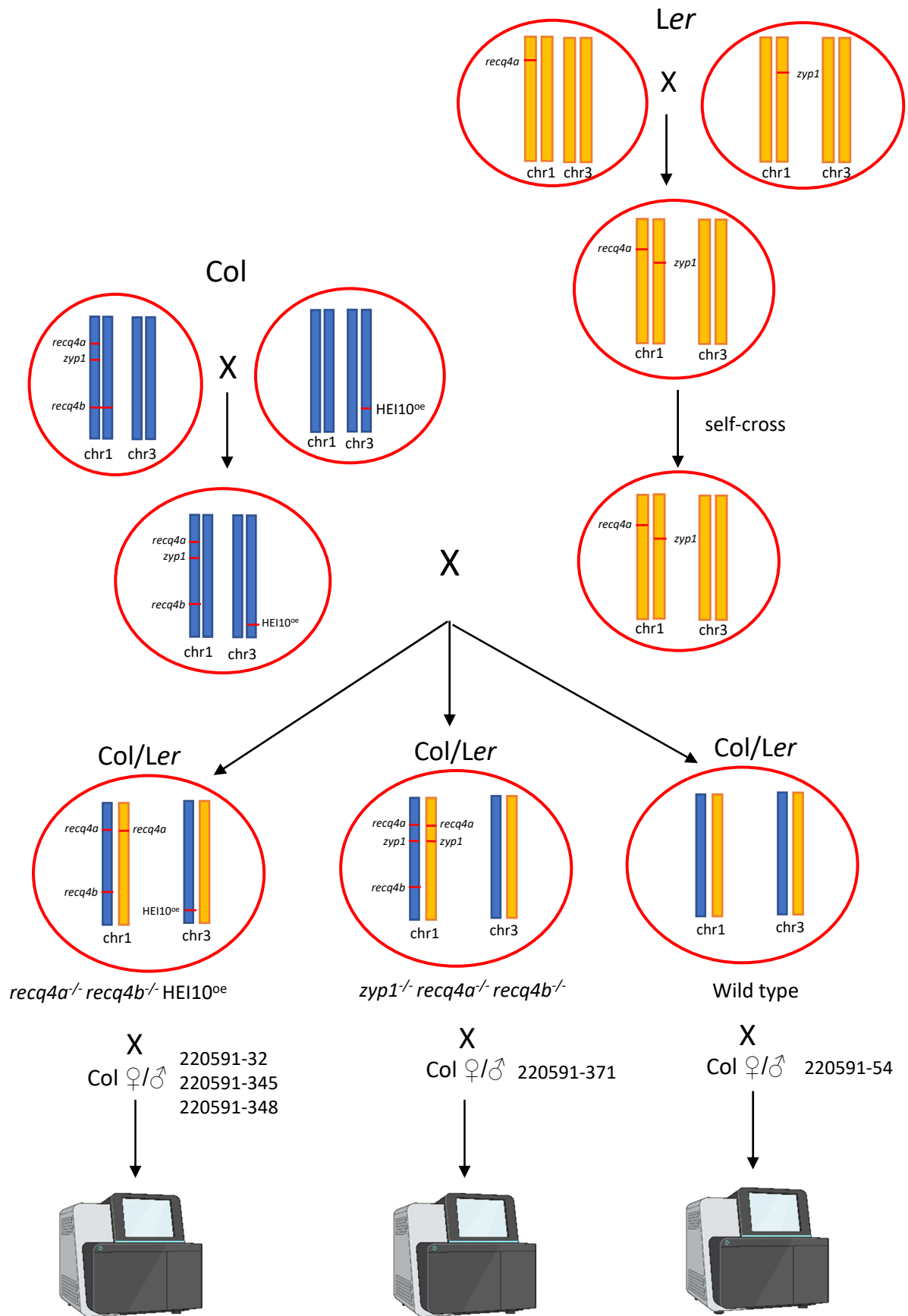


Fig S3. Generation of *recq4a*^{-/-} *recq4b*^{-/-} *HEI10^{oe}* (Col/Ler), *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} (Col/Ler), wild type (Col/Ler).

zyp1^{+/+} *recq4a*^{+/+} *recq4b*^{-/-} (Col) were crossed with *HEI10^{oe}* (Col) to get *zyp1*^{+/+} *recq4a*^{+/+} *recq4b*^{-/-} *HEI10^{oe}* (Col). *zyp1*^{+/+} *recq4a*^{+/+} *recq4b*^{+/+} *HEI10^{oe}* (Col, *zyp1*, *recq4a* and *recq4b* in cis) were crossed with *zyp1*^{+/+} *recq4a*^{+/+} (*Ler*, *zyp1* and *recq4a* in trans) to get *recq4a*^{-/-} *recq4b*^{-/-} *HEI10^{oe}* (Col/Ler), *zyp1*^{-/-} *recq4a*^{-/-} *recq4b*^{-/-} (Col/Ler) and wild type (Col/Ler). Created in BioRender. Lian, Q. (2025) <https://BioRender.com/m7vjhfg>

