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## **Absence of chromosome axis proteins recruitment prevents meiotic recombination chromosome-wide in the budding yeast *Lachancea kluyveri***

Sylvain Legrand, Asma Saifudeen, H el ene Bordelet, Julien Vernerey, Arnaud Guille, Amaury Bignaut, Agn es Thierry, Laurent Acquaviva, Maxime Gaudin, Aurore Sanchez, et al.

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1 **Absence of chromosome axis proteins recruitment prevents meiotic**  
2 **recombination chromosome-wide in the budding yeast *Lachancea kluyveri***

3  
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22 **Abstract**

23

24 Meiotic recombination shows broad variations across species and along  
25 chromosomes, and is often suppressed at and around genomic regions determining  
26 sexual compatibility such as mating type loci in fungi. Here we show that the absence  
27 of Spo11-DSBs and meiotic recombination on Lak10C-left, the chromosome arm  
28 containing the sex locus of the *Lachancea kluyveri* budding yeast, results from the  
29 absence of recruitment of the two chromosome axis proteins Red1 and Hop1, essential  
30 for proper Spo11-DSBs formation. Furthermore, cytological observation of spread  
31 pachytene meiotic chromosomes reveals that Lak10C-left does not undergo synapsis.  
32 However, we show that the behavior of Lak10C-left is independent of its particularly  
33 early replication timing and is not accompanied by any peculiar chromosome structure  
34 as detectable by Hi-C in this yet poorly studied yeast. Finally, we observed an  
35 accumulation of heterozygous mutations on Lak10C-left and a sexual dimorphism of  
36 the haploid meiotic offspring, supporting a direct effect of this absence of meiotic  
37 recombination on *L. kluyveri* genome evolution and fitness. Because suppression of  
38 meiotic recombination on sex chromosomes is widely observed across eukaryotes, the  
39 novel mechanism for recombination suppression described here may apply to other  
40 species, with the potential to impact sex chromosome evolution.

41

42

## 43 **Introduction**

44

45 DNA double strand breaks (DSBs) initiate recombination between homologous  
46 chromosomes during meiotic prophase, an important event that promotes  
47 chromosome segregation, and hence fertility and genome evolution (1–3). The type II  
48 topoisomerase-related protein Spo11 is the catalytic subunit of a multiprotein  
49 machinery that generates the meiotic DSBs (4–8). Such Spo11-DSBs occur within  
50 nucleosome-free DNA, typically found in gene promoters that constitute Spo11-DSB  
51 hotspots in the yeast model *Saccharomyces cerevisiae* (9–11). The Spo11-DSB  
52 hotspot landscape is relatively well conserved among *Saccharomyces* species, likely  
53 because gene promoters are conserved functional elements that are under selective  
54 constraints (12).

55 In *S. cerevisiae*, meiotic prophase chromosomes undergo compaction to form  
56 chromatin loops of ca. 20-100 kb (13–18). These chromatin loops are anchored on a  
57 protein axis composed of a coalescence of cohesins, the axis-core protein Red1 and  
58 the HORMA domain-containing protein Hop1 (19–22). The current model of axis  
59 proteins recruitment is that the meiotic specific kleisin cohesin subunit Rec8 ensures  
60 most of Red1 recruitment through direct interaction, and that Red1 then recruits its  
61 interacting partner, Hop1 (16, 23). A second parallel pathway relies on a direct Hop1-  
62 chromatin interaction within broad genomic islands with high gene density, leading to  
63 further recruitment of Red1 (24). Spo11-DSB hotspots are specifically located within  
64 chromatin loops, away from the chromosome axis attachment sites at the base of these  
65 loops (16). While Rec8 is dispensable for Spo11-DSBs, the absence of either Red1 or  
66 Hop1 strongly reduces Spo11-DSBs, notably because Hop1 participates in the  
67 recruitment of Mer2, a key Spo11 cofactor for DSB formation (16, 25–32).

68 After DNA cleavage, Spo11 is released from DNA ends by the Mre11/Rad50/Xrs2  
69 complex in combination with Sae2 but remains covalently attached to an  
70 oligonucleotide (33, 34). Spo11-oligo sequencing allowed the establishment of a  
71 nucleotide-level resolution Spo11-DSBs genome wide map (10). Once processed,  
72 Spo11-DSBs are primarily repaired by homologous recombination with the non-sister  
73 homologous chromatids, to eventually yield crossovers (35, 36). This promotes pairing  
74 and synapsis of the homologous chromosomes through the synaptonemal complex  
75 assembly. The main component of this proteinaceous complex is the transverse  
76 filament protein Zip1, and its lateral elements correspond to the chromosomes axes  
77 (37). The chromosome axis component Hop1 is eventually removed from the axis upon  
78 completion of synaptonemal complex formation, shutting down DSB formation (21, 38–  
79 40). Interestingly, the length of the synaptonemal complex covaries with the number of  
80 crossovers independently of the genome size and the species (41, 42). In *S.*  
81 *cerevisiae*, the major crossover formation pathway involves the ZMM proteins (Zip1,  
82 2, 3, 4, Spo16, Msh4, 5 and Mer3, (43, 44). Zip3 appears as a specific marker of the  
83 resulting “type I” crossovers that show interference (45, 46). Genome-wide distribution  
84 of Zip3 colocalizes with Spo11-DSBs and alternates with chromosome axis binding  
85 sites (46, 47).

86 Meiotic homologous recombination is commonly repressed around the loci determining  
87 sexual compatibility such as the mating-type loci in fungi, and sometimes this inhibition  
88 extends outside of this locus (48). Both the mechanism and the selective advantage of  
89 recombination suppression are unclear. Recombination inhibition within the *MAT* locus  
90 may prevent loss of heterozygosity at this specific locus and subsequent disequilibrium  
91 between the two mating types within the population. In addition, recombination  
92 inhibition around the *MAT* locus may ensure linkage between specific alleles that

93 present strong selective advantages when linked (49). As another example of  
94 recombination inhibition around the mating-type locus, we previously reported a  
95 complete inhibition of meiotic recombination over almost the entire chromosome arm  
96 containing the mating type locus of the budding yeast *Lachancea kluyveri* (50). The  
97 inhibition spreads over the region of the left arm of *L. kluyveri* chromosome C starting  
98 from the left telomere to a point about 10kb to the left of the centromere; we refer to  
99 this region as Lakl0C-left. This inhibition of recombination results from the repression  
100 of Spo11-DSBs within this ca. 1 Mb long region, corresponding to ca. 8% of *L. kluyveri*  
101 genome. So far, the mechanism inhibiting Spo11-DSB formation over such a large  
102 genomic region is unknown.

103 *L. kluyveri* is a *Saccharomycetaceae* yeast that diverged from *S. cerevisiae* prior the  
104 whole genome duplication that characterizes the *Saccharomyces* lineage (51). *L.*  
105 *kluyveri* has only eight chromosomes, with a genome size and a gene complement  
106 comparable to *S. cerevisiae* (52, 53). As in *S. cerevisiae*, meiosis is efficiently induced  
107 by nitrogen starvation and meiotic progression is rather synchronous (50).  
108 Interestingly, Lakl0C-left is characterized by a G + C frequency almost 10% higher than  
109 the rest of the genome (53). A population genomics survey of *L. kluyveri* led to the  
110 proposal that Lakl0C-left arose from an introgression from a yet unknown *Lachancea*  
111 species (54). Finally, Lakl0C-left contains almost exclusively early replication origins,  
112 in contrast to the rest of the genome (55). Whether any of these Lakl0C-left specificities  
113 are at the source of the repression of Spo11-DSBs formation is unknown.

114 Here, we confirm Spo11-DSB inhibition on Lakl0C-left using a genome wide DSB  
115 mapping method (56) and find that the Spo11-DSB hotspot landscape is poorly  
116 conserved between orthologous syntenic intergenic regions from *L. kluyveri* and *S.*  
117 *cerevisiae*. Using Hi-C, we showed that *L. kluyveri* chromosomes undergo compaction

118 during meiotic prophase, and that Lakl0C-left compaction is comparable to the rest of  
119 the genome, which correlates with a comparable binding of Rec8 as detected by ChIP-  
120 seq. By contrast, Hop1 and Red1 are completely absent from Lakl0C-left, likely  
121 explaining the lack of Spo11-DSBs. Finally, fluorescence visualization of Zip1, Rec8  
122 and the left and right arms of chromosome C recapitulated these molecular findings  
123 and revealed the lack of pairing of Lakl0C-left. Overall, *L. kluyveri* chromosome C  
124 appears as a prototypical young sex chromosome.

125

126

## 127 **Results**

128

### 129 **Genome wide Spo11-DSB mapping in *L. kluyveri***

130

131 Using pulsed-field gel electrophoresis (PFGE) and Southern blotting, we previously  
132 showed that Lakl0C-left is depleted of Spo11-DSBs but flanked by a Spo11-DSB  
133 hotspot in the *GPI18* promoter, located about 10 kb to the left of the centromere. We  
134 also identified by Southern blot two additional DSB hotspots in the promoters of the  
135 *RAS1* and *PIS1* genes that correlate with crossover hotspots (50). To confirm these  
136 results with an independent technique and to extend them to the whole genome, we  
137 used the Covalent Complexes-sequencing (CC-seq) technique that allows nucleotide-  
138 resolution mapping of protein-linked DNA breaks (56).

139 We performed two independent experiments. After background filtering (methods), we  
140 identified a specific signal that clustered into hotspots, as seen for *S. cerevisiae* Spo11-  
141 DSBs, and the two replicates showed good reproducibility (Figure 1A and S1). We  
142 used the MACS2 peak calling algorithm to define the Spo11-DSB hotspots (57). One

143 replicate showed better signal enrichment than the other (1,933 hotspots vs 1,025  
144 hotspots, Figure S1) and was used for subsequent analyses.

145 The average DSB hotspot density is 1 per 5.9 kb and the average width was 0.282 kb  
146 in *L. kluyveri* compared to 1 per 4.2 kb and 0.409 kb, respectively in *S. cerevisiae* (56).

147 As in *S. cerevisiae*, most DSB signal is in intergenic regions between divergent or  
148 tandemly orientated genes (Figure 1B and S2A). Only 13.2% of the DSB signal is within

149 genes and 3.1% is within intergenic regions between convergent genes. In addition,  
150 centromere-flanking regions, subtelomeres and the rDNA locus were depleted for

151 Spo11-DSBs compared to the rest of the genome (Figure S3). However, and in sharp  
152 contrast with the rest of the genome, a depletion of Spo11-DSBs was observed all

153 along Lak10C-left, which shows a 12-fold decrease in Spo11-DSBs per base pair  
154 compared to the rest of the genome (Figure 1A). Overall, genome-wide mapping of

155 Spo11-DSBs reveals similar properties of Spo11-DSBs distribution between *L. kluyveri*  
156 and *S. cerevisiae* chromosomes and confirms the depletion of Spo11-DSBs in Lak10C-

157 left.

158

159 **Spo11-DSBs hotspots are poorly conserved between *L. kluyveri* and *S.***  
160 ***cerevisiae***

161

162 Spo11-DSBs hotspot positions and strength are remarkably conserved within  
163 *Saccharomyces* species, likely because they are mainly found within gene promoters,

164 which are functional genetic elements (12). In *L. kluyveri*, Spo11-DSBs are also mainly  
165 located within gene promoters. To test the conservation of Spo11-DSBs hotspot

166 strength between *L. kluyveri* and *S. cerevisiae*, we compared Spo11-DSBs levels in  
167 syntenic intergenic regions (sIGRs) *i.e.* intergenic regions flanked by pairs of



168 orthologous genes. Although these two species diverged over 100 million years ago,  
169 they share 239 synteny blocks and 2,030 sIGRs (58). The correlation coefficient  
170 between the Spo11-DSB hotspots strength of the two species is  $r=0.256$  (Figure 2A),  
171 showing poor conservation between the two species. Nevertheless, it remains higher  
172 than expected by chance since randomization of the corresponding datasets yields no  
173 correlation at all (Figure S2B).

174

175 To assess the conservation of Spo11-DSB hotspot strength on an evolutionary scale  
176 broader than that of the *Saccharomyces* species, but independently of *L. kluyveri*, we  
177 focused on ohnologs (59). These are the subset of *S. cerevisiae* paralogs that  
178 emanated from the whole genome duplication that characterizes the *Saccharomyces*  
179 lineage, but that occurred after the divergence between the *Saccharomyces* and the  
180 *Lachancea* lineages (60). We identified 39 sIGRs among the 547 ohnolog pairs.  
181 Considering the Spo11-oligo data from (10) corresponding to these 39 sIGRs, the  
182 correlation coefficient between the Spo11-DSB hotspots strength of these sIGRs is  
183  $r=0.038$  (Figure 2B). This shows that, for this subset of intergenic regions, the strength  
184 of Spo11-DSBs is not conserved over an evolutionary scale smaller than that of the  
185 divergence between the *Lachancea* and the *Saccharomyces* lineages, and therefore  
186 suggests different evolutionary constraints on intergenic regions associated with  
187 ohnologs versus orthologs.

188

### 189 **Spo11-DSBs sites are enriched in the crossover factor Zip3**

190

191 In *S. cerevisiae*, the crossover factor Zip3 is enriched around Spo11-DSBs (46). To  
192 determine if this is also the case in *L. kluyveri*, we performed ChIP-seq experiments

193 using Zip3 FLAG-tagged on its carboxyl terminus as a bait. As in *S. cerevisiae*, Zip3 is  
194 specifically expressed after meiotic induction (Figure S4). We observed Zip3 ChIP-seq  
195 enrichment peaks genome wide, with the notable exception of Lakl0C-left and about  
196 250kb left and right of the rDNA locus on Lakl0H (Figure 1A). Piling up Zip3 ChIP-seq  
197 signals centered on the 500 strongest Spo11-DSBs hotspots revealed a specific Zip3  
198 enrichment around these Spo11-DSBs hotspots (Figure 1C). Importantly, the absence  
199 of Zip3 ChIP-seq signal on Lakl0C-left suggests a complete absence of crossovers in  
200 this chromosome arm in the nearly homozygous CBS10367 strain background used  
201 here. This population average analysis confirms and generalizes our original  
202 observation suggesting, from a limited number of meioses in the artificial NBRC10955  
203 x 67-588 hybrid background, a lack of recombination along this arm (50, 61).

204

205 **The lack of meiotic recombination on Lakl0C-left is conserved among *L. kluyveri***  
206 **isolates and results in a *MAT*-linked growth phenotype**

207

208 We attributed the complete absence of recombination on Lakl0C-left observed in a  
209 hybrid background to the strong depletion of Spo11-DSBs, as measured previously by  
210 Southern blot (50) and here by CC-seq in the natural CBS10367 diploid background  
211 (54). However, we cannot rule out that a low level of Spo11-DSBs would be enough to  
212 induce recombination within Lakl0C-left in this homozygous background, but not in a  
213 hybrid background, where sequence polymorphism would prevent recombination  
214 through the action of mismatch repair (62–64).

215 We therefore designed a reporter assay inserting drug resistance cassettes to monitor  
216 recombination within two intervals of *L. kluyveri* chromosome C in the nearly  
217 homozygous CBS10367 background (Figure 1D). From left to right, the first interval is

218 exclusively on Lakl0C-left and spans about 550 kb from *MAT* to the Lakl0C-899 locus  
219 positioned about 110 kb left of the centromere. The second interval starts from Lakl0C-  
220 899 to the *CHS3* locus located about 50 kb right of the centromere and is about 160  
221 kb long. The spore viability of the resulting strain is 76% (n=96 tetrads), similar to that  
222 of the parental strain (83%, n=39 tetrads). From 96 tetrads with four viable spores, we  
223 did not detect a single crossover on the Lakl0C-left 550 kb long interval and we  
224 detected 57 single crossovers and five double crossovers on the second 160 kb long  
225 interval (Figure 1D). The absence of recombinants on the Lakl0C-left interval in this  
226 nearly completely homozygous context shows that inter-homolog recombination is  
227 inhibited on Lakl0C-left independently of sequence polymorphism, and that the low  
228 Spo11-DSBs level detected does not promote recombination. The absence of meiotic  
229 recombination on Lakl0C-left therefore occurs in both the NBRC10955 x 67-588 hybrid  
230 and the CBS10367 strains. Given the *L. kluyveri* phylogeny (54), this shows that  
231 recombination cessation on Lakl0C-left is ancient and likely common to the entire  
232 population.

233 Remarkably, *MATa* colonies coming from CBS10367 spores were systematically  
234 smaller than *MATalpha* colonies (Figure 1D). One explanation could be that there is at  
235 least one DNA sequence polymorphism between the *MATa* and *MATalpha*-associated  
236 linkage groups responsible for this sexual dimorphism in the CBS10367 natural diploid.  
237 This would agree with the fact that non-recombining regions tend to accumulate  
238 heterozygous DNA sequence polymorphisms, as frequently observed for sex  
239 chromosomes ((48) and discussion). Alternatively, differentially expressed genes  
240 between *MATa* and *MATalpha* cells could be responsible for this growth phenotype,  
241 although this is not the case in other species including *S. cerevisiae*.

242

243 **Hi-C reveals similar compaction level of all *L. kluyveri* meiotic chromosomes**

244

245 We wondered if the lack of recombination on Lakl0C-left could result from a peculiar  
246 chromosome structure that would differ from the canonical compaction of  
247 chromosomes during meiosis prophase into cohesin-mediated chromatin loops (14).  
248 Using Hi-C (Lieberman-Aiden et al. 2009; Muller et al. 2018), we generated genome-  
249 wide contact maps of eight *L. kluyveri* meiotic timepoints harvested between zero- and  
250 eight-hours after meiosis induction. ~70% of cells passed the second meiotic division  
251 at the eight-hours timepoint (Figure S5A). Overall, the contact maps of *L. kluyveri*  
252 meiotic Hi-C resemble those from *S. cerevisiae* (Figure 3A) (15, 17). Centromere  
253 clustering is clearly visible at t=0 (Rabl configuration) and is progressively lost (Figure  
254 3A, S5B). Chromosomes gradually individualize, as shown by the increase and  
255 decrease of intra- and inter-chromosomal contacts, respectively. Compaction was  
256 maximal five hours following entry into meiosis (Figure S5C). Enrichment in  
257 intrachromosomal contacts involves pairs of loci within the 10 to 200 kb range, as  
258 illustrated both by the thickening of the main diagonals on the normalized contact maps  
259 (Fig. 3 A and B) and the shoulder made by the distance law, reflecting the genomic  
260 average contact frequencies over increasing genomic distances (Figure 3C). This  
261 compaction most likely results from the folding of chromatin into loops by the cohesin  
262 complex (15, 17, 18). Remarkably, the Hi-C pattern displayed by the Lakl0C-left was  
263 comparable to the other chromosomes. The intrachromosomal contact frequency and  
264 the distance law of the Lakl0C-left arm displayed nearly the same compaction  
265 throughout the meiotic time course than the other chromosome arms of similar lengths  
266 (Figure 3C, S5D and E). In addition, based on similar interchromosomal contact  
267 frequencies compared to the other chromosome arms, Lak0C-left is not isolated from

268 the other chromosomes (Figure 3A, S5C and D). Overall, Laki0C-left is folded into  
269 cohesin mediated loops, whose scores, length and kinetic of formation are similar to  
270 loops analyzed on other chromosomal arms (Figures 3B, D and E, S5E and F). In  
271 conclusion, this shows that the lack of recombination of Laki0C-left is not associated  
272 with any specific structure detectable by Hi-C, and that Laki0C-left undergoes similar  
273 compaction by cohesins as all other chromosomes.