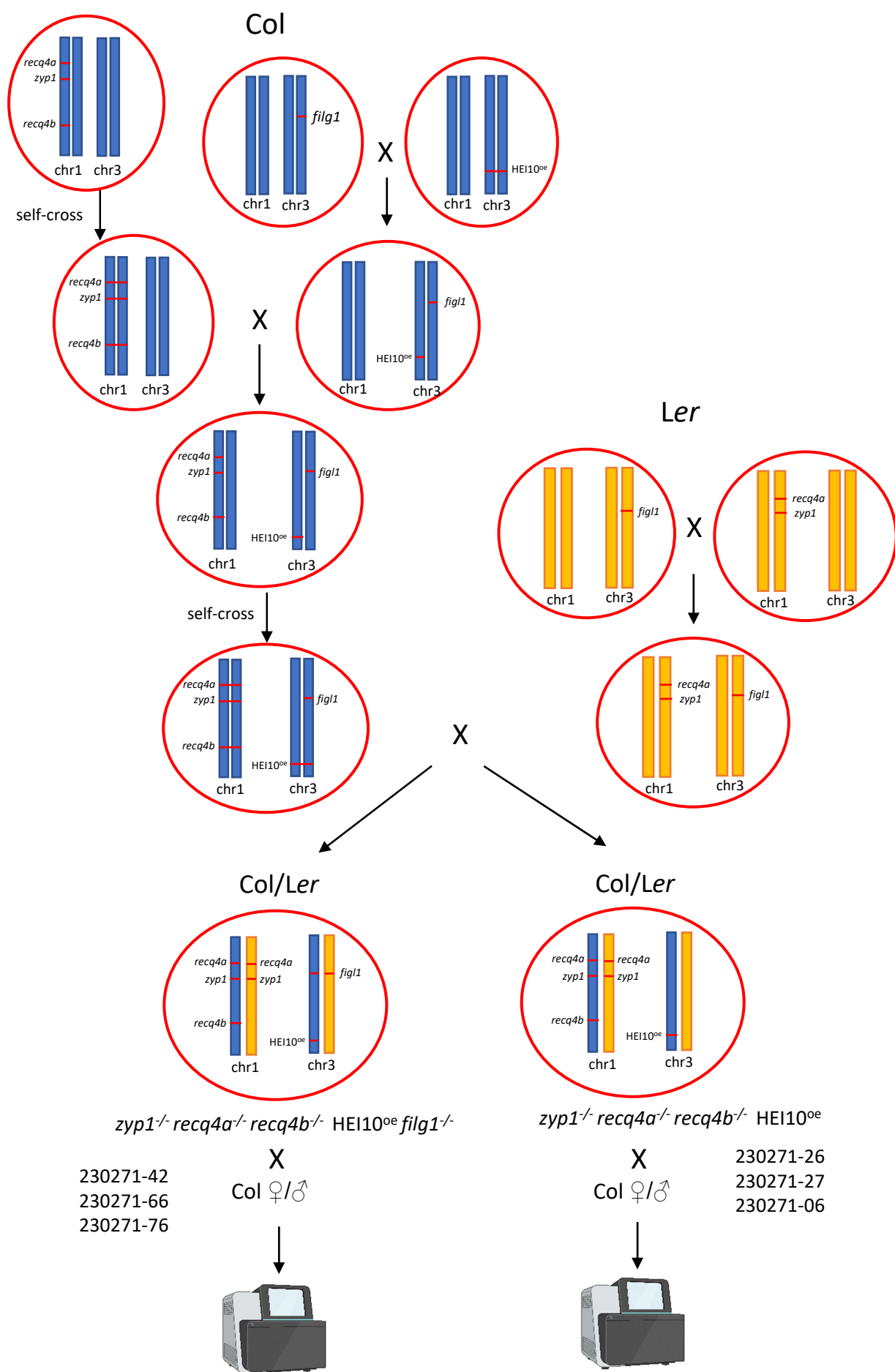


Fig S4.

Fig S4. Generation of *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe} figl1^{-/-} (Col/Ler)*, and *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe} (Col/Ler)*.

zyp1^{+/-} recq4a^{+/-} recq4b^{+/-} (Col. *zyp1*, *recq4a* and *recq4b* in cis) were self-crossed to get *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-}* (Col), *figl1^{+/-}* (Col) were crossed with HEI10^{oe} homo (Col) to get HEI10^{oe}hz *figl1^{+/-}* (Col). Then *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-}* (Col) were crossed with HEI10^{oe}hz *figl1^{+/-}* (Col, HEI10^{oe}hz and *figl1* in trans) to get *zyp1^{+/-} recq4a^{+/-} recq4b^{+/-} HEI10^{oe}hz figl1^{+/-}* (Col. *zyp1*, *recq4a* and *recq4b* in cis, HEI10^{oe}hz and *figl1* in trans we don't know), then this plant was self-crossed to get *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe}homo figl1^{+/-}* (Col). *zyp1^{+/-} recq4a^{+/-}* (Ler, *zyp1* and *recq4a* in cis) were crossed with *figl1^{+/-}* (Ler) to get *zyp1^{+/-} recq4a^{+/-} figl1^{+/-}* (Ler, *zyp1* and *recq4a* in cis). Then *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe}homo figl1^{+/-}* (Col) were crossed with *zyp1^{+/-} recq4a^{+/-} figl1^{+/-}* (Ler, *zyp1* *recq4a* in cis) to get *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe}hz figl1^{-/-}* (Col/Ler) and *zyp1^{-/-} recq4a^{-/-} recq4b^{-/-} HEI10^{oe} hz* (Col/Ler) (Fig. S4). Created in BioRender. Lian, Q. (2025) <https://BioRender.com/m7vjhfg>.

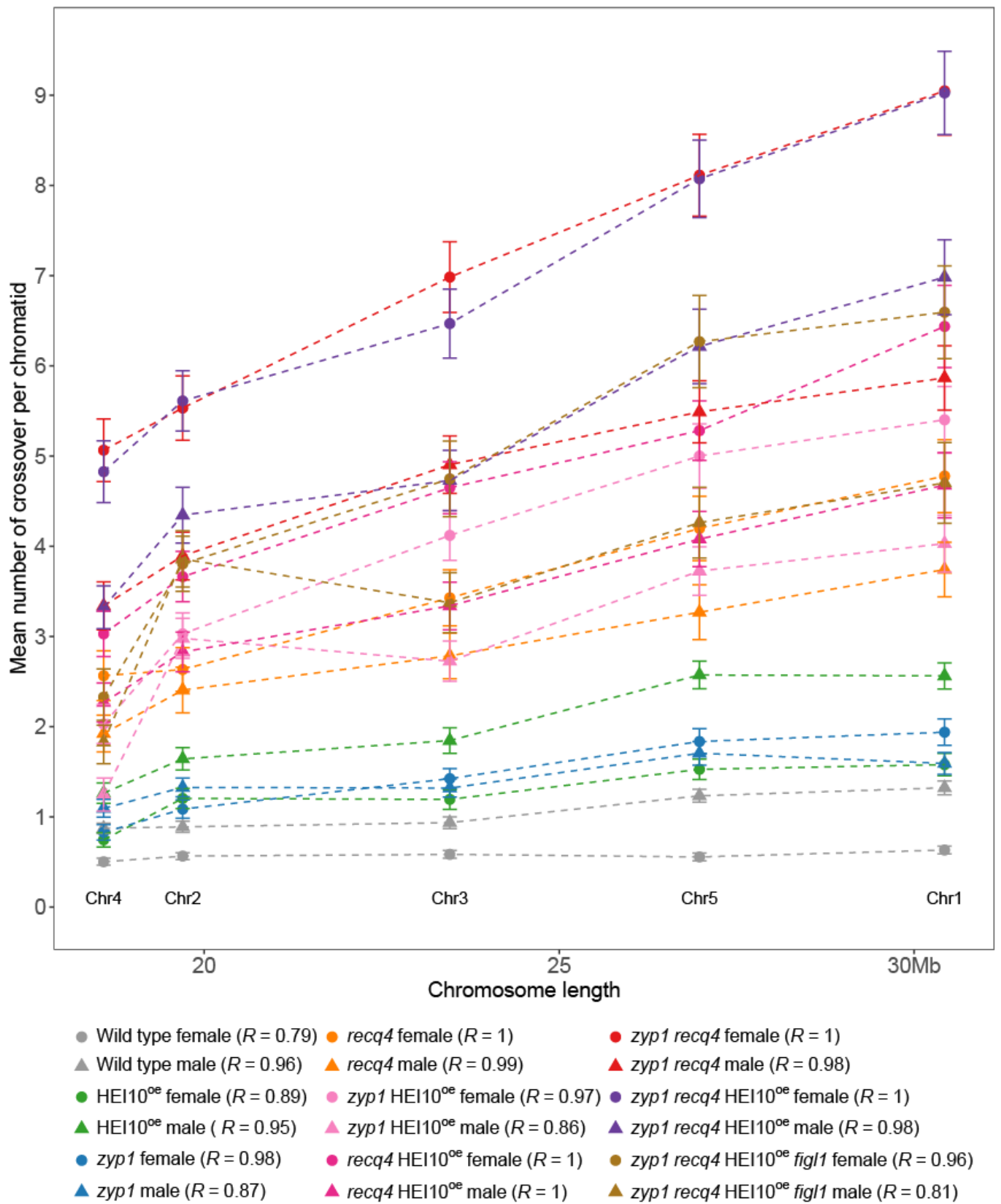


Fig S5. Correlation analysis between mean number of COs per transmitted chromatid and chromosome size.



Fig S6. Chromosome 1



Fig S6. Chromosome 2

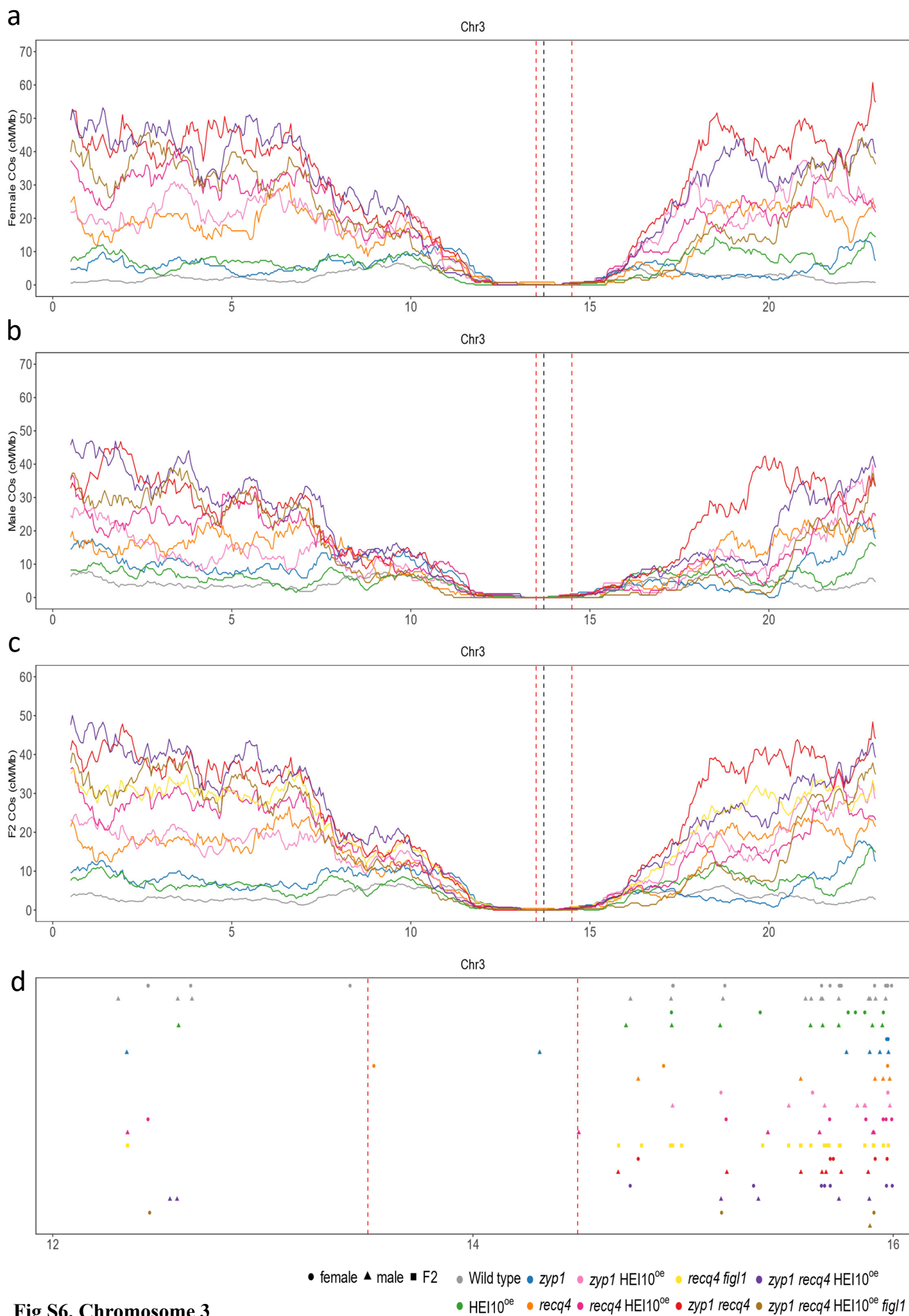


Fig S6. Chromosome 3



Fig S6. Chromosome 4

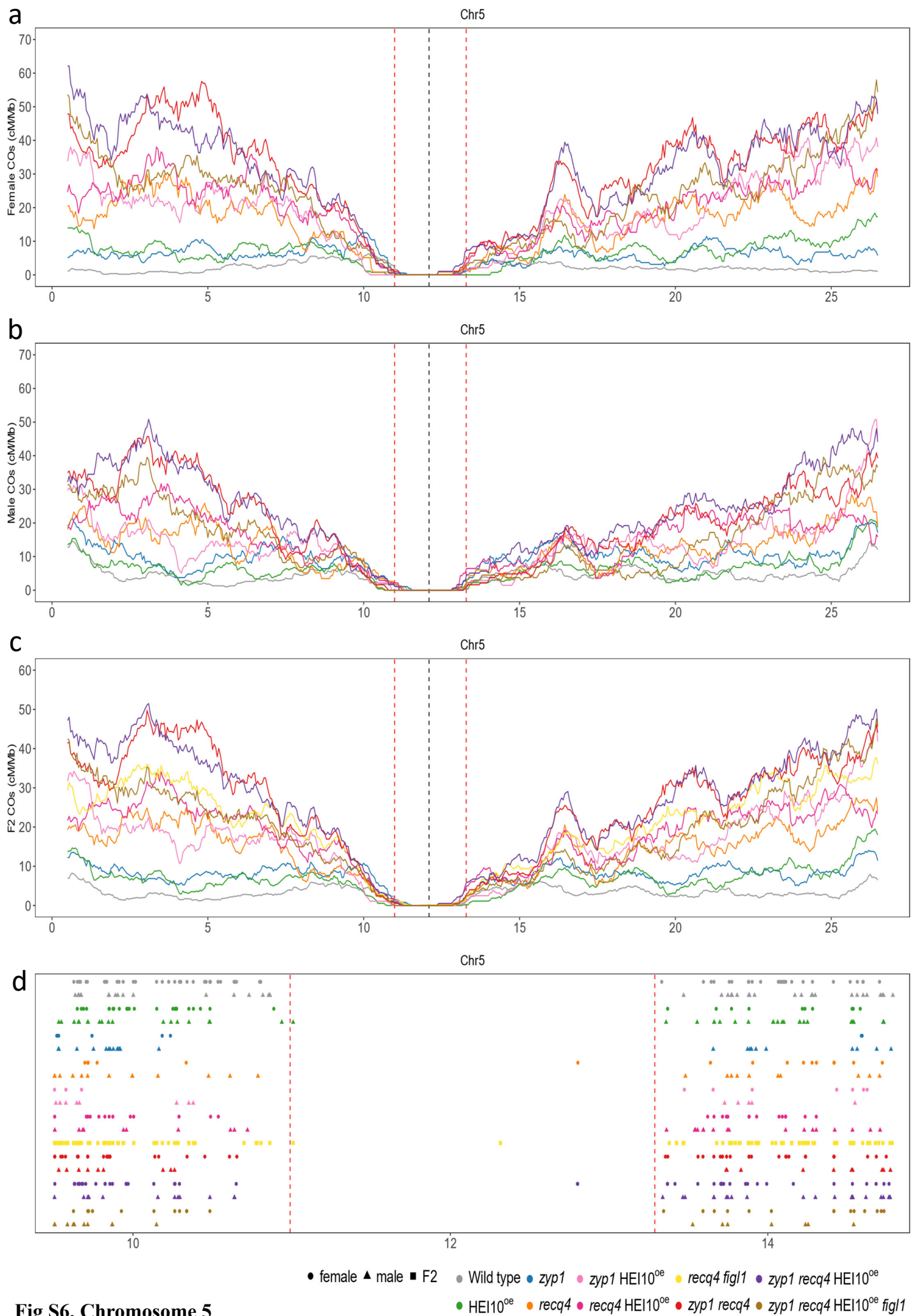


Fig S6. Chromosome 5

Figure S6. Chromosomal distribution of COs in female, male, and F2 contexts.