274

### 275 **Meiotic axis proteins Hop1 and Red1 are absent from Laki0C-left**

276

277 The compaction of Laki0C-left suggests the loading of cohesins, including the meiosis  
278 specific Rec8 kleisin subunit. To assess this, we performed ChIP-seq analysis of Rec8,  
279 FLAG-tagged at its carboxy-terminus, which was specifically expressed after meiosis  
280 induction and show retarded forms on western blots, as in *S. cerevisiae* (Figure S4)  
281 (Brar et al. 2006). *L. kluyveri* Rec8 ChIP-seq profiles are similar to those of *S.*  
282 *cerevisiae* (Figure 4). The Rec8 ChIP-seq signal first appears early (1h) after induction  
283 of meiosis around all centromeres (Figure S6) (32). Four hours after meiosis induction,  
284 the Rec8 ChIP-seq signal is then distributed in peaks throughout the genome and  
285 accumulates in convergent intergenic regions, likely as a result of transcription (Figures  
286 4, S6, S7A and B) (23, 66). In addition, Rec8 ChIP-seq peaks alternate with the peaks  
287 of Zip3 ChIP-seq and Spo11-DSBs CC-seq (Figure S7A and C). Such a distribution is  
288 compatible with the loop-axis structure of meiotic chromosomes, with Spo11-DSBs  
289 formed within the loops at the basis of which lie the axis proteins including Rec8 (16).  
290 Importantly, the loci at the basis of such chromatin loops detected as discrete dots  
291 away from the diagonal in the Hi-C experiments are enriched in Rec8 (Figures 3B and  
292 E) (67). Furthermore, Rec8 ChIP-seq signal is decreased in subtelomeric regions

293 (Figure S8). We noted a slight reduction of Rec8 ChIP-seq signal on Laki0C-left  
294 relative to the rest of the genome (Figure S7B, compare right and left panels,  
295 respectively). However, it comes from a nonspecific sequencing bias, also observed in  
296 the untagged control, that is likely due to the elevated GC content of Laki0C-left (68).  
297 Considering this sequencing bias, the overall Laki0C-left Rec8 ChIP-seq signal is  
298 comparable to the rest of the genome, which is in agreement with the Hi-C results  
299 showing comparable compactions.

300 Coalescence of cohesins participates in the formation of the chromosome axes that  
301 also comprise Hop1 and Red1, needed for Spo11-DSBs formation (26–28, 30, 31).  
302 Since Rec8 loads normally on Laki0C-left, we investigated if Red1 and Hop1 were also  
303 loaded. Like for Rec8, we tagged Red1 and Hop1 with FLAG at their carboxy-termini.  
304 As in *S. cerevisiae*, both proteins are specifically expressed after meiosis induction  
305 and show retarded forms on western blots (Figure S4) (69, 70). In addition, Red1 and  
306 Hop1 ChIP-seq signals form peaks that strongly colocalize with Rec8 ChIP-seq peaks  
307 (Figures 4, S6, S7A and D). However, several cases of Rec8 peaks without associated  
308 Hop1 or Red1 peaks were noted. First, Red1 and Hop1 are depleted around  
309 centromeres, unlike Rec8 (Figure S8). This depletion of Red1 and Hop1 coincides with  
310 the depletion of the Spo11-DSB signal (Figures 1A, S3 and S7A) and the decrease in  
311 recombination (50), as seen in *S. cerevisiae*. Second, around the rDNA locus, while  
312 neither Rec8 nor Red1 ChIP-seq signal shows any decrease compared to the rest of  
313 the genome, the Hop1 ChIP-seq signal is decreased (Figure S8, chromosome H).  
314 Strikingly, this decrease occurs on a much narrower region than that of Zip3. Finally,  
315 the most remarkable discrepancy between Rec8 and Hop1/Red1 signal was observed  
316 on Laki0C-left, where virtually no Red1 or Hop1 ChIP-seq signal was observed despite  
317 a normal Rec8 loading (Figure 4). While in *S. cerevisiae* Hop1- and Red1- rich or poor

318 regions have been described (16, 71), this is the first time, to our knowledge, that a  
319 complete depletion over an almost entire chromosome arm is observed. Given the  
320 importance of Hop1 and Red1 for Spo11-DSBs formation, it is tempting to speculate  
321 that their absence is directly responsible for the absence of Spo11-DSBs formation on  
322 Lakl0C-left.

323

### 324 **Spo11-DSBs formation inhibition on Lakl0C-left is independent of Tel1**

325

326 In the absence of Tel1, Spo11-DSBs increase in *S. cerevisiae*, although to a lesser  
327 extent than what is observed in the absence of its ATM homolog in mice, notably on  
328 the non-homologous parts of the sex chromosomes (72–74). We therefore tested by  
329 PFGE followed by Southern blot meiotic Spo11-DSBs formation in the *tel1* null mutant.  
330 The overall Spo11-DSB profile is similar in the reference and *tel1* backgrounds, notably  
331 on chromosome C (Figure S9). This shows that Spo11-DSB depletion on Lakl0C-left  
332 is independent of Tel1.

333

### 334 **The lack of recombination on Lakl0C-left is independent of its early replication** 335 **timing**

336

337 Spo11-DSB formation is coordinated with meiotic DNA replication and occurs on fully  
338 replicated chromosomes (75–77). Interestingly, Lakl0C-left is replicated earlier than  
339 the rest of the genome due to the prevalence of early firing replication origins in  
340 vegetative cells (55, 78). Although studies in *S. cerevisiae* suggest that this would  
341 result in early recruitment of DSB factors and early DSB formation on Lakl0C-left (27,  
342 77), we wondered whether, in *L. kluyveri*, early replication timing would prevent Spo11-

343 DSBs formation, and hence recombination. Therefore, we subdivided the genome in  
344 four replication quartiles of eight minutes each based on the known vegetative  
345 replication timing (55, 78) and assessed Spo11-DSBs and Zip3-ChIP-seq signals. This  
346 partitioning shows that the early replication quartile encompasses a smaller genomic  
347 fraction than the mid-early and the late replication quartiles, but a larger genomic  
348 fraction than the late replication quartile (Figure 5A). In addition, it shows that Lakl0C-  
349 left contains mainly early and mid-early replicated regions. Considering the genomic  
350 fraction encompassed by each quartile, it appears that both the early and mid-early  
351 replication quartiles contain a significant fraction of the Spo11-DSBs and the Zip3-  
352 ChIP-seq signals, which vary less than two-fold between the four quartiles (Figure 5B  
353 and C). This shows that the early and mid-early replicated regions from *L. kluyveri*  
354 undergo Spo11-DSBs and recombination to a comparable extent as the rest of the  
355 genome. Hence, the depletion in Spo11-DSBs and subsequent recombination on  
356 Lakl0C-left is not related to its early / mid-early replication timing. Further regulation  
357 therefore exists to explain the Spo11-DSBs depletion on Lakl0C-left. Interestingly, the  
358 earliest quartile shows slightly fewer Spo11-DSBs than the next two quartiles while it  
359 shows slightly more Zip3-ChIP-seq signal, suggesting potential differences in the  
360 outcome of early versus late Spo11-DSBs.

361

### 362 **Lakl0C-left does not synapse**

363

364 Recombination is required to initiate synaptonemal complex formation and homolog  
365 synapsis in *S. cerevisiae* (44). Based on this, the absence of recombination on Lakl0C-  
366 left may prevent synapsis in this chromosome arm, unless synaptonemal complex  
367 formation and synapsis could spread through its entire length after initiating either right



368 of it or at the very left telomere from which recombination cannot be genetically  
369 mapped. To test this prediction, we performed immunofluorescence staining of *L.*  
370 *kluyveri* meiotic chromosome spreads using a *S. cerevisiae* anti-Zip1 antibody that  
371 cross reacts in *L. kluyveri* and an anti-FLAG antibody directed against a FLAG tagged  
372 version of Rec8. Experiments were performed in the absence of Ndt80 to block cells  
373 at the pachytene stage. As expected, we observed continuous Zip1 staining of *L.*  
374 *kluyveri* meiotic chromosomes (Figure 6A and B). We observed on average nine Zip1  
375 continuous lines per nucleus in agreement with the chromosome number. Interestingly,  
376 the cumulated length of Zip1 lines in *L. kluyveri* is 13.86 micrometers on average  
377 compared to 28 micrometers in *S. cerevisiae* (Figure 6C). This ca. two-fold longer  
378 meiotic chromosome size agrees with the ca. four-fold higher recombination frequency  
379 in *S. cerevisiae* compared to *L. kluyveri* (50). It also suggests longer loops in *L. kluyveri*  
380 than in *S. cerevisiae*. Measurement of the width of the DAPI signal of pachytene  
381 chromosomes spread on the same slide showed this width was on average 1.26  
382 micrometers in *L. kluyveri* and 0.83 micrometers in *S. cerevisiae*, therefore supporting  
383 longer chromatin loops in *L. kluyveri* (Figure 6D and E).

384 Rec8 staining colocalized with Zip1 staining except at two regions that exhibited Rec8-  
385 only staining (Figure 6F). One of these regions exhibit a circular structure emanating  
386 from a chromosome which corresponds to the rDNA locus as demonstrated by staining  
387 with a specific FISH probe. The second region consists of a “V” shape emanating from  
388 a base stained by both Zip1 and Rec8. This overall “Y” shape chromosome could be  
389 compatible with chromosome C, with the base of the Y corresponding to the synapsed  
390 right arm, and the arms of the Y corresponding to the left arms entirely unsynapsed.  
391 Corroborating this hypothesis, using FISH probes specific of the left and the right arms  
392 of chromosome C, we could detect that the FISH probe specific of the right arm of

393 chromosome C stains a DAPI dense region, while the FISH probe specific of the left  
394 arm of chromosome C stains two nearby areas less DAPI dense (Figure 6G). Such  
395 signals support synapsed right arms of chromosome C and unsynapsed left arms.  
396 Consistently, at five hours post meiosis induction, the distance law of Lakl0C-left shows  
397 a higher probability of contact at longer range (> 100 kb) than other chromosome arms,  
398 which could reflect the ability of the unsynapsed arm to contact distant regions (Figure  
399 3C). In agreement with the ChIP-seq experiment that revealed the presence of Rec8  
400 along Lakl0C-left and the Hi-C experiment that revealed normal compaction of Lakl0C-  
401 left, the Y structure is also entirely stained by Rec8. Finally, the fact that the arms of  
402 the Y structure are separated further suggests that there is no recombination within the  
403 left telomere of chromosome C in regions that could not be genetically mapped nor  
404 assessed by our ChIP-seq or CC-seq approaches. Overall, our analysis of *L. kluyveri*  
405 meiotic chromosomes spreads reveals that Lakl0C-left does not synapse. This shows  
406 that recombination and subsequent synapsis initiated outside Lakl0C-left is not enough  
407 to spread through this entire region. Recombination seems therefore also required in  
408 *L. kluyveri* to locally initiate homolog synapsis, as in *S. cerevisiae*.

409

410

## 411 **Discussion**

412

413 The lack of meiotic recombination on Lakl0C-left implies an absolute genetic linkage  
414 and an absence of sequence homozygosity by gene conversion during meiosis. This  
415 chromosome arm that contains the *MAT* locus therefore relies exclusively on mitotic  
416 recombination to break genetic linkages and homogenize mutations. It is therefore  
417 expected that Lakl0C-left genes would exhibit a lower purifying selection than the other

418 genes of the genome. This is actually the case, since Lakl0C-left genes show a higher  
419 ratio of non-synonymous (dN) to synonymous (dS) substitution rates (dN/dS) than the  
420 rest of the genome (79). It is also expected to find a higher density of heterozygous  
421 sequence polymorphisms on Lakl0C-left than on the rest of the genome, with at least  
422 one possibly being responsible for the different growth phenotypes of *MATa* and *MATα*  
423 spores from the CBS10367 isolate. In agreement with this expectation, analysis of the  
424 CBS10367 diploid genome with respect to the CBS3082 reference genome revealed  
425 a significantly higher fraction of heterozygous SNPs and indels on Lakl0C-left  
426 compared to the other chromosomes excluding the highly variable subtelomeric  
427 regions, with 1.53 vs 0.11 heterozygous SNPs per kb and 0.33 vs 0.018 heterozygous  
428 indels per kb, respectively (Table 1 and Figure S10A). Despite this higher SNP density,  
429 the most telomeric ca. 200 kb left of chromosome C are almost completely devoid of  
430 heterozygous SNPs, potentially reflecting homozygosis by mitotic recombination (G2  
431 crossover or break-induced replication) in the recent history of the strain. Finally, the  
432 density of homozygous sequence polymorphisms on Lakl0C-left existing between  
433 CBS10367 and CBS3082 is comparable to the rest of the genome, with 25.29 vs 23.42  
434 homozygous SNPs per kb, respectively, and is much higher than the density of  
435 heterozygous sequence polymorphisms mentioned above (Figure S10B). Given the  
436 absence of meiotic recombination on Lakl0C-left that is likely common to all *Lachancea*  
437 *kluyveri* isolates, this suggests a leading role of mitotic recombination in yielding  
438 homozygous sequence polymorphisms in *L. kluyveri*.

439

440 Having determined the Spo11-DSBs landscape of *L. kluyveri*, we could assess the  
441 conservation of the strength of Spo11-DSBs hotspots between *L. kluyveri* and the  
442 reference *S. cerevisiae* by comparing the frequency of Spo11-DSBs within syntenic

443 intergenic regions as in (12). Unlike within the *Saccharomyces* species, the strength  
444 of Spo11-DSBs hotspots is only poorly conserved between *L. kluyveri* and *S.*  
445 *cerevisiae*, and does not result in conserved crossover hotspots (50). We figured out  
446 that the ohnologs provide another relevant dataset to look at the conservation of  
447 Spo11-DSBs hotspots within a single genomic environment at a broad evolutionary  
448 scale, larger than that of the *Saccharomyces* clade but shorter than that separating *S.*  
449 *cerevisiae* and *L. kluyveri*. Although the sample size is small, the strength of the Spo11-  
450 DSBs signal within syntenic inter-ohnolog regions is not conserved at all. In both cases  
451 it is not possible to distinguish between hotspot erosion, already visible within the  
452 *Saccharomyces* clade, and different chromosomal environments or selective  
453 constraints as a source of this lack of Spo11-DSBs hotspot strength conservation.  
454 However, this shows that the strength of Spo11-DSBs hotspots that do not rely on  
455 sequence specific elements tend to be not conserved at such an evolutionary scale  
456 within budding yeasts, which is about 100 million years (80).

457  
458 In most species studied so far, the number of meiotic crossovers per chromosome is  
459 between one and three. *S. cerevisiae* and *S. pombe* are among the outliers with much  
460 more crossovers per chromosome (81, 82). With a comparable genome size and twice  
461 fewer chromosomes, *L. kluyveri* has ca. four-fold fewer crossovers per meiosis than *S.*  
462 *cerevisiae* (50). *L. kluyveri* therefore resembles most species, with the exception of  
463 Lak10C-left that likely involves an additional mechanism preventing recombination. We  
464 observed a ca. two-fold longer synaptonemal complex length in *S. cerevisiae* and a  
465 shorter chromosome width as measured by DAPI staining. Given the evolutionary  
466 conservation of the dimensions of the “loop-base module” (42) and the similar genome  
467 size of *S. cerevisiae* and *L. kluyveri*, this suggests less but longer loops in *L. kluyveri*

468 compared to *S. cerevisiae*. The correlation of chromosome axis length and frequency  
469 of crossovers between these two closely related species is reminiscent of the  
470 covariation of the frequency of crossovers per nucleus with the chromosome axis  
471 length (41, 83). While this latter point suggests a constant crossover frequency per  
472 chromatin loop within a given species, the actual crossover frequency per chromatin  
473 loop might be different between *L. kluyveri* and *S. cerevisiae*. Overall, given the strong  
474 difference in terms of recombination frequency and chromosome axis length between  
475 *S. cerevisiae* and *L. kluyveri*, the detailed comparison of their meiotic chromatin loops  
476 and associated genes and proteins may be a way to understand what governs the  
477 meiotic recombination frequency of a given species. Notably, the Pds5 cohesion  
478 maintenance factor and the NuA4 histone acetyltransferase complex catalytic subunit  
479 Esa1 were shown to contribute significantly but independently to meiotic chromosome  
480 axis length (84, 85). It will be interesting to determine their contributions in the  
481 difference in meiotic chromosome axis length between *L. kluyveri* and *S. cerevisiae*.

482  
483 The Y structure of Lak10C chromosome during pachytene, with only the base of the Y  
484 stained by Zip1, suggests that the synaptonemal complex does not form along Lak10C-  
485 left, although it is present on the right arm of this chromosome. While recombination is  
486 essential to initiate synaptonemal complex formation in *S. cerevisiae* and likely in *L.*  
487 *kluyveri* (44), our results suggest that either the extent of polymerization of the  
488 synaptonemal complex in *L. kluyveri* is limited and requires initiation points not too far  
489 apart to cover chromosomes entirely, and/or that something is specifically inhibiting  
490 synaptonemal complex polymerization on Lak10C-left after its priming outside of  
491 Lak10C-left. Alternatively, the failure to polymerize could be due to the absence of pre-  
492 existing Red1 or Hop1, as discussed below.

493

494 Our most striking finding is the complete absence of the meiotic axis proteins Hop1  
495 and Red1 from Lakl0C-left. The abundance of these proteins is known to vary along  
496 chromosomes. Hop1 is notably enriched in regions with higher recombination activity  
497 (16). However, the complete absence of Hop1 and Red1 from a chromosome region  
498 that encompasses ca. 8% of the entire genome has not been reported so far, to our  
499 knowledge. Despite their phylogenetic divergence, the regulation of meiotic  
500 recombination is highly similar between *S. cerevisiae* and *L. kluyveri*. Most if not all  
501 relevant meiotic recombination genes are conserved between the two species. This  
502 contrasts with all the other *Lachancea* species that lost most genes from the ZMM  
503 pathway right after the divergence of *L. kluyveri* from the rest of the clade (58, 86).  
504 Notably, Hop1, Red1 and Rec8 display similar properties between *S. cerevisiae* and  
505 *L. kluyveri*. These include the early recruitment of Rec8 around centromeres and the  
506 subsequent recruitment of Rec8 in discrete peaks between convergent genes  
507 throughout the genome that strongly colocalize with both Hop1 and Red1 peaks. This  
508 suggests conserved recruitment regulation of these proteins. In *S. cerevisiae*, the main  
509 Hop1 and Red1 recruitment pathway is through Rec8 that first recruits Red1 which  
510 subsequently recruits Hop1 (16, 23). A second recruitment pathway independent of  
511 Rec8 and relying on the PHD domain of Hop1 was recently reported (24). The  
512 complete absence of both Hop1 and Red1 from Lakl0C-left suggests that these two  
513 pathways are inhibited in this entire 1 Mb long region. Whether this relies on the  
514 presence of an inhibitory factor, or the absence of an activating factor is so far  
515 unknown. Although the specific early replicating profile was a plausible explanation for  
516 this chromosome-wide behavior, we showed that other early replication regions are  
517 not significantly depleted in Spo11-DSBs and Zip3 ChIP-seq signals. Another

518 hypothesis is that this behavior is related to the ca. 12% higher GC content of Lakl0C-  
519 left than the rest of the genome, *i.e.* 52.9 vs 40.4% respectively (53, 87). Interestingly,  
520 Heldrich et al (24) showed that the direct recruitment of Hop1 to genomic islands relied  
521 on the local DNA sequence. Therefore, one possibility is that Lakl0C-left sequence is  
522 incompatible with direct Hop1 recruitment, for reasons that remain to be determined.  
523 However, Rec8 is properly loaded on Lakl0C-left as detected by ChIP-seq where it  
524 ensures proper chromosome condensation as detected by Hi-C and directly by Rec8  
525 immuno-staining. Hence, while the unusual composition bias of Lakl0C-left could  
526 explain the absence of direct recruitment of Hop1, it likely does not explain the  
527 inhibition of Hop1 recruitment by Rec8.