(zoom of figure 4)

a-b. The distribution (sliding window-based, window size 1 Mb, step size 50 kb) of COs along chromosomes in female (**a**) and male (**b**) of wild type, *HEI10^{oe}*, *zyp1*, *recq4*, *zyp1 HEI10^{oe}*, *recq4 HEI10^{oe}*, *zyp1 recq4*, *zyp1 recq4 HEI10^{oe}* and *zyp1 recq4 HEI10^{oe} figl1*. **c.** The distribution (sliding window-based, window size 1 Mb, step size 50 kb) of COs along chromosomes in F2 or pseudo F2 of wild type, *HEI10^{oe}*, *zyp1*, *recq4*, *zyp1 HEI10^{oe}*, *recq4 HEI10^{oe}*, *recq4 figl1*, *zyp1 recq4*, *zyp1 recq4 HEI10^{oe}* and *zyp1 recq4 HEI10^{oe} figl1*. **d.** The zoom of the CO position in the centromere proximal regions (Non-Recombining Zones, NRZs). Each point is a CO, circles, triangles and squares are females, males and F2s, respectively. The vertical dashed lines in red indicates the position of marker COs of NRZs, lines in black shows the middle position of centromeres

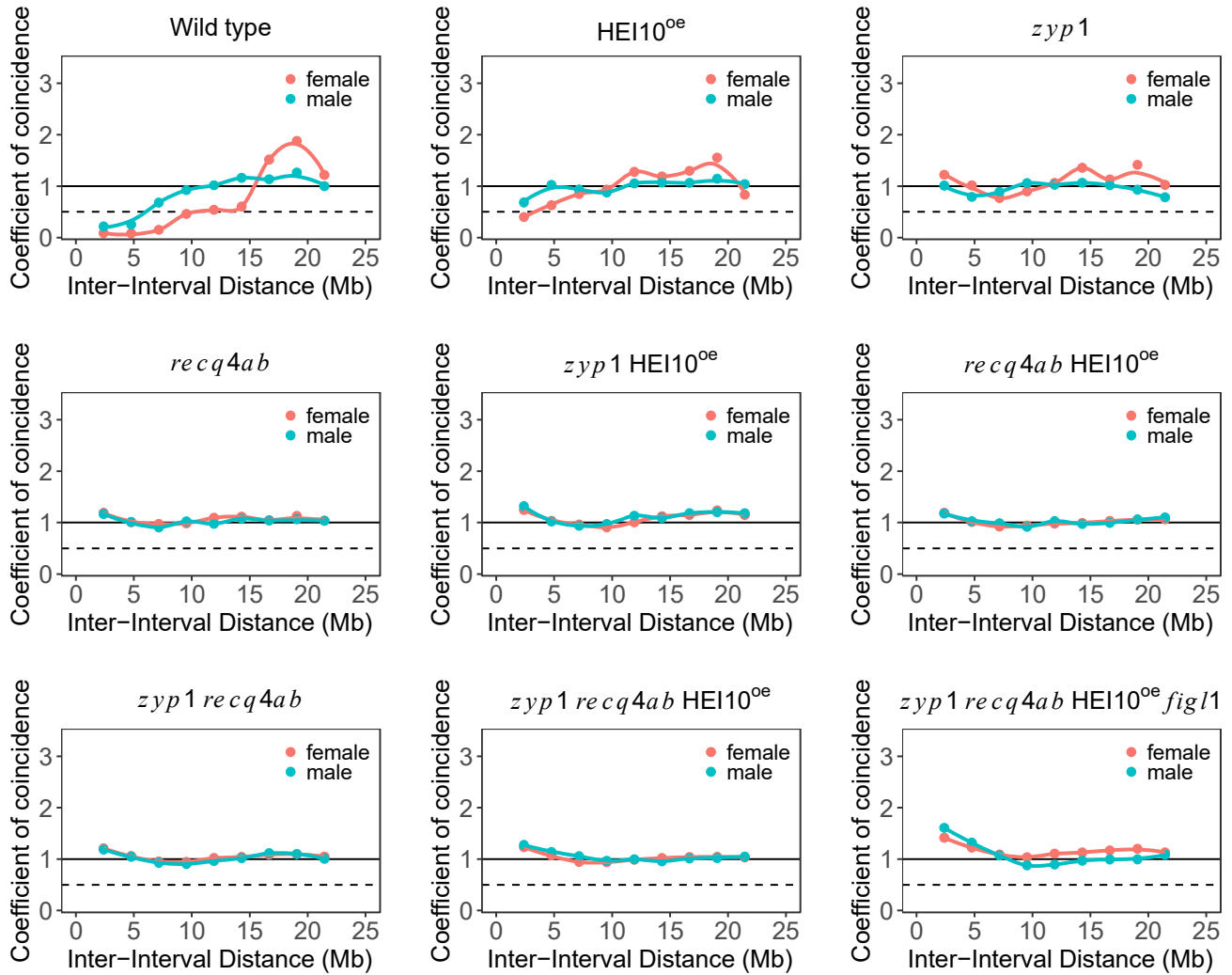


Fig S7. Analysis of CO interference.

CoC curves in the female and male meiosis of wild type, *HEI10^{oe}*, *zyp1*, *recq4*, *zyp1 HEI10^{oe}*, *recq4 HEI10^{oe}*, *zyp1 recq4*, *zyp1 recq4 HEI10^{oe}* and *zyp1 recq4 HEI10^{oe} figl1*, respectively. Chromosomes were divided into 10 intervals, for calculating the mean coefficient of coincidence of each pair of intervals. A CoC close to 1 means the absence of CO interference. A CoC close to 0 reveals an absence of double COs and is thus the presence of CO interference. The crossover interference is undetectable in both female and male meiosis of hyper recombination mutants with *ZYP1* and/or *RECQ4* mutations, consistent with previous studies (Séguéla-Arnaud et al. 2015; Serra et al. 2018; Fernandes et al. 2018; France et al. 2021; Capilla-Pérez et al. 2021; Durand et al. 2022).

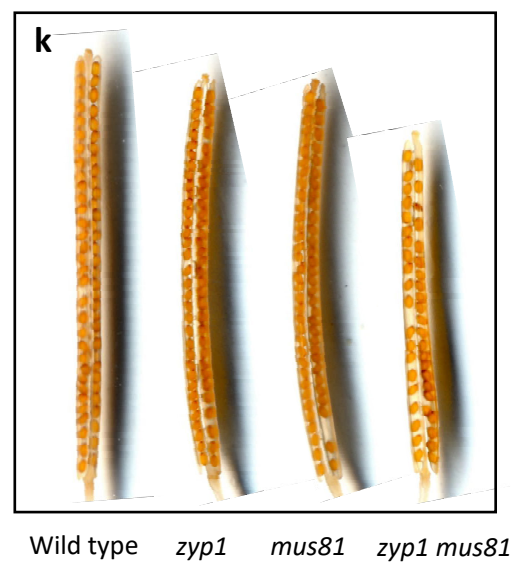
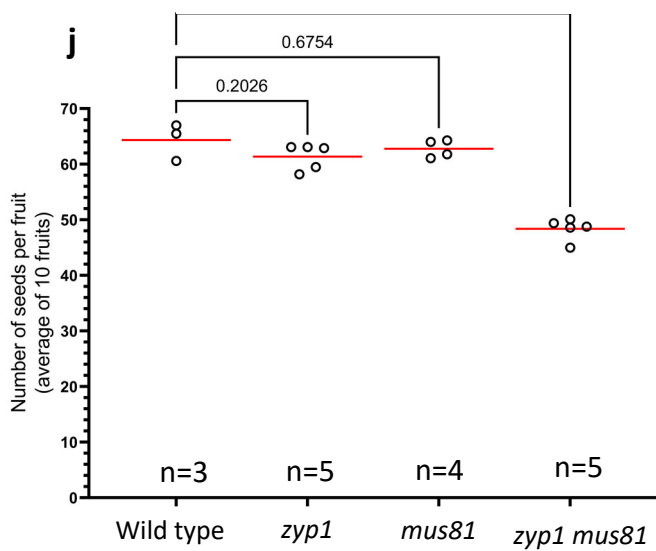
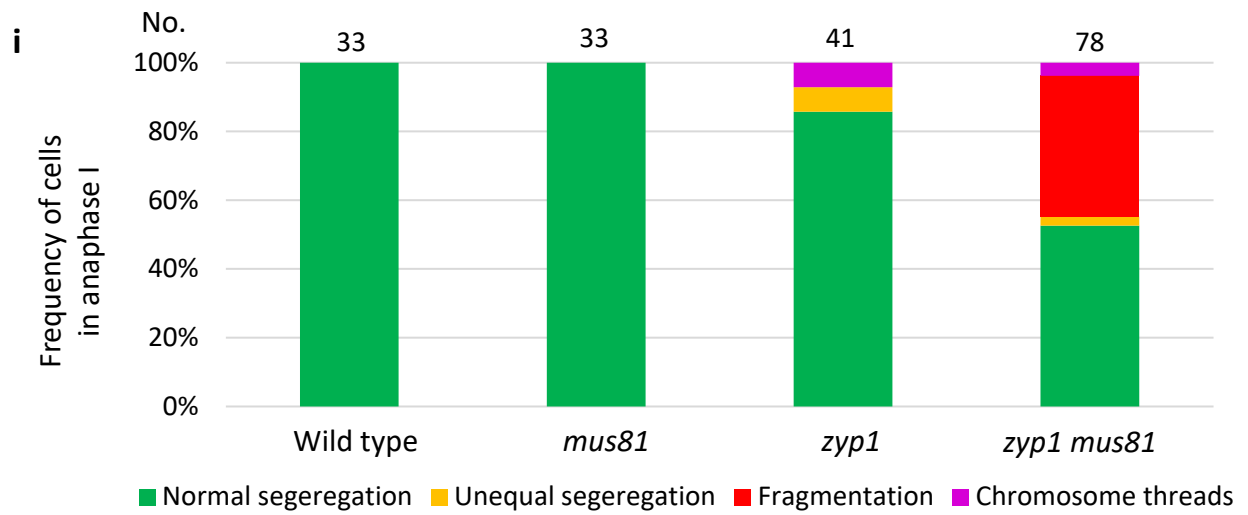
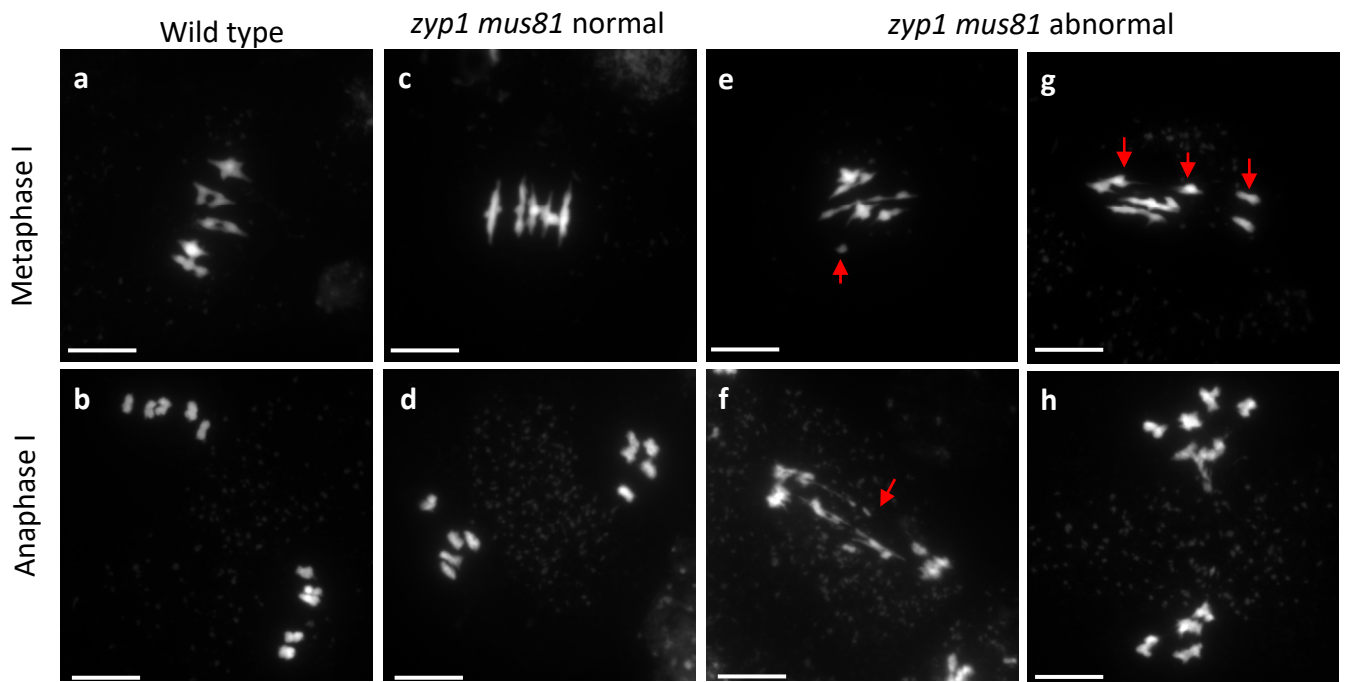


Fig S8.