528

529 Extensive recombination suppression is seen on sex chromosomes, self-  
530 incompatibility loci in plants and mating type loci in fungi and algae (48). In the case of  
531 fungi, the rationale for the initial recombination suppression at the mating type loci is  
532 probably to avoid breaking linkage between genes controlling correct mating. In the  
533 budding yeasts *S. cerevisiae* and *L. kluyveri*, such genes include *MAT ALPHA1* and  
534 *ALPHA2* genes in the *Mata* genotype, and *MATA1* and *MATA2* in the *Mata* genotype,  
535 although no known role of *MATA2* has been determined so far. Interestingly,  
536 recombination cessation extends beyond the *MAT* locus in many fungi, but the  
537 selective advantage is not always clear (48). In the case of *L. kluyveri*, the  
538 recombination cessation is likely concomitant with the introgression of Lakl0C-left,  
539 therefore relatively recent and includes only one evolutionary stratum covering 80% of  
540 chromosome C. No such recombination inhibition is observed in neighbor species  
541 including *L. waltii* and *S. cerevisiae*. Interestingly, although meiotic recombination is  
542 inhibited, the accumulation of homozygous sequence polymorphisms between isolates

543 suggests that mitotic recombination is taking place equally in Lakl0C-left and the rest  
544 of the genome, and that it plays the major role in *L. kluyveri* genome evolution. Overall,  
545 while no clear selective advantage has been identified so far for the recombination  
546 cessation on Lakl0C-left, the mechanism reported here, *i.e.* the lack of recruitment of  
547 meiotic chromosome axis proteins, could be responsible for the extension of  
548 recombination cessation around mating type loci in many fungal genomes and play an  
549 important role in the emergence of sex chromosomes that rely on the cessation of  
550 meiotic recombination (48, 88).

551

552

### 553 **Figures legends**

554

555 **Figure 1. Lakl0C-left is depleted in Spo11-DSBs, Zip3 and crossovers. A.**  
556 Genome-wide CC-seq (Spo11-DSBs, top) and Zip3-FLAG ChIP-seq (bottom) profiles  
557 of *L. kluyveri* CBS10367 at six hours post meiosis induction. The eight chromosomes  
558 are concatenated in the alphabetical order from A (left) to H (right) and separated by  
559 vertical dotted lines. Black discs indicate centromeres. The black triangle indicates the  
560 rDNA locus position, which corresponding signal has been masked. The y axis of the  
561 CC-seq profile represents the Spo11-DSB count after denoising of a single experiment.  
562 The y axis of the Zip3-FLAG ChIP-seq profile represents the mean ChIP-seq signal of  
563 five independent experiments. A zoom in a representative region of chromosome E  
564 with superimposition of the CC-seq and Zip3-FLAG ChIP-seq signals is shown. Coding  
565 regions are represented on the x axis (chromosome coordinates in kilo bases).  
566 Transcription is left to right for red boxes and right to left for blue boxes. Maximum  
567 values are lower in the genomic view due to visualization constraints. **B.** Box plot



568 representing the distribution of the CC-seq signal according to the type of genomic  
569 location. Intergenic regions comprise regions flanked by convergent genes, divergent  
570 genes, and genes that are in a head to tail (tandem) configuration. Horizontal bars  
571 show the medians and crosses show the means. Boxes delimit the first and third  
572 quartiles. The error bars represent minimum and maximum values. **C.** Average signals  
573 of five independent Zip3-FLAG ChIP-seq experiments performed 6 hours after meiosis  
574 induction piled up around the centers of the 500 strongest Spo11-DSBs hotspots  
575 defined by MACS2 from the CC-seq experiment. **D.** Recombination assay in the *L.*  
576 *kluyveri* CBS10367 diploid strain. The two copies of chromosome C are represented  
577 as a horizontal black line. Relevant loci are indicated as well as their chromosome  
578 coordinates in kb. *PHLEO* was integrated at the Laki0C-899 locus. The black ovals  
579 represent centromeres. The table indicates single and double crossovers (CO)  
580 between *MAT* and *PHLEO* and between *PHLEO* and *CHS3* out of 96 tetrads. The  
581 picture on the right is representative of a full tetrad with the two *MAT $\alpha$*  colonies  
582 exhibiting a smaller size than the two *MAT $\alpha$*  colonies.

583

584 **Figure 2. Spo11-DSB signal conservation across syntenic intergenic regions**  
585 **(IGR) between *L. kluyveri* and *S. cerevisiae*, and between *S. cerevisiae* ohnologs.**

586 **A.** Top panel represents syntenic intergenic regions (highlighted by grey boxes) in *L.*  
587 *kluyveri* and *S. cerevisiae* that are flanked by orthologous genes couples. The bottom  
588 graph shows the comparison of the CC-seq Spo11-DSB signal from 1,956 syntenic  
589 IGR between *L. kluyveri* (x axis) and *S. cerevisiae* (y axis) with the correlation  
590 coefficient  $r$  indicated. **B.** Same as in A except that 39 *S. cerevisiae* intergenic regions  
591 flanked by couples of ohnologs are considered.

592

593 **Figure 3. Lakl0C-left compaction along meiotic prophase is similar to other**  
594 **chromosomal arms.**

595 **A.** Contact maps of the whole genome of *L. kluyveri* before (left) and five hours (middle)  
596 after induction of meiosis. Ratio maps of five hours over premeiotic chromosomes  
597 (right). Bin size, 10 kb.

598 **B.** Contact maps of chromosome C (left), D (middle) and E (right) at five hours after  
599 meiosis induction. Bin size, 1 kb. Magnified insets show portions of 200 kb, with the  
600 corresponding Rec8 ChIP over untagged ratio profiles, determined at four hours post  
601 meiosis induction.

602 **C.** Distance laws of Lakl0C-left compared to the mean of ~1Mb of different  
603 chromosome arms before, three hours and five hours post meiosis induction.

604 **D.** Spectrum of loop score detected at five hours post meiosis induction at the  
605 intersection of Rec8 peaks detected at four hours post meiosis induction, as a function  
606 of the distance separating the pair of Rec8 peaks (kb).

607 **E.** Aggregated ratio maps of 29 kb windows (five hours post meiosis induction)  
608 centered on pairs of Rec8 peaks (four hours post meiosis induction) over randomly  
609 chosen positions in the same inter-distance range. Bin size: 1 kb.

610

611 **Figure 4. ChIP-seq of chromosome axis proteins Rec8-FLAG, Hop1-FLAG and**  
612 **Red1-FLAG.** The top panel represents the genome-wide ChIP-seq signals from two  
613 independent experiments six hours after meiosis induction for the three proteins. Same  
614 legend as in Figure 1. The bottom panel is a close-up view of a representative genomic  
615 region of chromosome E illustrating the strong similarity between the ChIP-seq profiles  
616 of the three proteins. Genes coding regions are represented as in Figure 1A.

617

618 **Figure 5. Spo11-DSBs and Zip3-FLAG ChIP-seq enrichment as a function of the**  
619 **replication timing. A.** *L. kluyveri* genome was partitioned in four groups  
620 encompassing the entire vegetative replication phase. Each group corresponds to a  
621 period of eight minutes. The left graph represents the fraction of the genome (in kb)  
622 encompassed by each replication group. The right graph represents the fraction of  
623 each replication group within each chromosome. W/O Lakl0C-left: region of *L. kluyveri*  
624 chromosome C excluding Lakl0C-left. **B.** Fraction of the Spo11-DSBs signal at six  
625 hours post meiosis induction included in each replication group normalized by the  
626 genomic fraction of each group. **C.** Fraction of the Zip3-FLAG ChIP-seq signal at six  
627 hours post meiosis induction included in each replication group normalized by the  
628 genomic fraction of each group.

629

630 **Figure 6. Cytology of *L. kluyveri* meiotic chromosomes. A.** Pachytene spread  
631 nuclei of *S. cerevisiae ndt80* mutant strain (BLY1808) 12h after sporulation induction  
632 stained with DAPI (blue) and anti-Zip1 antibody (green). **B.** Pachytene spread nuclei  
633 of *L. kluyveri ndt80* mutant strain (LAKL220) 13.5h after sporulation induction stained  
634 with DAPI (blue) and anti-Zip1 antibody (green). **C.** Quantification of the total length of  
635 Zip1 lines per cell in  $\mu\text{m}$ . Black lines show means  $\pm$  SD. *L. kluyveri*:  $13.86 \pm 2.81 \mu\text{m}$   
636 ( $n= 170$ ), *S. cerevisiae*:  $28 \pm 3.2 \mu\text{m}$  ( $n= 139$ ). The average number of Zip1 lines  
637 observed and quantified per cell is  $8.7 \pm 0.76$  for *L. kluyveri* and  $14.24 \pm 1.32$  for *S.*  
638 *cerevisiae*. **D.** Pachytene spread nuclei of (i) *S. cerevisiae ndt80* mutant strain  
639 (BLY1808) 12h after sporulation induction and (ii) *L. kluyveri ndt80* mutant strain  
640 (LAKL220) 13.5h after sporulation induction stained with DAPI (blue) and Zip1 (green).  
641 *S. cerevisiae* and *L. kluyveri* spread nuclei were mixed on the same slide to make sure  
642 they undergo the same treatments. **E.** Quantification of DAPI signal width from panel

643 D which reflects the chromosome loops size. Black lines show mean  $\pm$  SD. *L. kluyveri*:  
644  $1.26 \pm 0.37 \mu\text{m}$  (n= 100), *S. cerevisiae*:  $0.83 \pm 0.19 \mu\text{m}$  (n= 104). In panel C and E,  $p <$   
645  $0.0001$ , Mann-Whitney U test. **F and G.** Pachytene spread nuclei of *L. kluyveri ndt80*  
646 mutant strain (LAKL227) 13.5h after sporulation induction. In F, staining was performed  
647 with DAPI (blue), anti-FLAG antibody against Rec8-FLAG (red), anti-Zip1 antibody  
648 (cyan) and an rDNA FISH probe (green). The left arrowhead points at the rDNA locus.  
649 The right arrowhead points at the inferred chromosome C right unsynapsed arms. In  
650 G, staining was performed with DAPI (blue), Lakl0C-left FISH probe (green), Lakl0C-  
651 right FISH probe (red) and an rDNA FISH probe (white). The arrowhead points at  
652 chromosome C. The structure of chromosome C inferred from staining experiments is  
653 drawn (not to scale) on the right of panels F and G. Scale bars =  $1 \mu\text{m}$ .

654

655 **Figure S1: Spo11-DSB identification by CC-seq.**

656 **A.** Test for locus specific enrichment of Spo11-DSBs. The CC-seq Spo11-DSB  
657 purification protocol was applied to  $6.5 \times 10^8$  *L. kluyveri* cells at six hours post meiosis  
658 induction. Here, the DNA was digested by PflMI instead of sheared by sonication.  
659 Aliquots of the indicated fractions were analyzed by 0.8 % agarose gel electrophoresis  
660 followed by Southern blot using the indicated probe (vertical black bar) as in (50). The  
661 right side of the blot shows the gene (arrows) organization around the *GPI18* locus  
662 with arrow heads indicating the relative transcription orientations. M: molecular weight  
663 ladder (kb). A Spo11-DSB signal specific to the GPI18 promoter is detected in the  
664 elution fractions.

665 **B.** Two independent CC-seq profiles after background removal as in Figure 1B. The  
666 signal/noise ratio of the top experiment is lower than that of the bottom experiment.  
667 Only the bottom experiment was kept for further analyses.

668 **C.** Correlation of the two independent CC-seq experiments after background removal  
669 inside the intersected hotspots detected by MACS2 (n = 2038). Normalized in hit per  
670 million reads of the corresponding sequencing library.

671

672 **Figure S2: Characteristics of Spo11-DSB signal**

673 **A.** Box plot representing the distribution of the CC-seq signal (mean DSB count in hits  
674 per million reads of the sequencing library per kb) according to the type of genomic  
675 location as in Figure 1B in *L. kluyveri* and *S. cerevisiae*.

676 **B.** Pearson correlation coefficient of 1,000 pairwise comparisons of randomized  
677 Spo11-DSB signal from syntenic intergenic regions (IGR) between *L. kluyveri* and *S.*  
678 *cerevisiae* (left), and between *S. cerevisiae* intergenic regions flanked by pairs of  
679 ohnologs (right). The red lines represent the actual coefficients from Figure 2. The  
680 small-dots lines represent the mean coefficients from the comparisons of the  
681 randomized datasets.

682

683 **Figure S3: Spo11-DSB CC-seq chromosome-wide signal smoothed with a Hann**  
684 **sliding window of 50kb.** The dotted lines represent the genome average.

685

686

687 **Figure S4: Western blot analysis of Red1, Hop1, Rec8 and Zip3.** Protein extracts  
688 from cultures of the indicated time points after sporulation induction were run on  
689 acrylamide gels, transferred to a nitrocellulose membrane, incubated with an anti-  
690 FLAG antibody (mouse) which is common to the four proteins analyzed and revealed  
691 with an anti-mouse-HRP antibody. M: molecular weight marker with sizes in kDa  
692 indicated on the left. Estimated molecular weight are: 99 kDa for Red1, 69 kDa for

693 Hop1, 79 kDa for Rec8 and 37 kDa for Zip3. Each of these proteins is fused with six  
694 histidines and three copies of the FLAG tag, which adds about 4 kDa.

695

696 **Figure S5: Meiotic progression and evolution of chromosomal contacts.**

697 **A.** Fraction of cells with two to four nuclei detected by DAPI staining as a function of  
698 time post meiosis induction (in hours). Error bars represent standard deviation among  
699 the four cultures pooled together for the Hi-C experiment.

700 **B.** Inter-centromere contact frequencies at different time points of a meiotic time-  
701 course.

702 **C.** The number of chromosomal contacts sequenced per each time point is measured  
703 on the right y axis. The left y axis represents the fraction of each type of contact, i. e.  
704 intra-chromosomal, inter-chromosomal or undetermined.

705 **D.** Frequency of interchromosomal contacts for several ~1Mb chromosome arms from  
706 zero to five hours of a meiotic time course.

707 **E.** Distance laws of the several ~1Mb chromosome arms from zero to six hours of a  
708 meiotic time course.

709 **F.** Spectra of loop scores detected at the intersection of Rec8 peaks as a function of  
710 the distance separating the pairs of Rec8 peaks (kbp) for different time points of a  
711 meiotic time-course and for several ~1Mb chromosomal arms.

712

713 **Figure S6.** ChIP-seq individual replicates of Zip3-FLAG, Rec8-FLAG, Red1-FLAG and  
714 Hop1-FLAG. Same legend as in Figure 1A.

715

716 **Figure S7: Comprehensive ChIP-seq analysis of the axis proteins Rec8, Red1**  
717 **and Hop1, with the crossover marker Zip3 and the Spo11-DSBs.** **A.** Combination

718 of CC-seq data and ChIP-seq data from Figure 1A and Figure 4. ChIP-seq data are  
719 the means of all the replicates presented in Figure S6 (five replicates for Zip3 and two  
720 replicates for Rec8, Red1 and Hop1). **B.** Pileup of Rec8-FLAG ChIP-seq signals at six  
721 hours post meiosis induction overlapping intragenic regions, convergent intergenes,  
722 divergent intergenes, and intergenes in a tandem configuration, centered on the center  
723 of the region of interest. Left, whole genome; right, Lakl0C-left only. The lower Rec8-  
724 FLAG ChIP-seq signal on Lakl0C-left (right panel) likely results from a lower  
725 sequencing efficiency of this region due to an elevated GC content (see methods). **C.**  
726 Background-corrected pileup of Rec8-FLAG, Red1-FLAG, Hop1-FLAG and Zip3-  
727 FLAG ChIP-seq signals centered on Rec8-FLAG ChIP-seq peaks centers (left) and  
728 Zip3-FLAG ChIP-seq peaks centers (right). Rec8 and Zip3 peak coordinates  
729 overlapping rDNA coordinates were removed from the analysis. **D.** Overlaps between  
730 Hop1-FLAG, Red1-FLAG and Rec8-FLAG ChIP-seq peaks at four (left) and six (right)  
731 hours post meiosis induction. \*\*\*P < 0.001 (hypergeometric test).

732

733 **Figure S8: Rec8, Red1 and Hop1 ChIP-seq chromosome-wide signals smoothed**  
734 **with a Hann sliding window of 50kb.** The dotted lines represent the genome  
735 average.

736

737 **Figure S9.** The absence of Tel1 does not restore Spo11-DSBs on Lakl0C-left. A.  
738 PFGE analysis of *L. kluyveri* meiotic chromosomes. Left: ethidium bromide-stained gel.  
739 M: *S. cerevisiae* SK1 chromosomes were used as molecular weight markers with their  
740 sizes indicated in Mb on the left. Right. Southern blot analysis using a chromosome D  
741 left probe. The vertical grey bar represents chromosome D and the grey oval indicates

742 the centromere location. B. Same as in A except that a chromosome C right probe was  
743 used.

744

745 **Figure S10. A.** Number of heterozygous SNPs per 10 kb windows. **B.** Number of  
746 heterozygous (dark grey) and homozygous (light grey) SNPs per 10 kb windows.

747

748

## 749 **Methods**

750

### 751 **Strains and media**

752 All yeast strains used in this study are derivatives of *L. kluyveri* CBS10367 natural  
753 diploid isolate used for its efficient meiotic entry and progression (54, 79). Strain  
754 genotypes are listed in Table S1 as well as plasmids used. Gene editing was performed  
755 by PCR-mediated gene targeting followed by PCR analysis. Long homology fragments  
756 (ca 500 bp long) were used because short homology fragments are not efficient for  
757 gene targeting in *L. kluyveri*. Combining long homology fragments with selective  
758 markers was made by overlapping PCR or Gibson cloning (Cat# E5520S, NEB).  
759 Oligonucleotides are listed in Table S2. Because CBS10367 derived haploids tend to  
760 diploidize, all gene editing experiments were performed in a diploid background. This  
761 required two successive gene targeting experiments with different selective markers  
762 to modify the two alleles of a gene.

763 Growth conditions and media, as well as detection of meiotic DSBs by PFGE followed  
764 by Southern blot are as in (50). Briefly, YPD medium (yeast extract 1%, peptone 2%,  
765 glucose 2%) was used for vegetative growth at 30 °C and sporulation was induced on



766 1% potassium acetate. Inactivation of both alleles of the chitin synthase III gene *CHS3*  
767 is a prerequisite for tetrad dissection in *L. kluyveri*.

768

### 769 **CBS10367 genome sequencing**

770 Genomic DNA from CBS10367 was prepared using Qiagen tips according to the  
771 manufacturer instructions and sequenced at BGI. Reads were mapped to the *L.*  
772 *kluyveri* reference genome (downloaded from  
773 [http://gryc.inra.fr/storedData/download/Lachancea\\_kluyveri/CBS\\_3082/](http://gryc.inra.fr/storedData/download/Lachancea_kluyveri/CBS_3082/)) with BWA  
774 (v.0.7.4), using mem option (89). Duplicated reads were marked with Picard-tools  
775 (v.2.10.5) ([broadinstitute.github.io/picard](https://broadinstitute.github.io/picard)) and local realignment around indels and  
776 variant calling were performed with GATK (v.3.7-0) (90). Default parameters were  
777 applied except for the realignment step (GATKIndelRealigner), in which the following  
778 parameters were set: '-maxReadsForConsensuses 500 -maxReadsForRealignment  
779 40000 -maxConsensuses 60 -maxPositionalMoveAllowed 400 -entropyThreshold 0.2'.  
780 Variants were called with GATK HaplotypeCaller function, before running GATK  
781 Variant Annotator to add allele balance information in the vcf file.

782 The complete genome sequence of the CBS10367 isolate was inferred both from the  
783 homozygous variants (SNPs and indels) and specifically from the homozygous SNPs  
784 (by removing indels with --remove-indels option from vcftools) into the reference  
785 sequence with GATK FastaAlternateReferenceMaker. This yielded the  
786 CBS10367\_SNP\_inferred and CBS10367\_SNP\_indels\_inferred genome sequences.  
787 For SNPs and indels density analysis along chromosomes, the following variants were  
788 excluded: those located in the subtelomeric regions (30 kb from the beginning/end of  
789 the chromosome), and heterozygous variants for which the alternative residue vs  
790 (reference + alternative residues) allele ratio deviates from 0.4 to 0.6.

791

## 792 **CC-seq experiments**

793 The *sae2* CBS10367 strain (LAKL 221) was used to perform meiotic time courses.  
794  $3 \times 10^{10}$  cells were harvested for each CC-seq experiment six hours after meiosis  
795 induction, which corresponds to the peak of Spo11-DSBs (50). This amount of cells  
796 corresponds to ca. 10 times the amount of cells used for *S. cerevisiae* by (56) because  
797 of lower recovery yields in *L. kluyveri*. Likely because of different cell wall compositions,  
798 spheroplasting using zymolyase treatment takes longer in *L. kluyveri* compared to *S.*  
799 *cerevisiae*. We therefore used mechanical cell lysis instead of enzymatic lysis. Cells  
800 were lysed in 2 ml screw cap tubes with 300 microliters of glass beads (425-600  
801 micrometer of diameter, Sigma) per tube using a Fast prep (MP Biomedicals). We used  
802 three cycles of 30 seconds at 4 m/s, with five minutes on ice between the cycles. Cell  
803 lysates were harvested by centrifuging the screw cap tubes after drilling a hole at their  
804 base for 2 minutes at 950g at 4°C. From this point, cell lysates were treated as in (56).  
805 The efficiency of the CC-seq purification procedure was confirmed by Southern blot  
806 analysis of the previously characterized GPI18 Spo11-DSB hotspot using PflMI  
807 digested DNA (Figure S1A).

808

## 809 **CC-seq data analysis**

810 Sequences were analyzed as in (56). *L. kluyveri* reference genome is  
811 CBS10367\_SNP\_inferred. The terminalMapper script from (56) generates a file with  
812 four columns corresponding to (i) the chromosome, (ii) the coordinate, (iii) the number  
813 of DSBs mapped on the Watson strand at this position and (iv) the number of DSBs  
814 mapped on the Crick strand at this position. The number of DSBs mapped on the

815 Watson and Crick strands showed a good correlation and were pooled together for our  
816 subsequent analyses.

817 Unlike *S. cerevisiae* CC-seq data, *L. kluyveri* CC-seq data contained important  
818 background signal (about 60% of the entire signal) that needed to be filtered to extract  
819 the specific signal. We empirically determined a background signal threshold after  
820 signal smoothing with a Hann sliding window function over 15 units, a unit being a  
821 coordinate with a number of associated DSBs >0. Since intragenic regions are  
822 expected to be depleted in specific signal, we considered the mean signal per base  
823 pair over the entire intragenic regions as the background threshold that we subtracted  
824 to any genomic position. Only positive signals were kept for subsequent analyses. This  
825 denoising procedure suppressed around 81% and 73,5% of the total smoothed signal  
826 in experiment #1 and 2, respectively (Figure S1B). Experiment #2 showed the best  
827 enrichment. The number of DSB counts per bp is normalized by the total number of  
828 DSB counts per experiment and expressed as Hit per Million Hits mapped (HpM).

829 All the analyses were performed using R (3.5 version or later) and RStudio (1.1.456 or  
830 later). Spo11-DSBs hotspots detection was performed using SAM files with MACS2  
831 (2.1.1.20160309 version) (57) and the following parameters: callpeak -f SAM -g  
832 11.345814e6 --nomodel --extsize 100 --shift 50 -q 0.01 for *L. kluyveri* and -g  
833 12.157105e6 for *S. cerevisiae*.

834 The randomizations were performed by shuffling the Spo11-DSB counts associated to  
835 the intergenic regions in each dataset 1,000 times using the sample command from R.  
836 1,000 comparisons between the randomized Spo11-DSB counts were performed, and  
837 the corresponding “Pearson” correlation coefficient  $r$  was calculated.

838

839 **Onholog analysis**

840 Spo11-DSB counts of S288C *S. cerevisiae* strain were extracted from (10) supp data  
841 “FullMap.Spo11.S288C\_chrX.txt”. The sum of the DSBs within intergenic regions was  
842 determined using the S288C reference genome (<https://www.yeastgenome.org>,  
843 downloaded March 2023). Pairs of syntenic intergenic regions flanked by ohnologs  
844 were identified with the list available at <http://wolfe.gen.tcd.ie/ygob> (60).

845

### 846 **ChIP-seq experiments**

847 ChIP-seq experiments were performed as in (47, 91). All immuno-precipitations used  
848 the mouse anti-FLAG M2 antibody (Sigma). The expression of the FLAG-tagged  
849 proteins was verified by western blotting (Figure S4). ChIP-sequencing was performed  
850 with a HiSeq2500 sequencer (Illumina) using a 50-nt paired-end protocol. Two  
851 independent replicates of each condition were analyzed except for Zip3-FLAG that  
852 contained five replicates (Figure S6).

853

### 854 **ChIP-seq data analysis**

855 **Alignment.** Sequencing quality control was determined using FastQC tool  
856 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and aggregated across  
857 samples using MultiQC (v1.7) (92). Reads with Phred quality score less than 30 were  
858 filtered out. Paired-end reads were mapped to CBS10367\_SNP\_inferred reference  
859 genome using Bowtie2 (v2.3.4.3) (93) with default parameters. Only mapped reads  
860 were filtered in using samtools (v1.9) (94) (*view; --exclude-flags 4*). Duplicate tags were  
861 removed using Picard Tools (v2.20.2) (<https://broadinstitute.github.io/picard/>)  
862 (*MarkDuplicates; REMOVE\_DUPLICATES=true*) and mapped tags were processed  
863 for further analysis. Reads mapping to the mitochondrial genome and ribosomal DNA  
864 were removed from any analysis.

865

866 **BigWig files.** For visualization purpose, mapped tags were converted into bigwig and  
867 normalized by their library size using the deepTools (v3.2.1) (95) (*bamCoverage –*  
868 *scaleFactor library\_size\_factor –binSize 5*). Reads were extended using values  
869 previously computed by deepTools (*bamPEFragmentSize*) and used through the –  
870 *extendReads* option. Untagged-subtracted bigwig files were computed from the  
871 normalized bigwig files using deepTools (*bigwigCompare –operation subtract*). BigWig  
872 files from multiple replicates were merged into a single file using a custom bash script  
873 (*bigWigMerge, bedGraphToBigWig*). All sequencing data were visualized using the  
874 Integrative Genomics Viewer (IGV; v2.5.2) (96). Immunoprecipitated over untagged  
875 ratio profiles were generated with bamCompare using the –operation ratio (Figure 3B).

876

877 **Peak calling.** High confidence binding sites were determined using MACS2 callpeak  
878 (v2.1.2) (57) in broad mode (*--broad; --broad-cutoff 0.05; --qvalue 0.01*). Each sample's  
879 fragment size was previously computed by deepTools (*bamPEFragmentSize*) and  
880 used through the *--extsize* option. Untagged strains at the same timepoint were used  
881 as controls.

882

883 **ChIP-seq pileup analyses.** ChIP-seq signals were piled up around specific features  
884 by processing bigwig and untagged-subtracted bigwig files through the deepTools  
885 (*computeMatrix; --referencePoint center; --binSize=50*) and using custom R scripts for  
886 visualization. Background signals from plots containing signals of experiments done  
887 by immunoprecipitating different proteins have been adjusted for better comparison  
888 (Fig S7C). The background signal of a given condition was determined as the average  
889 of the extreme left and right values of the plot profile. The differences between

890 background signals were used to correct signals by making their background levels  
891 similar.

892

893 **Venn diagrams.** Venn diagrams analyses were performed from peak coordinates bed  
894 files obtained from MACS2 peak caller and using python matplotlib\_venn package  
895 (v0.11.5) (<https://github.com/konstantint/matplotlib-venn>) for visualization. Peaks were  
896 intersected using bedtools *intersect* (v2.28.0) (97) with a minimum overlap of 1-base.  
897 Overlaps significance was determined by hypergeometric test (*phyper*; *lower.tail* =  
898 *false*). Since a given peak can overlap multiple peaks of other proteins, some peaks  
899 were merged during the intersect processes leading to small variations in total peak  
900 numbers.

901

## 902 **Hi-C**

903 Hi-C experiments were performed using the CBS 10367 isolate. To harvest enough  
904 cells per time point (ie ca  $2 \times 10^9$  cells), four independent 200 ml cultures in 2 l flasks  
905 were used. Equal samples were harvested from the four flasks at 0, 2, 3, 4, 5, 6, 7  
906 and 8 hours post meiosis induction and treated with 3% formaldehyde for 20 minutes  
907 under constant shaking. Samples were quenched using 0.35M glycine at 4°C for 20  
908 minutes. Cells were harvested by centrifugation at 4000 g for 4 minutes at 4°C, washed  
909 once with potassium acetate 1% (sporulation medium), transferred to 1.5 ml tubes and  
910 pellets were flash frozen using liquid nitrogen.

911

## 912 **Generation and normalization of contact maps and ratio maps**

913 Alignment of paired-end reads and processing of the contact data were done with  
914 Hicstuff pipeline on the CBS10367\_SNP\_inferred reference genome, using with the

915 following parameters: aligning with bowtie2, filtering out spurious 3C events and PCR  
916 duplicates based on read positions. Contact maps were normalized with the ICE  
917 function as described in (98) and generated with the 'view' mode of Hicstuff (99).  
918 Contact maps were binned at 10 kb for the whole genome or 1 kb for single  
919 chromosomes.

920

### 921 ***Contact probability as a function of the genomic distance***

922 Contact probability as a function of genomic distance  $P(s)$  and its derivative were  
923 computed using the 'distance law' function of Hicstuff on the following ~1Mb  
924 chromosomal arms : Lakl0A-L, Lakl0B-R, Lakl0C-L, Lakl0E-R and Lakl0G-L arms.

925

### 926 ***Generation of aggregated cohesin-centered contact maps and spectrum of*** 927 ***loop score as a function of distance***

928 Loops at the intersection of cohesin enrichment sites located on ~1Mb of Lakl0A-L,  
929 Lakl0B-R, Lakl0C-L, Lakl0E-R and Lakl0G-L arms were scored with chromosight in  
930 quantify mode. Lowess-smoothed loop scores were plotted as a function of loop size  
931 data.

932 Cohesin pile-up contact maps were generated with chromosight (67) dividing averaged  
933 29 kb windows centered on pairs of cohesin-enriched sites by the average of 10  
934 windows centered on 10 random pairs of positions maintaining the same inter-distance  
935 distribution. Cohesin peaks whose enrichment determined by MACS2 was higher than  
936 2 were selected.

937

### 938 **Meiotic chromosome spreads**

939 Chromosome spreads from meiotic cultures were prepared from  $\sim 6 \times 10^8$  cells  
940 harvested at the indicated time-points following the protocol described in (100) with  
941 some modifications. The addition of 0.005% of Antifoam (Sigma) to the pre-sporulation  
942 and sporulation media helped avoid the clustering of cells in the cultures. Yeast cells  
943 were first processed into spheroplasts by incubation at 30°C for 15 (for *S. cerevisiae*  
944 strains) to 40 (for *L. kluyveri* strains) minutes with (400  $\mu\text{g/ml}$  final) Zymolyase 100T  
945 (120493-1, AMS Biotechnologie). Spheroplasts were washed and resuspended in cold  
946 MES(2-(N-morpholino)ethanesulfonic acid)/sorbitol buffer to which equal volumes of  
947 100mM sucrose solution was added and incubated for at least 10-30 minutes before  
948 spreading.

949 For chromosome spreading, slides were divided into two squares using an ImmEdge  
950 hydrophobic pen (Vector Labs) and placed horizontally in a humid chamber followed  
951 by the addition of 10  $\mu\text{l}$  of cell suspension per square. The cell suspension was  
952 subsequently lysed with 1% NP40 (IGEPAL CA-630, Sigma) and fixed with 3%  
953 paraformaldehyde (PFA) containing 3.4% sucrose and 0.04% Sodium Dodecyl Sulfate  
954 (SDS). In addition, 0.001M dithiothreitol (DTT) was added to MES/sorbitol buffer,  
955 sucrose and PFA solutions. The slides were gently swirled for homogenization, before  
956 incubation in the closed humid chamber overnight. The humid chamber was opened  
957 for a few hours the next day to allow almost complete drying of the cell suspension.

958

### 959 **Immunostaining**

960 Slides with spread nuclei were sequentially incubated with the proper primary and  
961 secondary antibodies. Slides were washed before and in the intermittent steps in a  
962 Coplin jar for 10 min each in PBS-Tween 0.05% containing 0.24% Photo-Flo (Kodak)  
963 and 0.01% Triton X-100, in that order, and 10 min in 0.01% Photo-Flo aqueous solution



964 on a shaker. Primary antibodies used were affinity purified rabbit anti-*S. cerevisiae*  
965 Zip1 antibody (Gift from A. Macqueen, dilution 1:100), mouse monoclonal anti-FLAG  
966 primary antibody M2 (Sigma, dilution 1:300). Secondary antibodies used were Alexa  
967 Fluor488 donkey anti-mouse (Life technologies, A21202), Alexa Fluor488 goat anti-  
968 rabbit (A-11008, Thermo Fischer Scientific), Alexa Fluor568 goat anti-mouse (A-  
969 11004, Thermo Fischer Scientific), Alexa Fluor568 donkey anti-rabbit (Life  
970 technologies, A10042), Alexa Fluor647 donkey anti-rabbit (Abcam, ab150067), Alexa  
971 Fluor647 donkey anti-mouse (Abcam, ab150107), all at 1:200 dilutions. Slides were  
972 mounted using an anti-fade mounting medium with DAPI (Vectashield with DAPI, H-  
973 2000-10, Vector Laboratories).

974

#### 975 **ImmunoFISH and DNA probe preparation**

976 Immunostained chromosome spreads were re-fixed using PFA 2% in PBS for 10 min  
977 then washed twice in PBS for 5 min. Slides were then dehydrated for 4 min in ethanol  
978 70%, 4 min in ethanol 90% and 5 min in ethanol 100% before air drying for 10 min.  
979 To generate FISH probes, we used the nick translation kit from Abbott Molecular  
980 following the manufacturer's instructions and using CF dye-conjugated dUTP  
981 (Biotium), on a pool of PCR products. We pooled 4 PCR products for the *LakI0C*-left  
982 and 3 PCR products for the *LakI0C*-Right, each being about 6 kb long. For the rDNA  
983 region we used 1 PCR product of 8 kb. Each product was amplified using LongAmp  
984 Taq polymerase (NEB #M0323L). To avoid non-specific hybridization of the *LakI0C*-  
985 left and *LakI0C*-right probes to the rDNA locus, slides were digested with RNase A  
986 (Sigma Aldrich/MERCK) during the secondary antibody incubation, and rDNA probes  
987 (labelled or not) were always hybridized during FISH.

988 Labeled DNA (500 ng) was precipitated during 30 min incubation at  $-20^{\circ}\text{C}$  after adding  
989 0.5 volume of 7.5 M ammonium acetate and 2.5 volumes of cold 100% ethanol. After  
990 washing with 70% ethanol and air drying in the dark, the pellet was dissolved in 15  $\mu\text{l}$   
991 of hybridization buffer. Chromosomal DNA and probes were co-denatured for 6 min at  
992  $78^{\circ}\text{C}$  and left overnight at  $37^{\circ}\text{C}$ . Washes were performed using PBS-Tween 0.1% for  
993 5 min twice and once in SSC 0.4X-NP40 0.3%. Finally, slides were air dried and  
994 mounted using Vectashield with DAPI.

995

### 996 **Image Acquisition**

997 Fluorescence microscopy was performed on a ZEISS IMAGER Z2 upright microscope,  
998 equipped with a CoolSNAP HQ<sup>2</sup> camera (PHOTOMETRICS, roper Scientific), DAPI,  
999 GFP, TEXAS red and Cy5 filter sets and controlled by METAMORPH Software.  
1000 Images were acquired using a 100X/1.46 NA oil immersion objective and a tubelens  
1001 at 1.25X. Images shown are projections of two to three individual focal planes along  
1002 the z axis.

1003

### 1004 **Measurement of chromosome axis length and DAPI width**

1005 Original, unmodified spread images of pachytene nuclei (DAPI-stained regions) were  
1006 used to measure axis length and DAPI width. The measurements were done manually  
1007 as described in (84). They showed that the chromosome axes can be reliably  
1008 visualized and measured by immunostaining either Zip1 or Rec8 as their abundance  
1009 are highly correlated in the synapsed regions during pachytene. In this study, we used  
1010 Zip1 length as a proxy for axis length measurement and DAPI width as a proxy for the  
1011 loop-size estimation.

1012 Image analysis and quantification was performed using the Fiji software. Zip1 length  
1013 was measured by tracing its immunofluorescence signal. The beginning and the end  
1014 of each Zip1 line is determined manually from the pixel intensity at the edges of each  
1015 line. For DAPI width estimation, a straight line perpendicular to the DAPI-stained  
1016 chromosome fragment, covering its entire width, was drawn at three different locations  
1017 (middle and closer to the ends) on the same chromosome. The width of each  
1018 chromosome was calculated as the average length of three perpendicular lines drawn  
1019 across it. DAPI signal intensities of the pixels along each perpendicular line were used  
1020 to differentiate the background from the exact length of the line spanning the width of  
1021 the chromosome. Graphs and statistical analyses were performed using GraphPad  
1022 Prism 9 software.