Fig S8. Analysis of meiosis and fertility in wild type, *zyp1*, *mus81* and *zyp1 mus81*.

a-g. DAPI-stained meiotic chromosome spreads from male meiocytes in wild type (**a, b**), *zyp1*(**c, d**), *mus81* (**e,f**) and *zyp1 mus81* (**g, h**). **a, c, e, g** Metaphase I, **b, d, f, h** Anaphase I. Red arrows pointed out abnormal chromosome connections, fragments and chromosome threads. **i.** Quantification of different chromosome behaviors at metaphase I in wild type, *zyp1*, *mus81* and *zyp1 mus81* in Col background. Cells were categorized according to normal (5 bivalents) and abnormal chromosome behavior (unequal segregation, fragmentation and chromosome threads). The number of analyzed cells are indicated above the bar. **j.** Quantification of fertility. Each dot represents the fertility of an individual plant, measured as the number of seeds per fruits averaged on ten fruits. The red bar shows the mean. All plants were grown in parallel, and the wild-type controls are siblings of the mutants. The number n of analyzed plants is indicated and p values are one-way ANOVA followed by Fisher's LSD test. **k.** Representative cleared fruits of wild type, *zyp1*, *mus81* and *zyp1 mus81* in Col background.

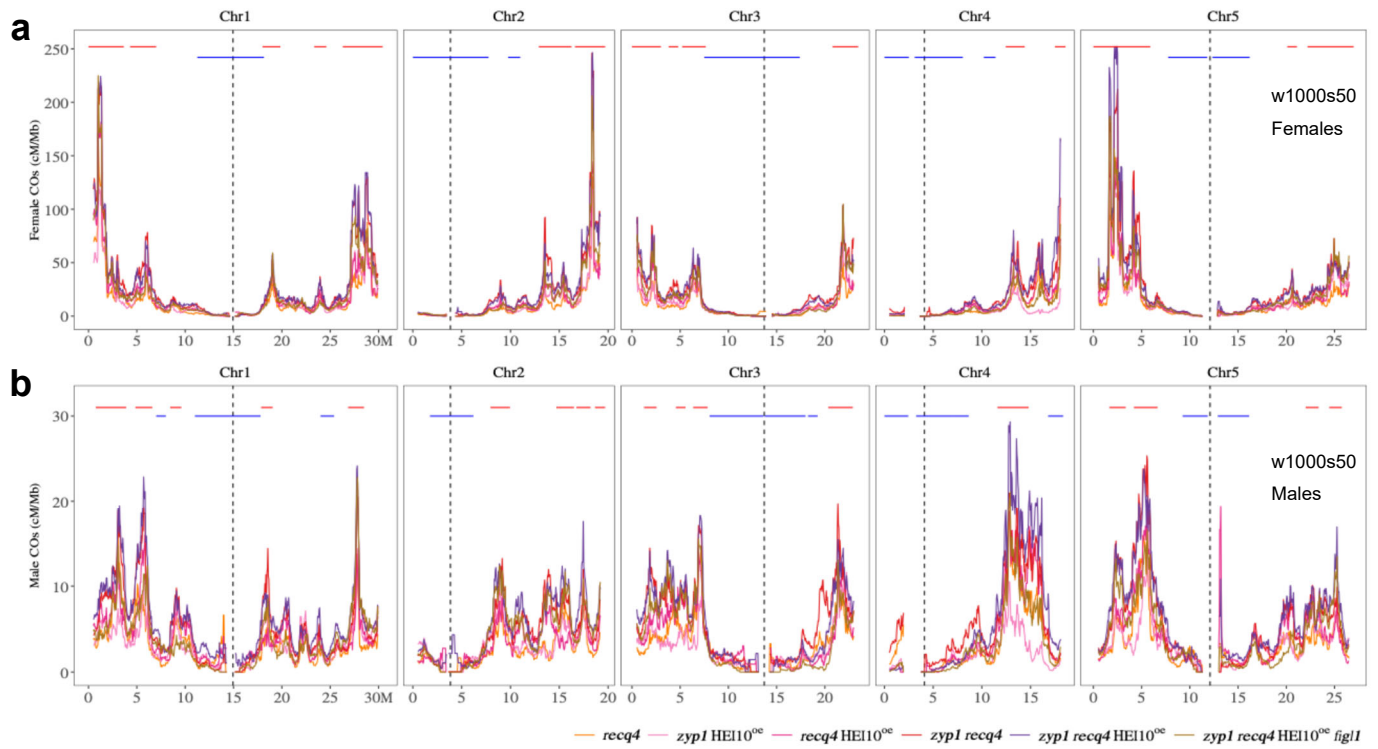


Fig S9. The fold-change analysis of CO frequencies along chromosomes in females and males.

The chromosomal distribution of fold-changes of CO frequencies (sliding window-based, window size 1 Mb, step size 50 kb) against wild-type in females (**a.**) and males (**b.**), respectively. The hot and cold zones of CO burst from hyper recombination mutants were colored by red and blue horizontal lines, separately.

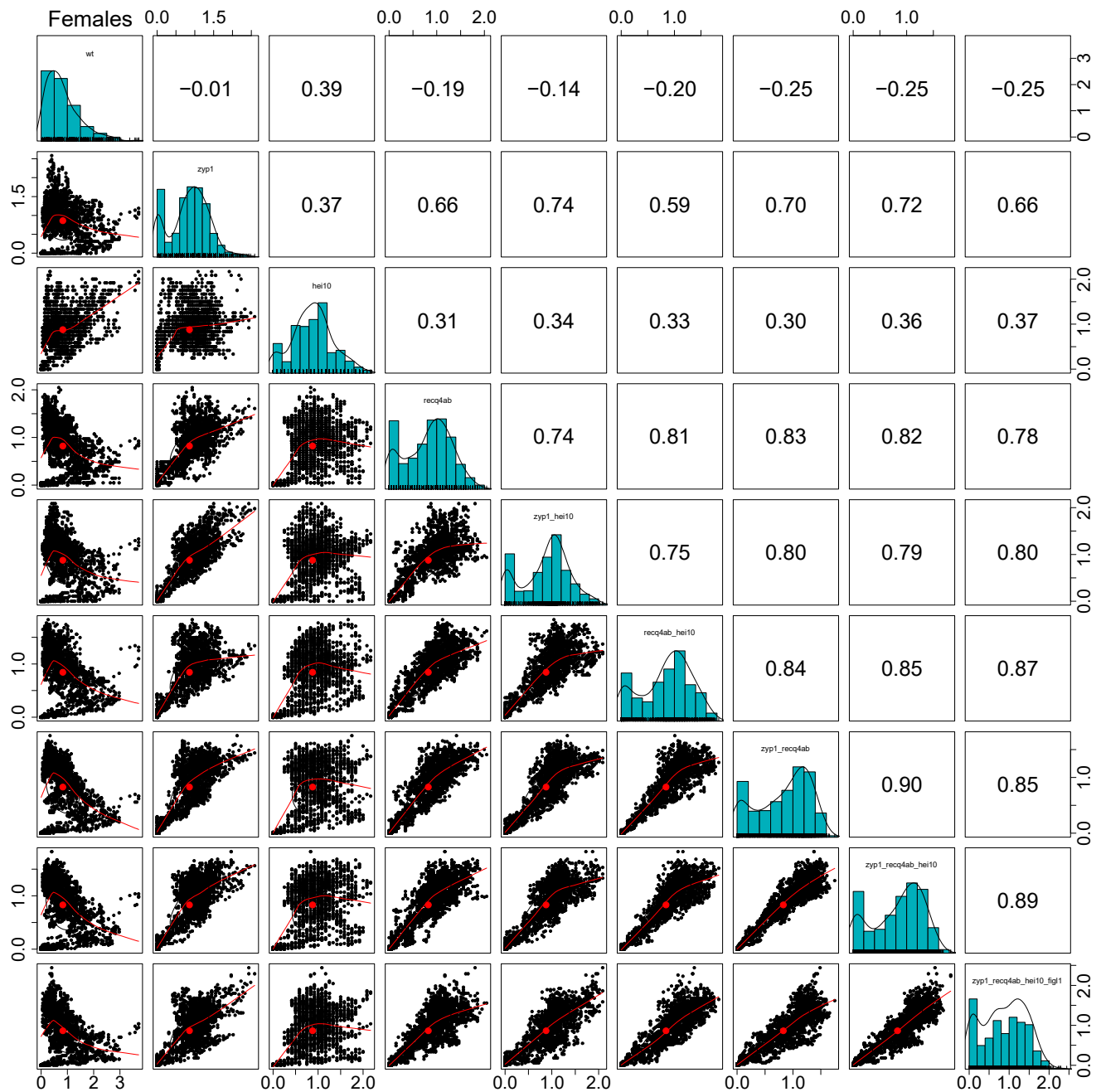


Fig S10. Correlation analysis of the relative distribution of COs in females.