1023

#### 1024 **Accession numbers**

1025 The Illumina reads related to the CBS10367 whole-genome sequencing are available  
1026 in the Sequence Read Archive under the BioProject PRJEB60460.

1027 The CC-seq, ChIP-seq and Hi-C data discussed in this publication have been  
1028 deposited in NCBI's Gene Expression Omnibus (101) and are accessible through GEO  
1029 SuperSeries accession number GSE237708

1030 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE237708>).

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1036

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1038

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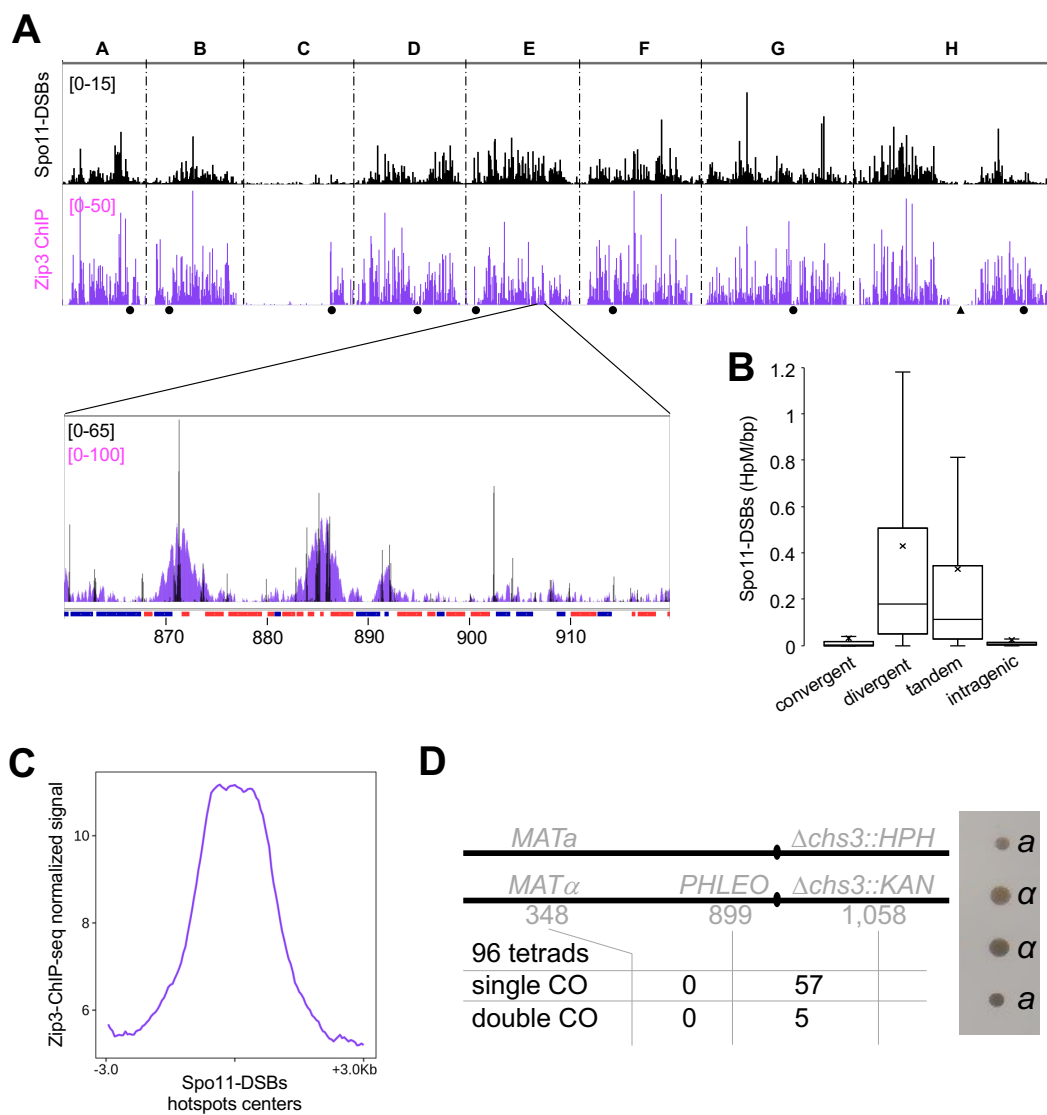
1286

Table 1

chromosome	chromLength	chromLengthWithout Subtelo(30kb)	#HomSNPs	homSNPs/kb	#Het(0.4-0.6)SNPs	hetSNPs/kb	#homozIndels	homIndels/kb	#heterozIndels	hetIndel/kb
Laki0A	951467	891467	21621	24.25	134	0.15	1211	1.36	17	0.0191
Laki0B	1119477	1059477	26752	25.25	128	0.12	1660	1.57	19	0.0179
Laki0C	1252455	1192455	30493	25.57	1512	1.27	2616	2.19	332	0.2784
Laki0C-left	989693	959693	24270	25.29	1467	1.53	2238	2.33	319	0.3324
Laki0C-right	262762	232762	6223	26.74	45	0.19	378	1.62	13	0.0559
Laki0D	1289366	1229366	29680	24.14	62	0.05	1790	1.46	9	0.0073
Laki0E	1295560	1235560	28269	22.88	302	0.24	1649	1.33	50	0.0405
Laki0F	1385275	1325275	31077	23.45	76	0.06	1847	1.39	13	0.0098
Laki0G	1737269	1677269	38845	23.16	189	0.11	1987	1.18	22	0.0131
Laki0H	2314951	2254951	49574	21.98	173	0.08	2691	1.19	35	0.0155
whole genome	11345820	10865820	256311	23.59	2576	0.24	15451	1.42	497	0.0457
whole genome except Laki0C-left	10356127	9906127	232041	23.42	1109	0.11	13213	1.33	178	0.0180



Figure 1



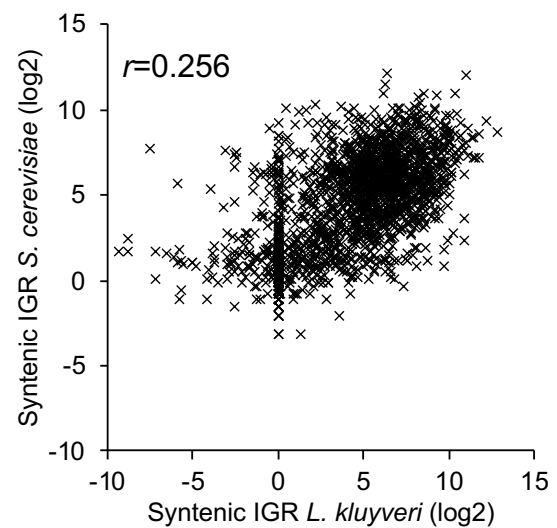
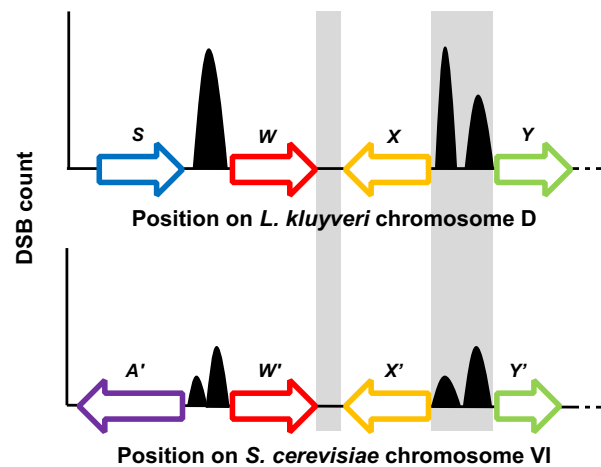
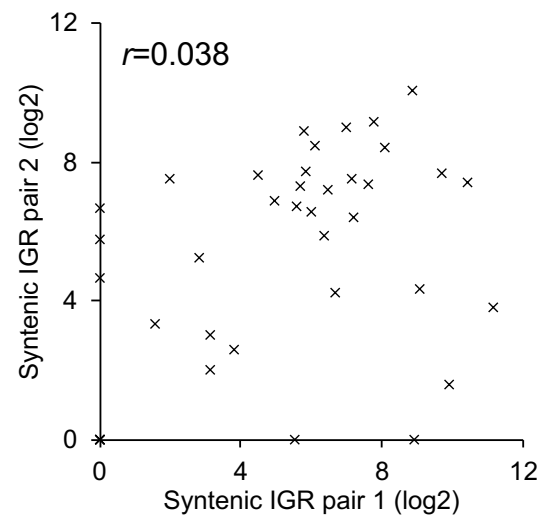
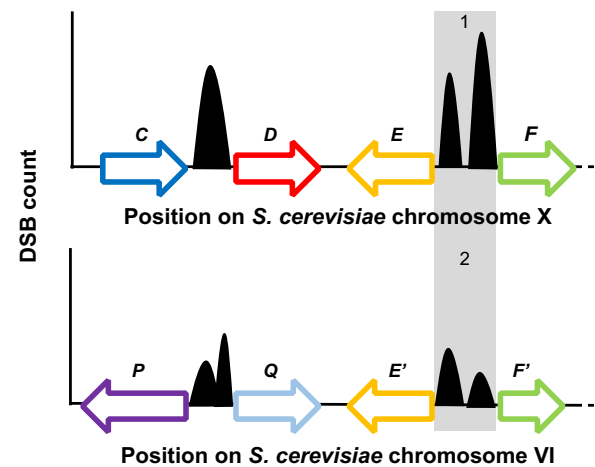
**A****B**

Figure 2

Figure 3

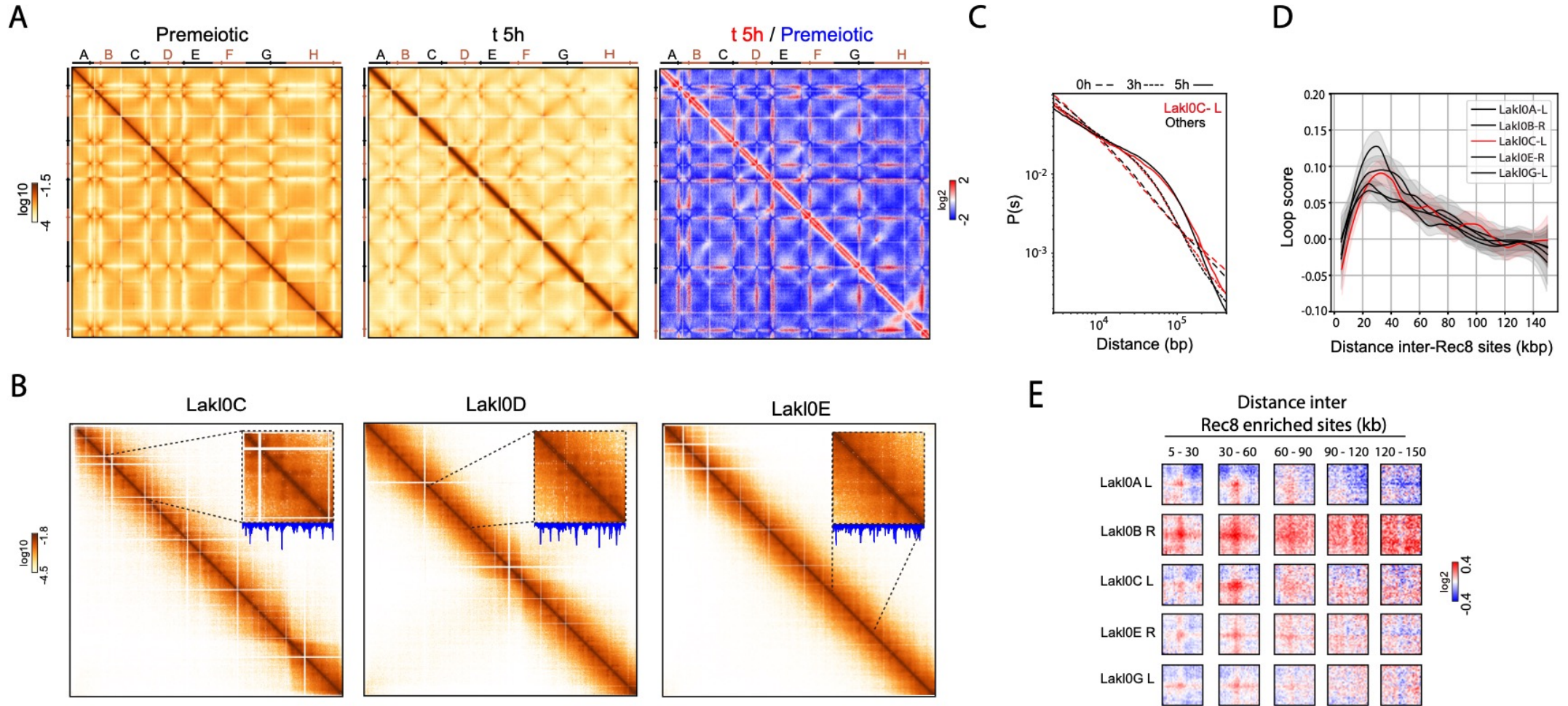


Figure 4

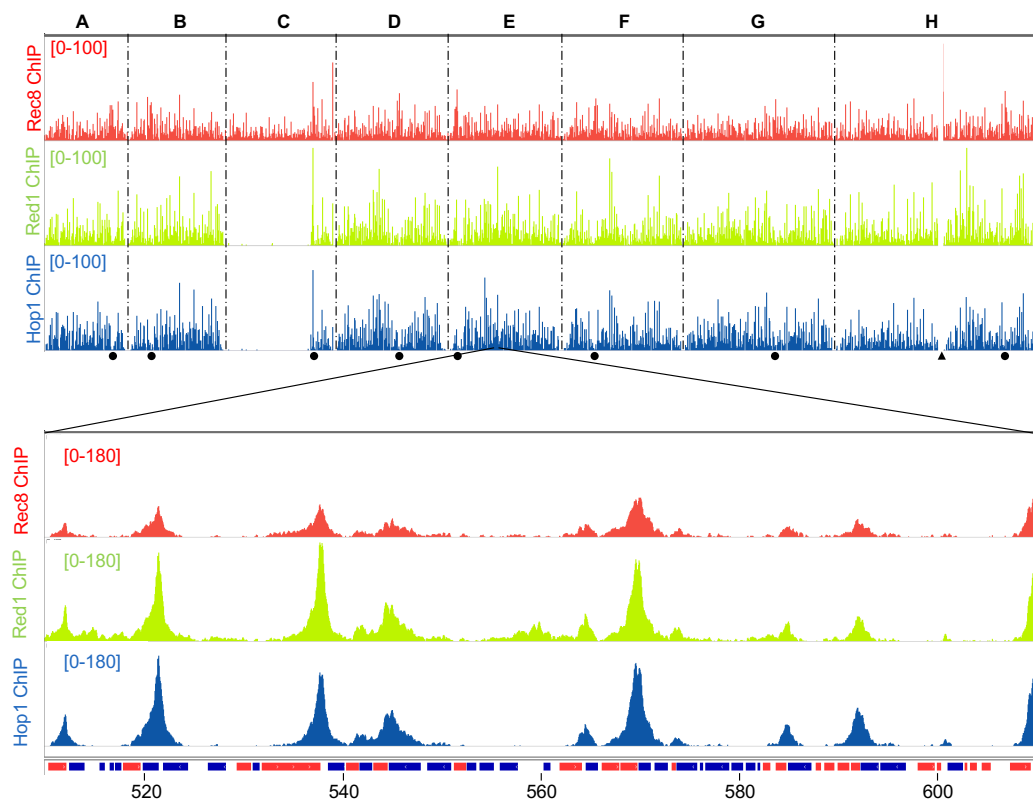
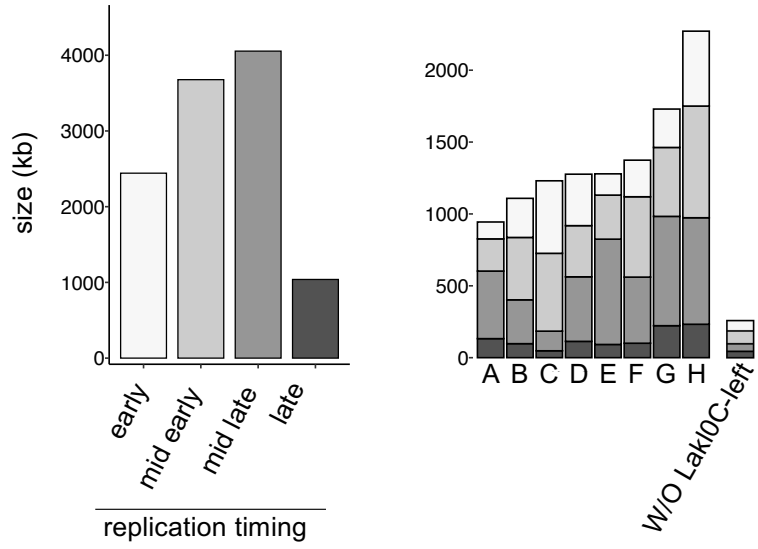
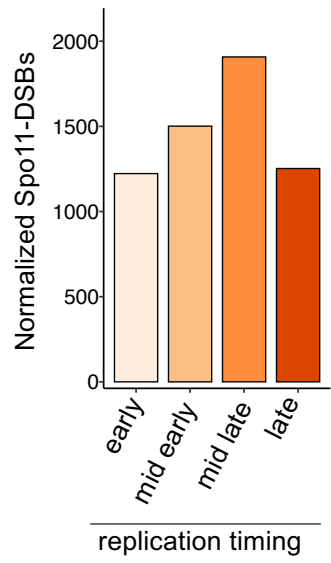


Figure 5

**A**



**B**



**C**

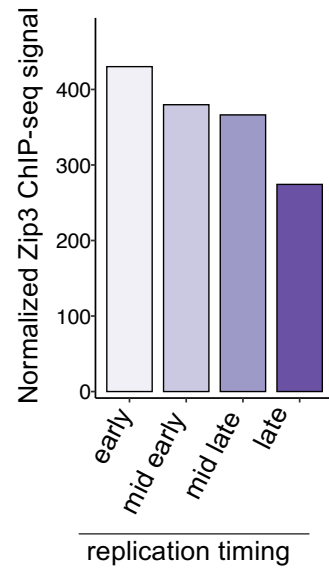


Figure 6

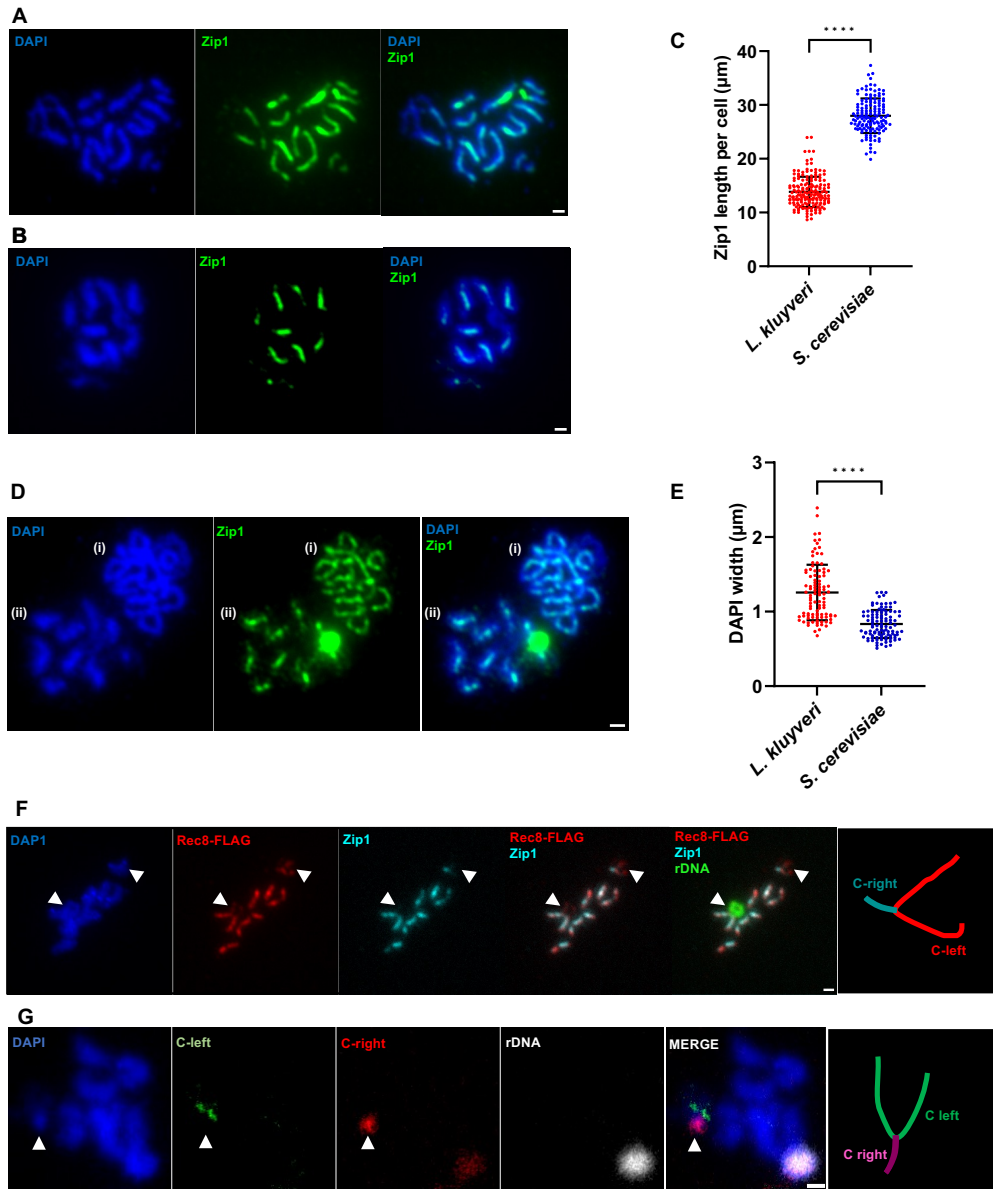
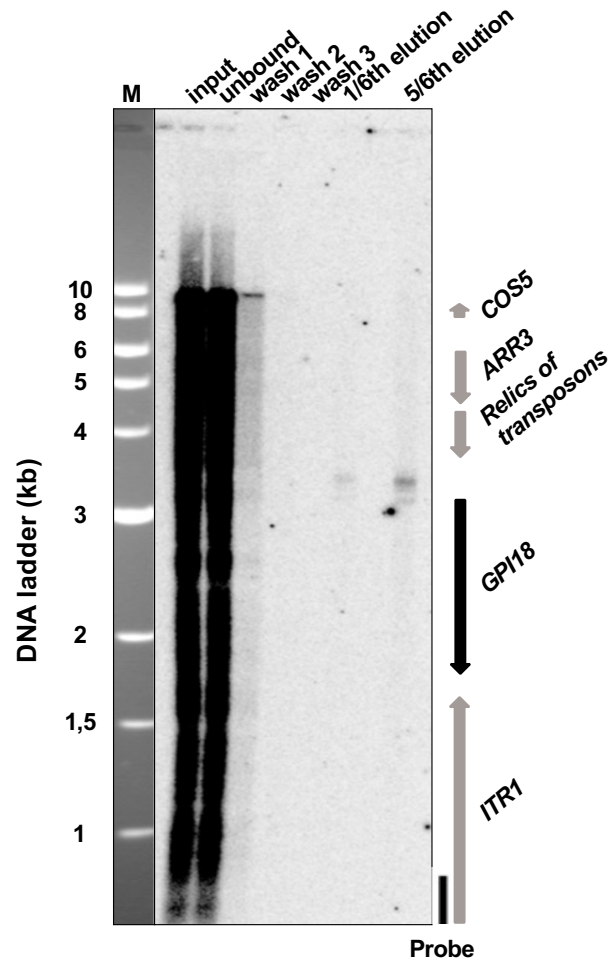
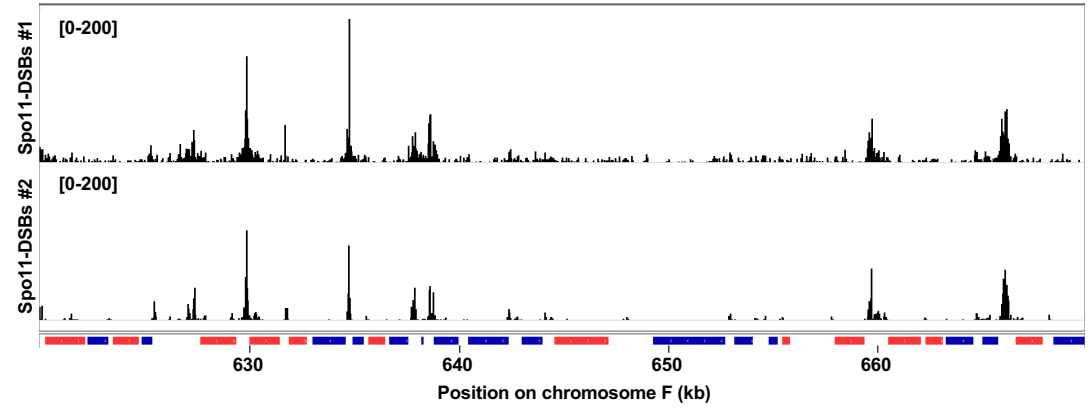


Figure S1

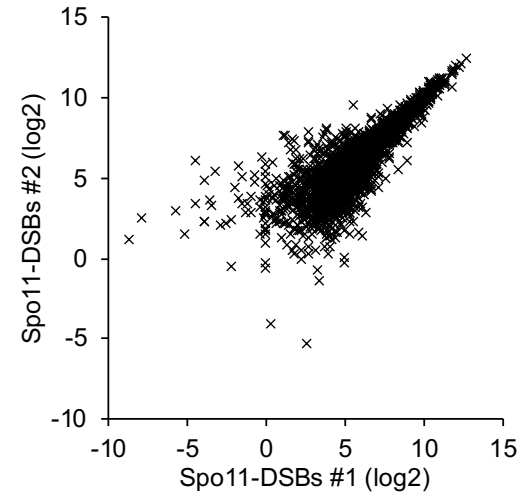
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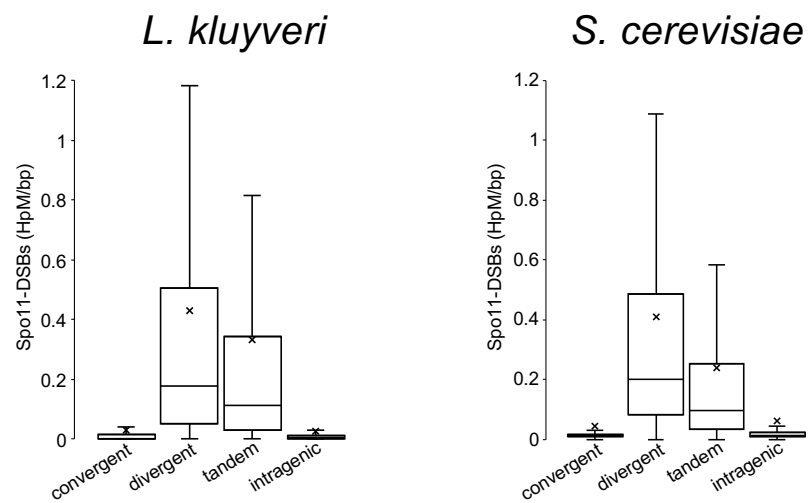
**B**



**C**



**A**



**B**

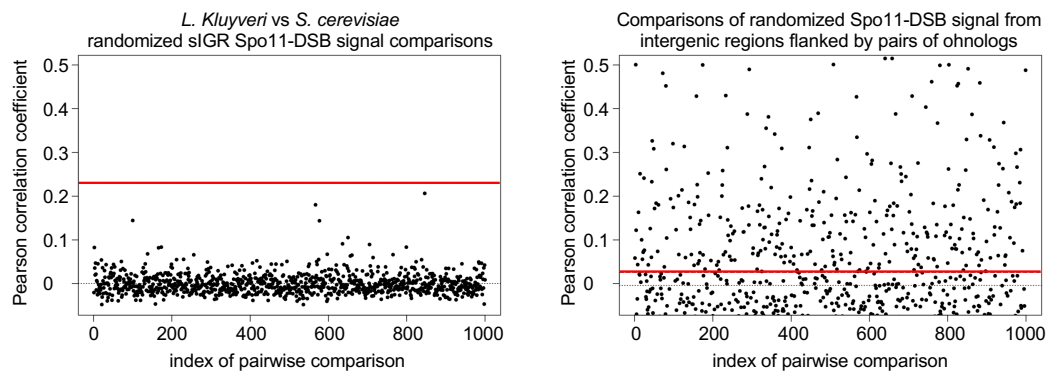
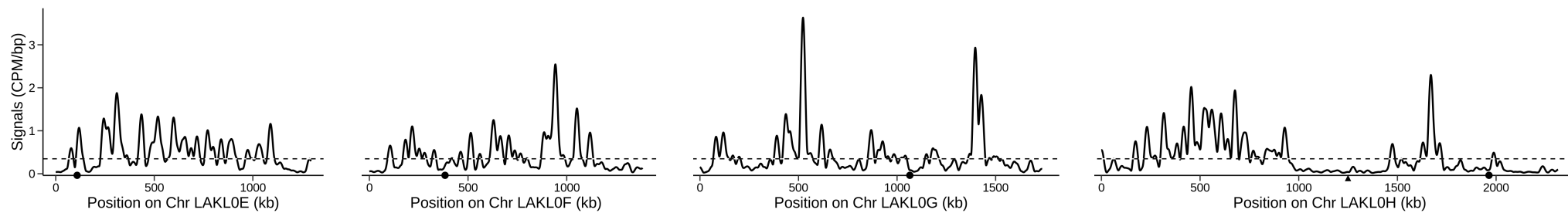
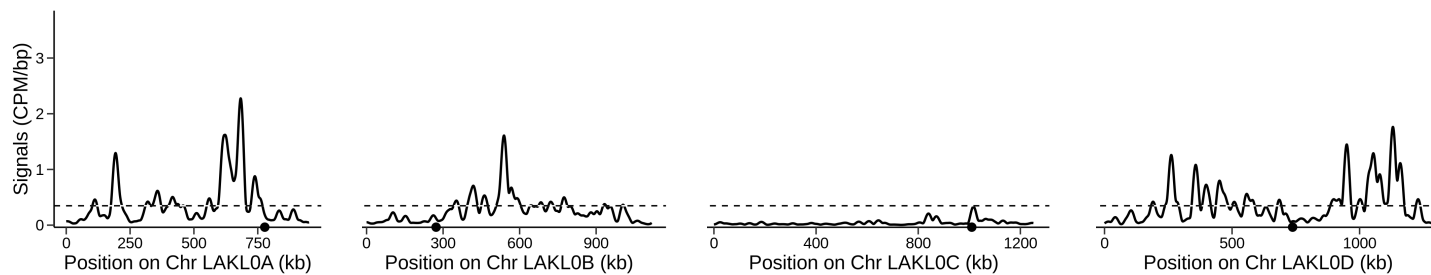




Figure S3



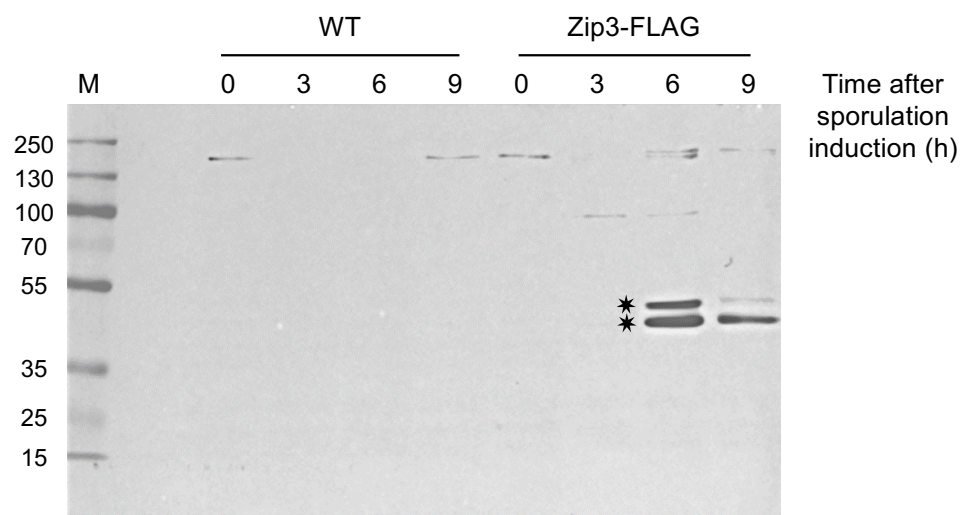
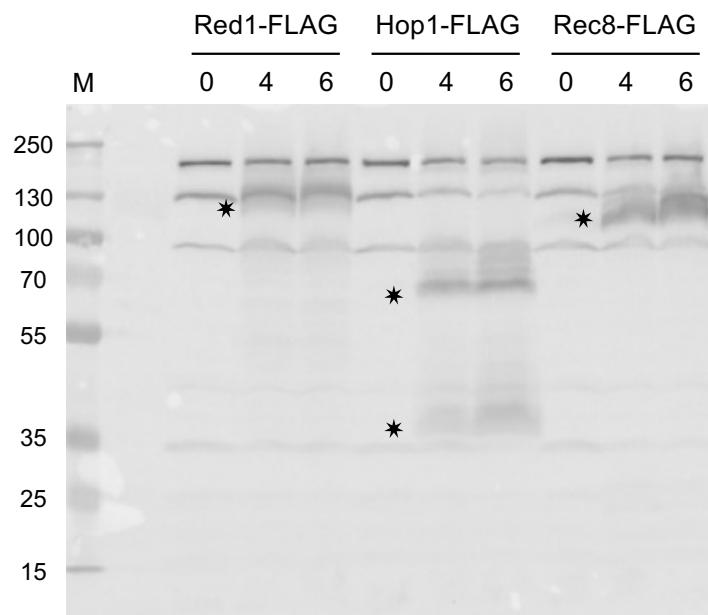
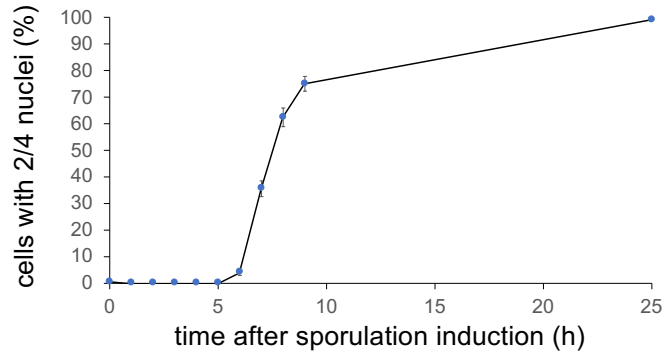
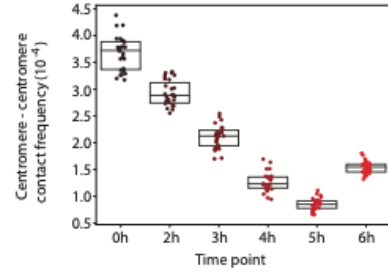


Figure S4

A.



B.



D.

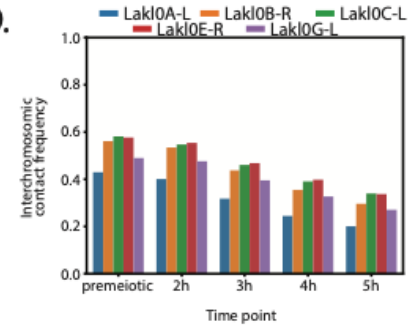
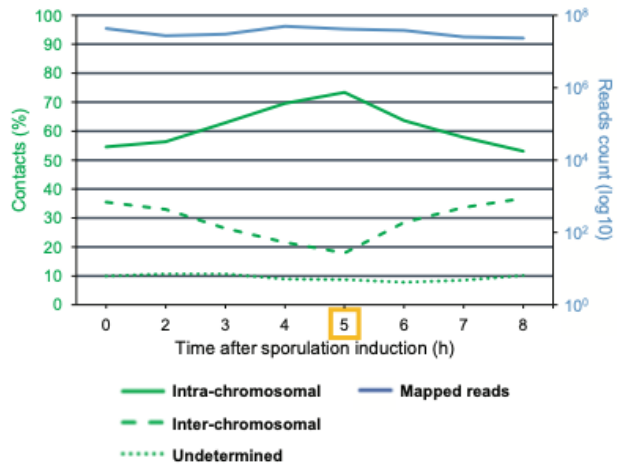


Figure S5 (1/2)

C.



E.

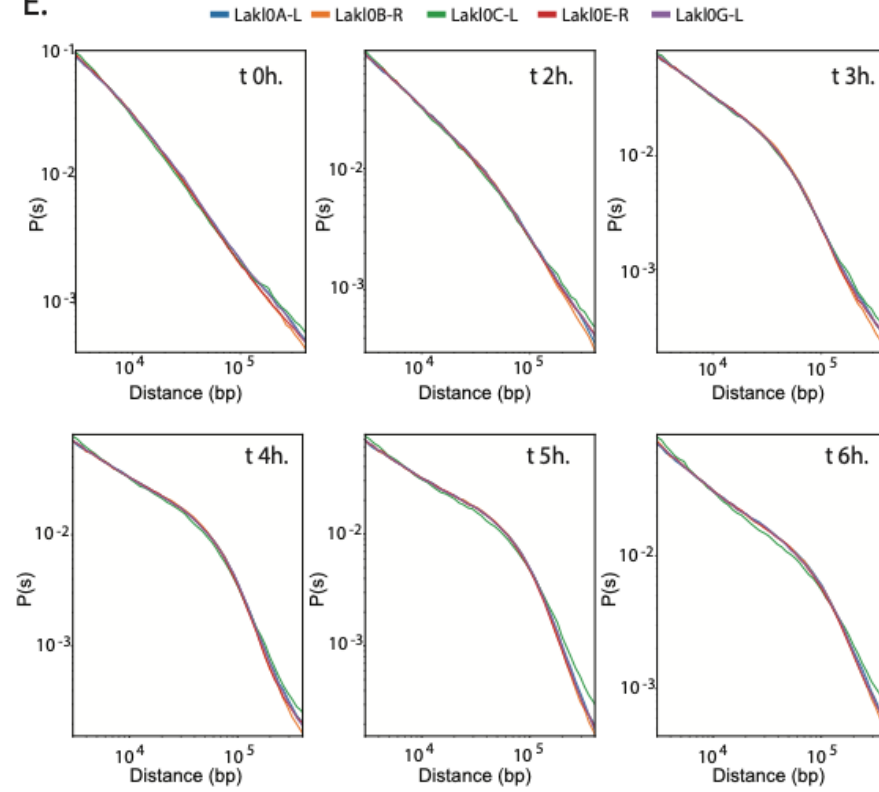


Figure S5 (2/2)

F.