Spearman's correlation between each pair of genotypes is shown in the upper triangle panel. The chromosome 4 is ignored in the analysis.

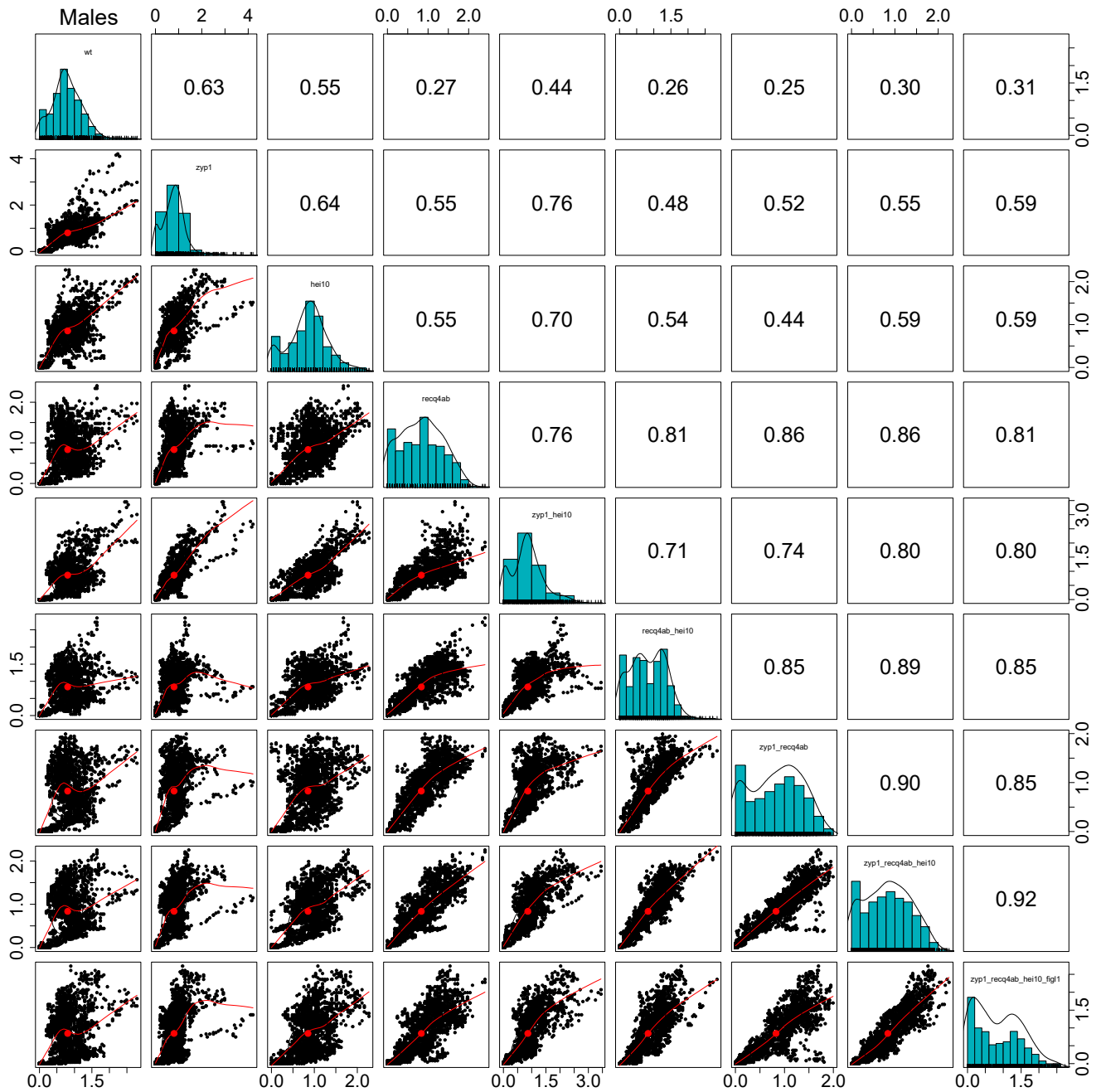


Fig S11. Correlation analysis of the relative distribution of COs in males.

Spearman's correlation between each pair of genotypes is shown in the upper triangle panel. The chromosome 4 is ignored in the analysis.