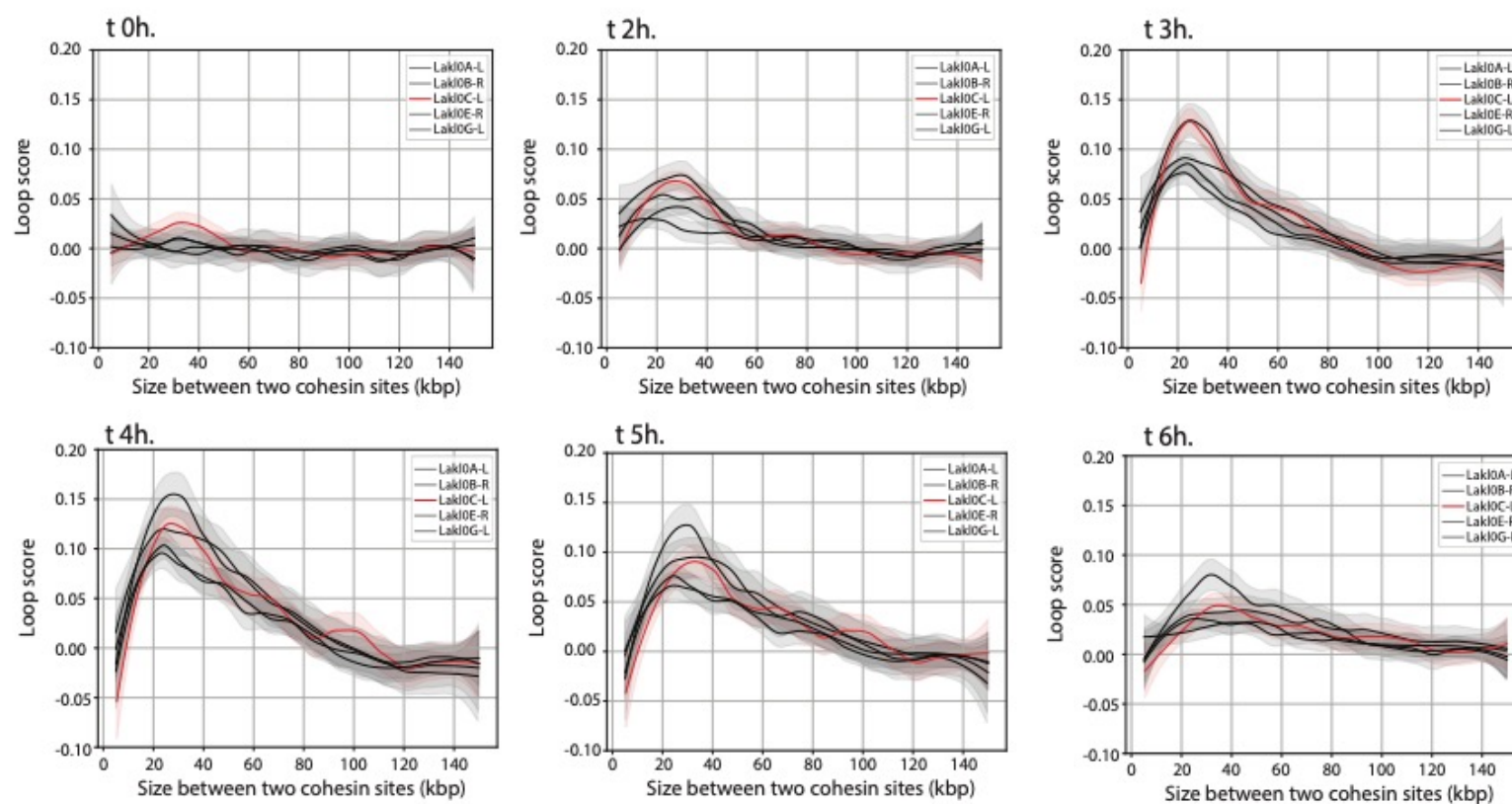
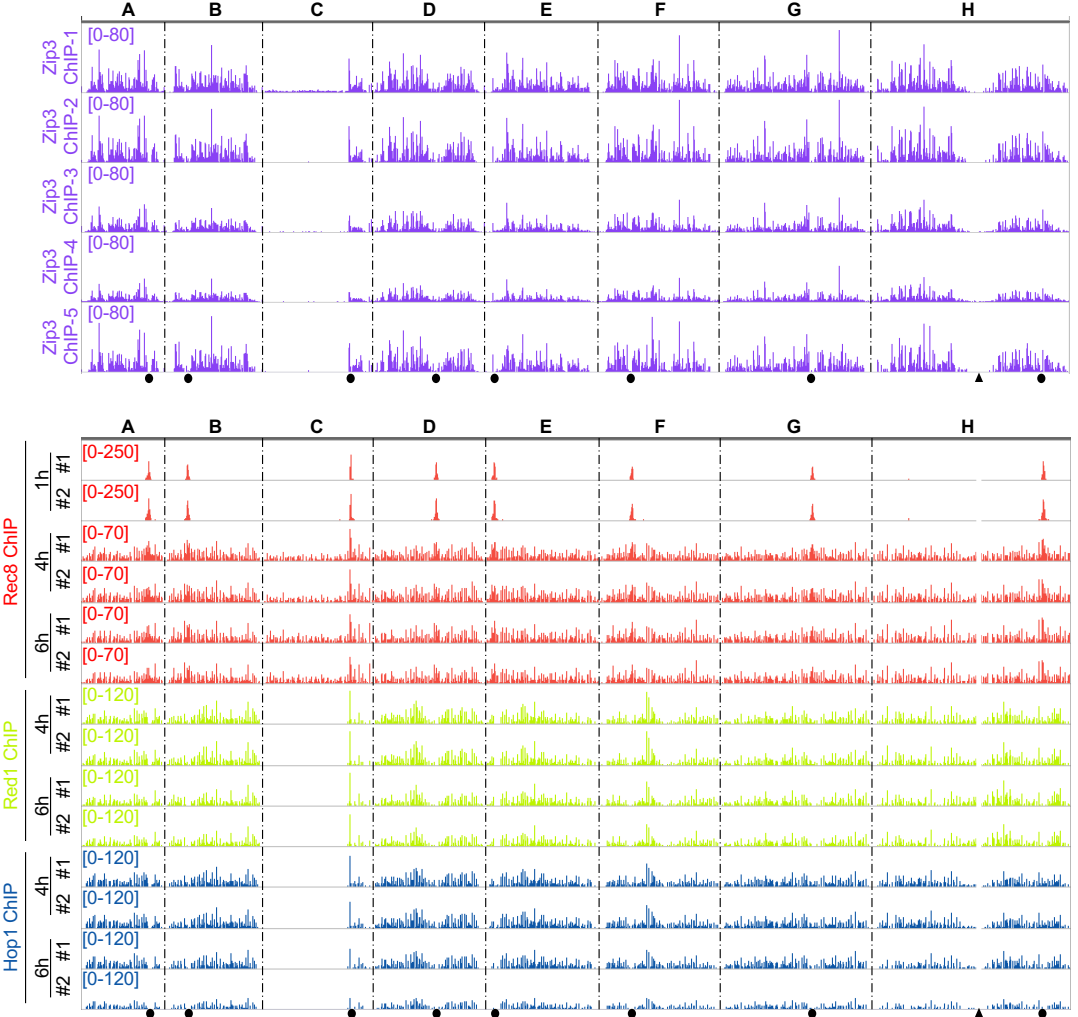


Figure S6



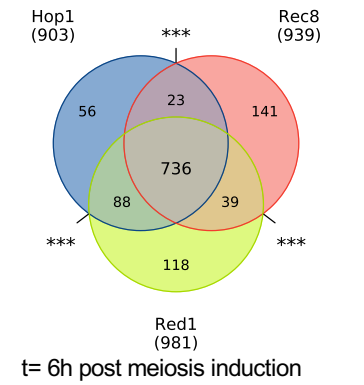
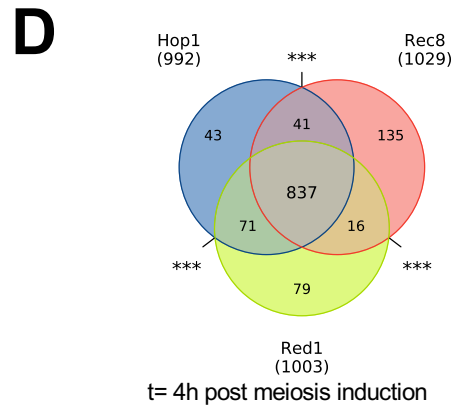
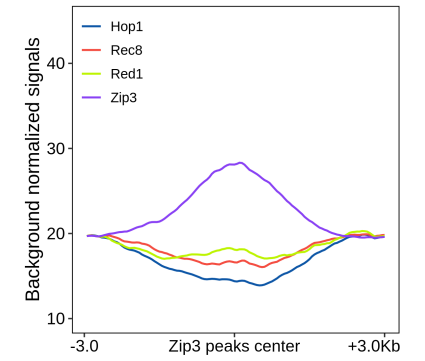
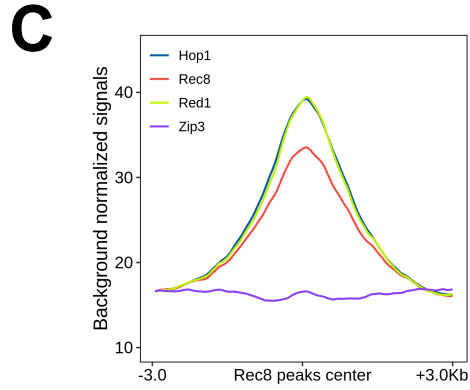
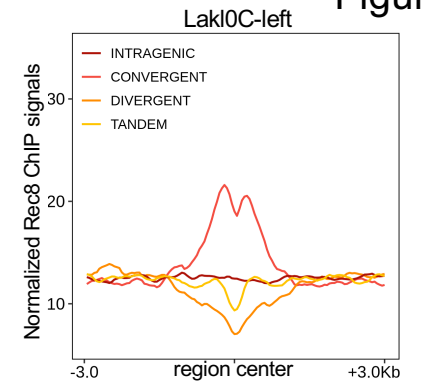
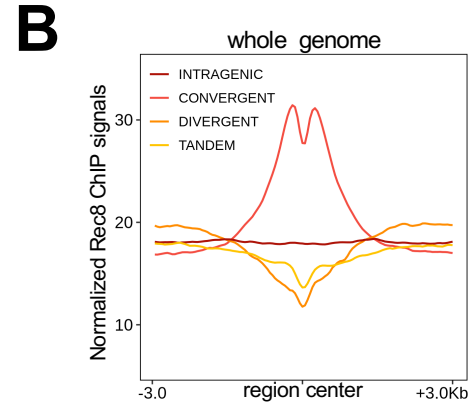
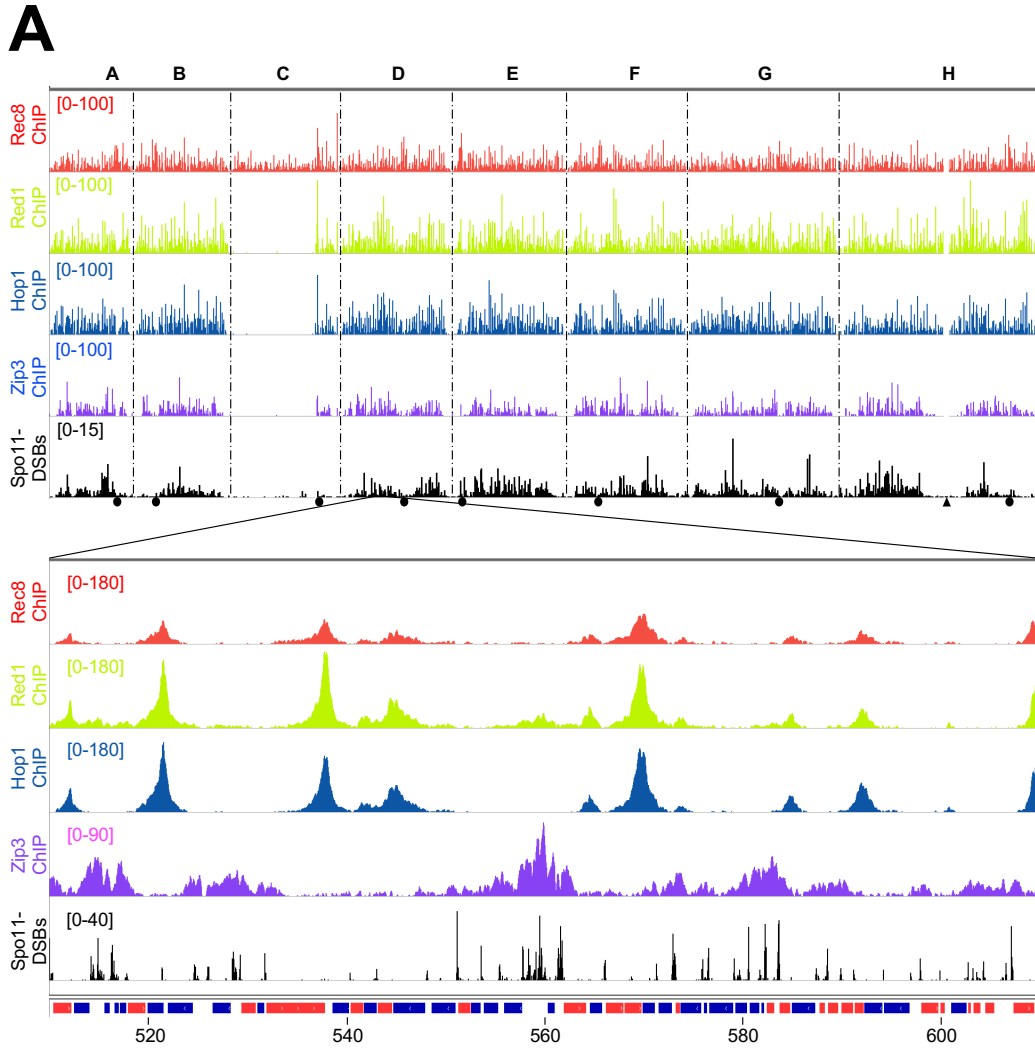


Figure S8 (1/2)

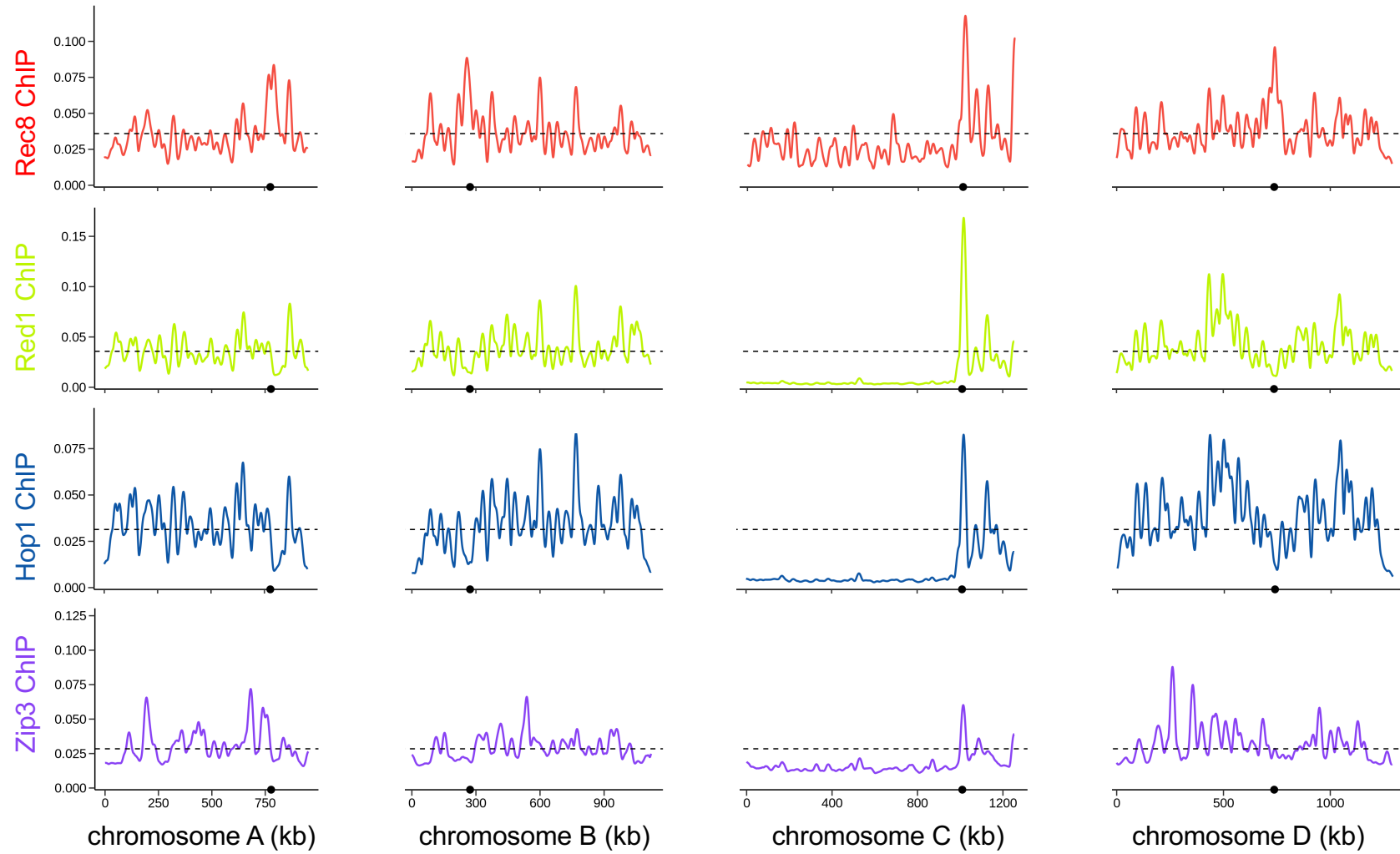


Figure S8 (2/2)