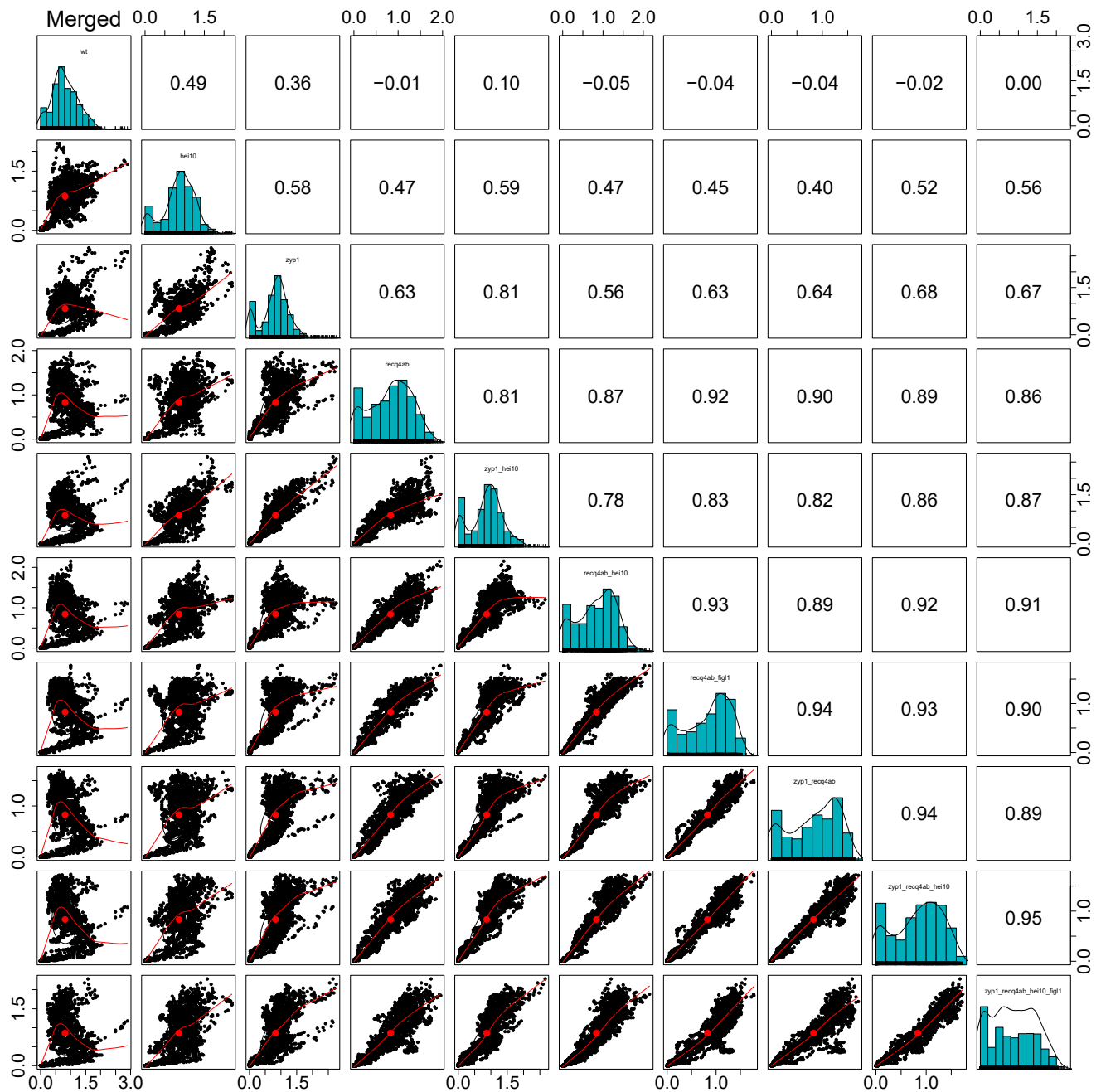


Fig S12. Correlation analysis of the relative distribution of COs in F2s or pseudo F2.

Spearman's correlation between each pair of genotypes is shown in the upper triangle panel. The chromosome 4 is ignored in the analysis.

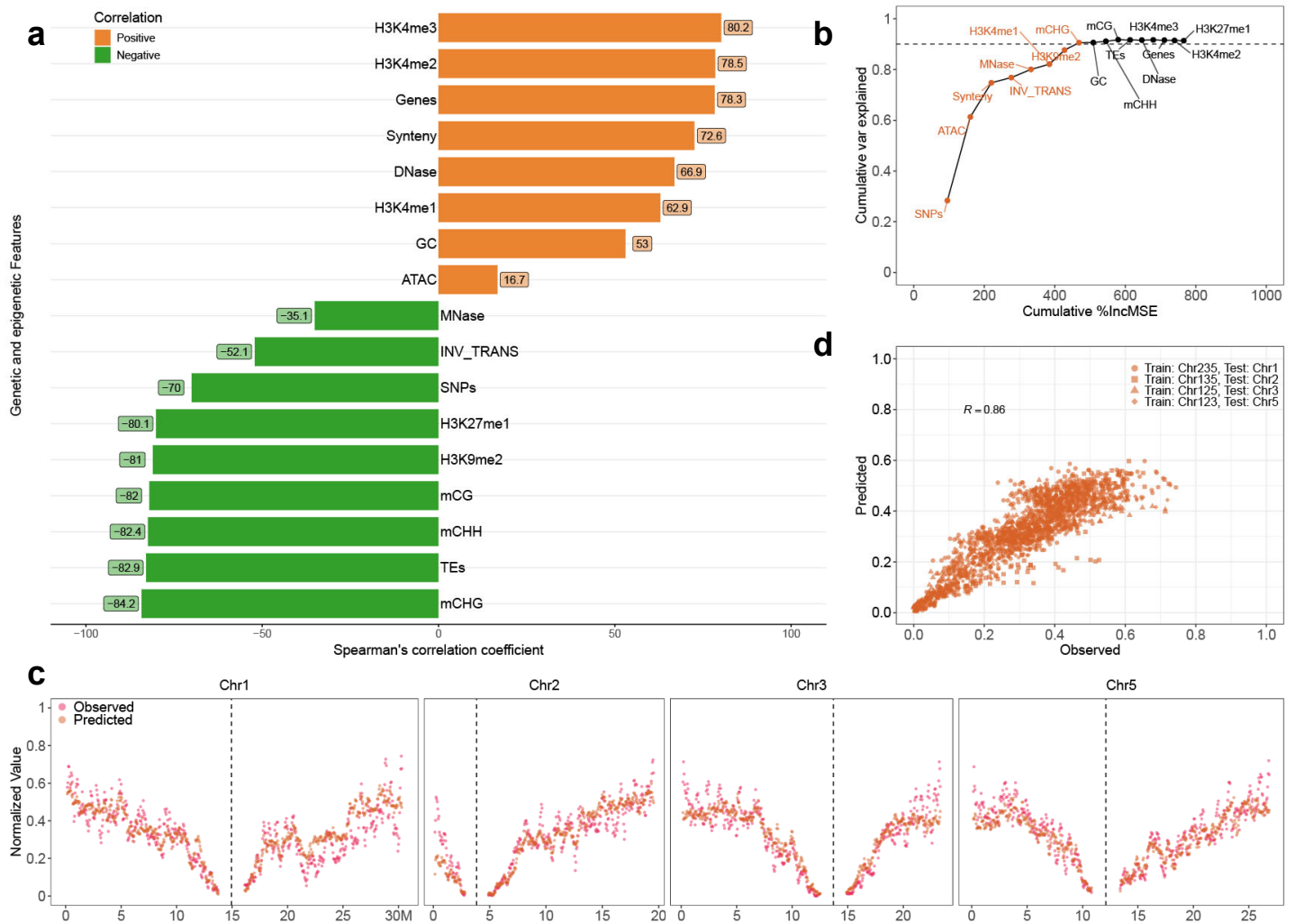


Fig S13. Association and prediction of precursor distribution with genetic and epigenetic features on chromosome arms.

a. Spearman's correlation test shows the comparison with features along chromosome arms, with differences in colour and length according to the correlation scale. SNPs (SNPs density between Col and Ler), INV_TRANS (inversions and translocations between Col and Ler), Synteny (collinearity between Col and Ler), Genes, TEs and GC (expressed gene in meiocytes, TE and GC density), ATAC and DNase (chromatin accessibility, ATAC-seq and DNase-seq, $\log_2(\text{Tn5/gDNA})$ and $\log_2(\text{DNase/gDNA})$), H3K4me1/2/3, H3K9me2, H3K27me1 (euchromatin, heterochromatin, and Polycomb histone marks, ChIP-seq, $\log_2(\text{ChIP/input})$), mCG, mCHG and mCHH (DNA methylation in CG, CHG, and CHH contexts, proportion methylated cytosine), MNase (nucleosome occupancy, MNase-seq, $\log_2(\text{MNase/gDNA})$). **b.** The cumulated proportion of variation that can be explained with the features at the chromosome arm scale. The top eight most important features are coloured, for which the cumulative proportion of variation that can be explained reaches the plateau. **c.** The chromosomal distribution of observed and predicted precursor maps. The precursor profiles of individual chromosomes were predicted using profiles of the top eight most important features from the other three chromosomes. **d.** The Spearman's correlation test between the predicted and observed precursor distributions. The training-testing dataset is differentiated in shapes.