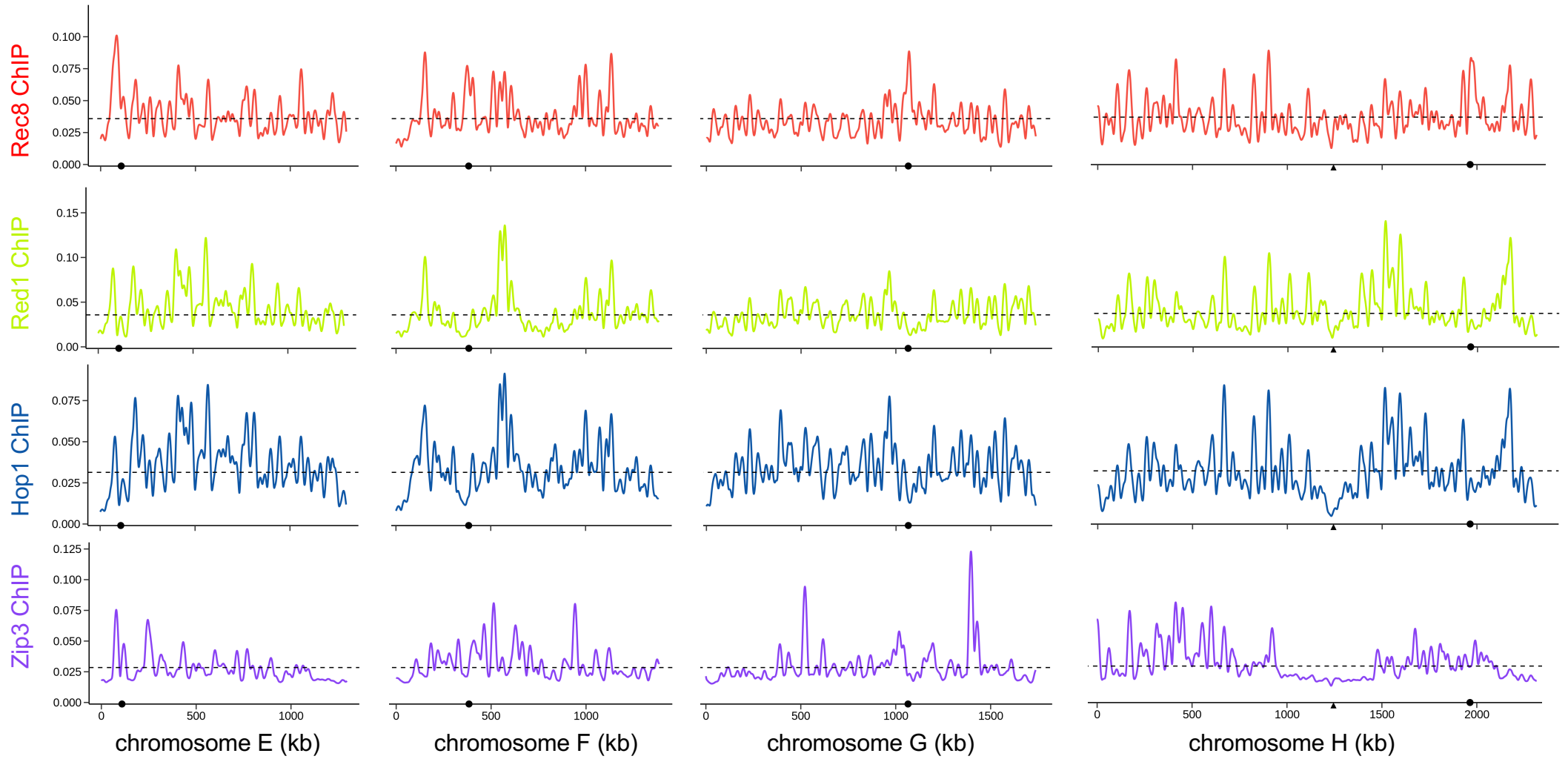




Figure S9

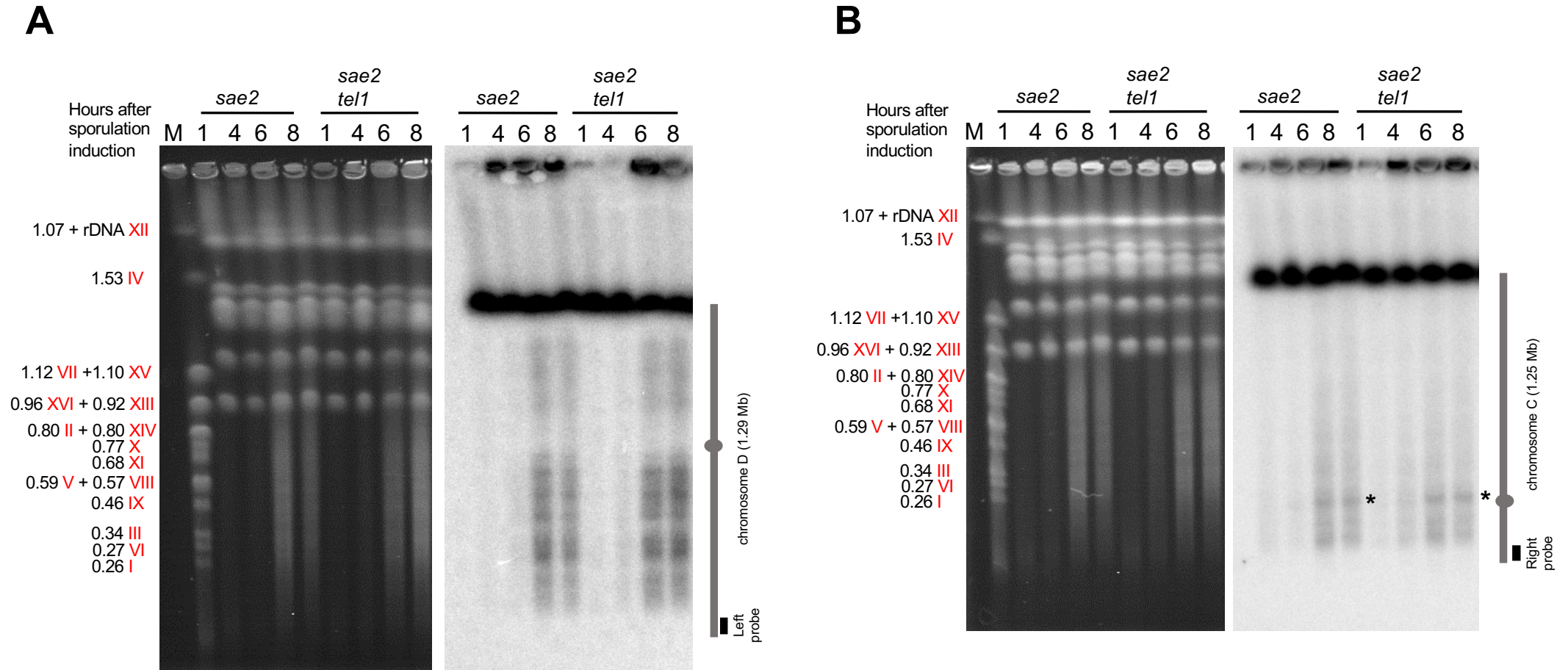


Figure S10A

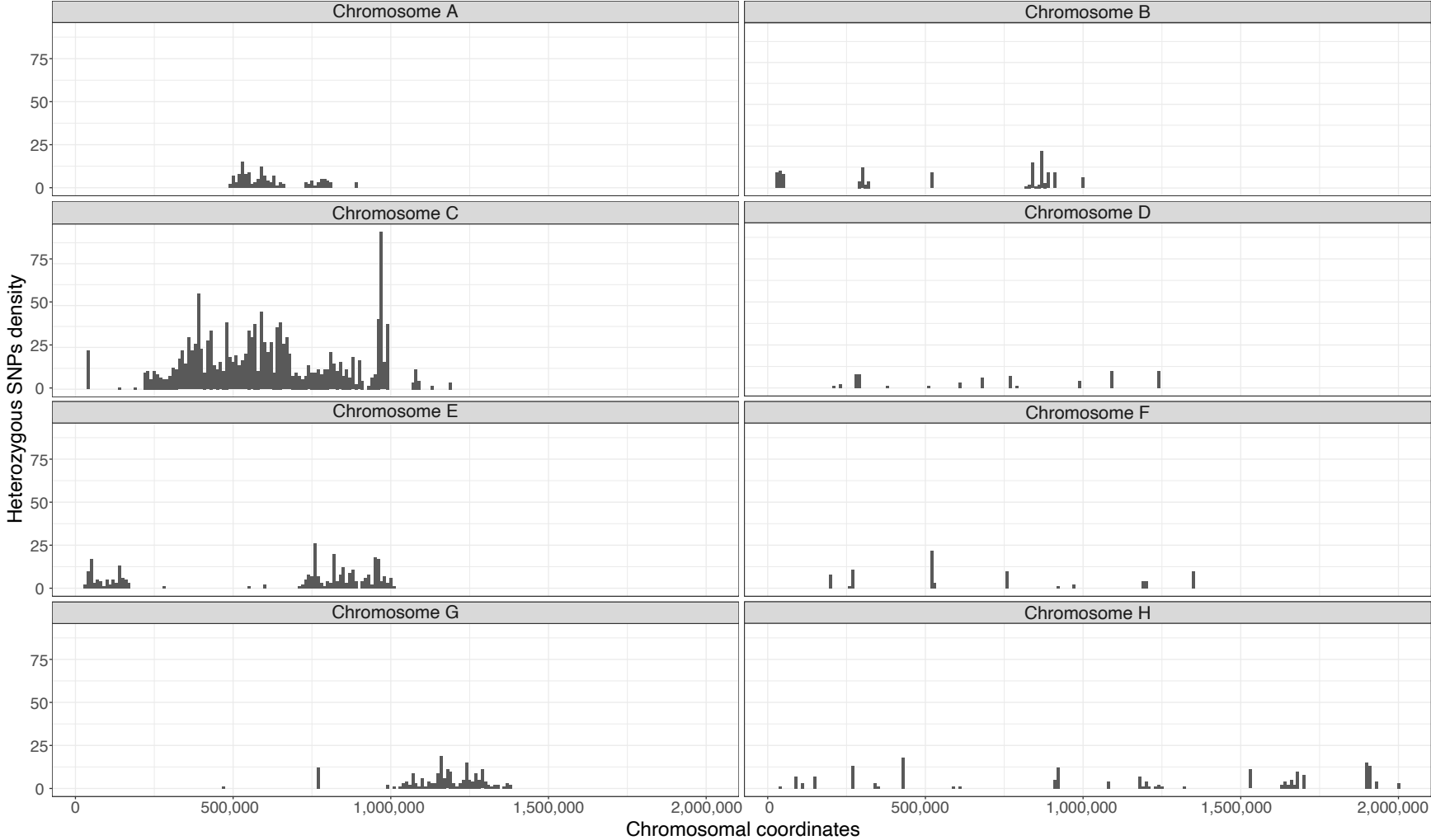
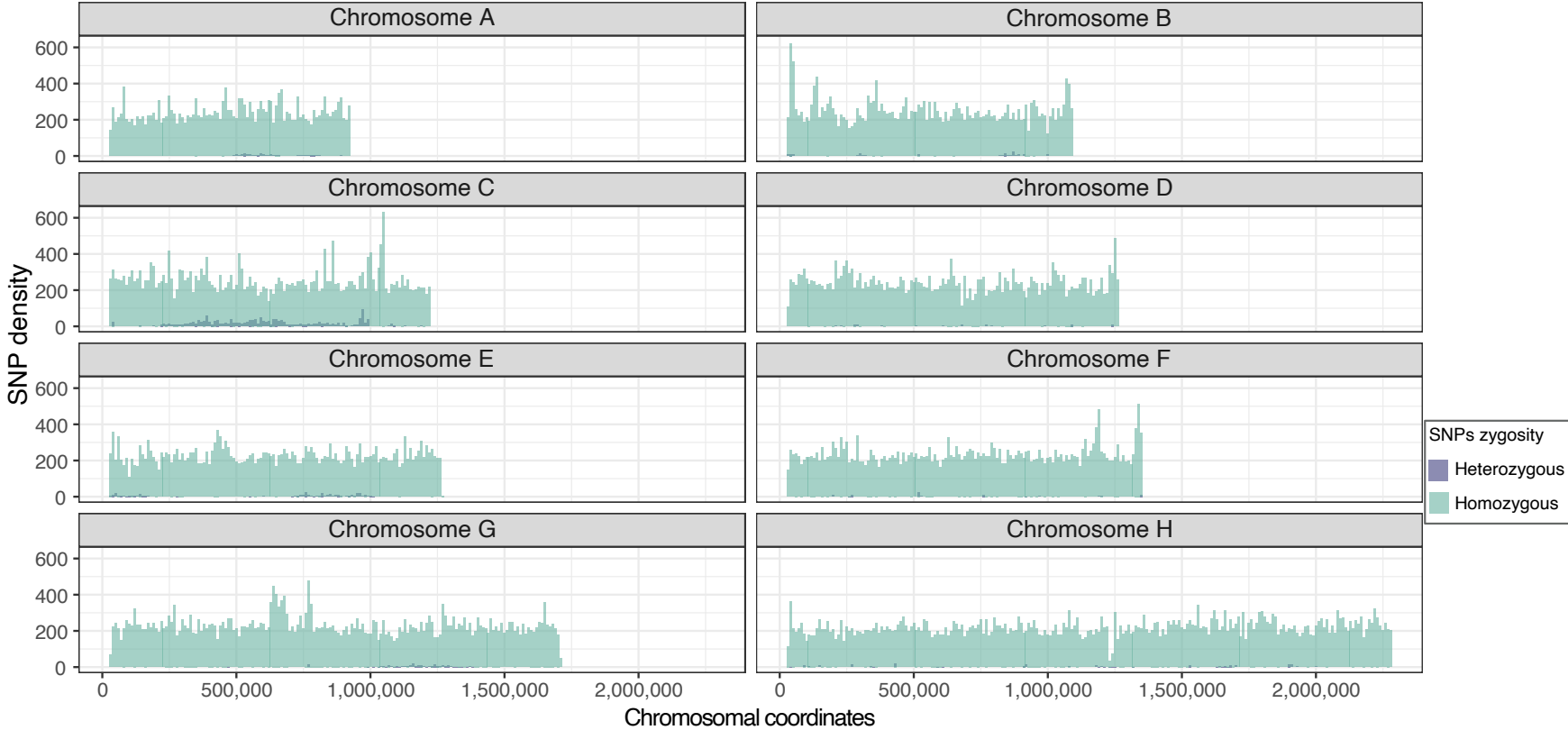


Figure S10B



**Table S1. Yeast strains table**

name	genetic background	genotype	source	experiment
CBS10367 ( <i>L. kluyveri</i> )	wilde type natural isolate	<i>MAT a/a</i>	CBS collection	HiC
LAKL221 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>sae2::kanMX6/sae2::hphMX6</i>	This study	PFGE/SB*
LAKL184 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>chs3::kanMX6/chs3::hphMX6</i> ; <i>Lak10C899::bleMX6/WT</i>	This study	Rec Assay
LAKL222 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>tel1::bleMX6/tel1::natMX6</i> ; <i>sae2::kanMX6/sae2::hphMX6</i>	This study	PFGE/SB
LAKL223 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>spo11::bleMX6/spo11::natMX6</i> ; <i>sae2::kanMX6/sae2::hphMX6</i>	This study	PFGE/SB
LAKL220 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>ndt80::bleMX6/ndt80::hphMX6</i>	This study	Cytology
LAKL224 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>REC8-6xHIS-FLAG(3x)-</i> <i>TAA(stop)-kanMX6/REC8-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-hphMX6</i>	This study	ChIP-seq Cytology
LAKL227 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>REC8-6xHIS-FLAG(3x)-</i> <i>TAA(stop)-kanMX6/REC8-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-hphMX6</i> , <i>ndt80::bleMX6/ndt80::natMX6</i>	This study	Cytology
LAKL235 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>HOP1-6xHIS-FLAG(3x)-</i> <i>TAA(stop)-kanMX6/HOP1-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-hphMX6</i>	This study	ChIP-seq
LAKL236 ( <i>L. kluyveri</i> )	CBS10367	<i>MAT a/a</i> ; <i>RED1-6xHIS-FLAG(3x)-</i> <i>TAA(stop)-kanMX6/RED1-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-hphMX6</i>	This study	ChIP-seq
LAKL056 ( <i>L. kluyveri</i> ) *obtained by crossing	CBS10367	<i>MAT a/a</i> ; <i>CST9(ZIP3)-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-</i> <i>kanMX6/CST9(ZIP3)-6xHIS-</i> <i>FLAG(3x)-TAA(stop)-kanMX6</i>	This study	ChIP-seq
LA166 ( <i>L. kluyveri</i> ) *obtained by crossing	CBS10367	<i>MAT a/a</i> ; <i>sae2::kanMX6/sae2::kanMX6</i>	Brion et al 2017	CC-seq
BLY1808 ( <i>S. cerevisiae</i> )	SK1	<i>MAT a/a</i> ; <i>ho::LYS2''</i> ; <i>lys2''</i> ; <i>ura3''</i> ; <i>arg4-nsp''</i> ; <i>leu2::hisG''</i> ; <i>his4X::LEU2''</i> ; <i>nuc1::LEU2''</i> ; <i>FLAG-</i> <i>TEL1''</i> ; <i>ndt80::HphMX''</i>	V. Garcia	Cytology

\* SB : Southern blot

**Plasmids table**

Plasmid name	Comments
pFA6a-natMX6	Amplification of the <i>natMX6</i> resistance cassette
pUG66	Amplification of the <i>bleMX6</i> resistance cassette
pFa6-hphNT1	Amplification of the <i>hphMX6</i> resistance cassette
pFa6-3HA-kanMX6	Amplification of the <i>kanMX6</i> resistance cassette
pUC19	Vector for Gibson cloning
pVB94 (from Valérie Borde's lab)	Amplification of the <i>6xHIS-FLAG(3x)-TAA(stop)-kanMX6</i> cassette

**Table S2. Primers table**

Name	Sequence 5'-3'	Primer description	Primer target
C4 1F	TCACCTTTCTACGACACGGC	PCR amplification of <i>LakI0C899</i> upstream homology region	Construction of LAKL 184 by 3 way PCR and verification
C4 1R	CATATACGCACCCGTC AAGG		
C4 2F	GTGGTTTGGTGTGCAGTGAC	PCR amplification of <i>LakI0C899</i> downstream homology region	
C4 2R	GCAAACCATTTCGGAGCACA		
C4 3F PHLEO	CCTTGACGGGTGCGTATATGCTGTTTAGCTTGCCTCGTCC	PCR amplification of <i>bleMX6</i> resistance cassette from pUG66 plasmid	
C4 3R PHLEO	GTCACTGCACACCAAACCACTCGACAGCAGTATAGCGACC		
C4-external D	AGCGTCTTTGTGCTGTGTCT	Verification of resistance cassette insertion	
C5 1F	ATCTTTTGC GAAGTTAGAAA	PCR amplification of <i>CHS3</i> upstream homology region	
C5 1R	AAGCATTCCAGACATGGTTCGTT		
C5 2F	TGCTGTATCAATGGATATGGCTTGA	PCR amplification of <i>CHS3</i> downstream homology region	
C5 2R	ACAATAAGGCCTGAGGCAT		
C5 3F	AACGAACCATGTCTGGAATGGCTTCGATCCCCGGGTTAATTAA	PCR amplification of <i>hphMX6</i> or <i>kanMX6</i> resistance cassette from pFa6-hphNT1 or pFa6-3HA-kanMX6 plasmids	
C5 3R	TCAAGCCATATCCATTGATACAGCAGAATTCGAGCTCGTTTAAAC		
C5 4F	GATTCATGCTCAAGTTTACA		
C5 4R	GACCAAACTTTCTCAAGAT	Verification of resistance cassette insertion	
ndt80 1F NAT	ATGATTACGCCAAGCTTGATGCCTGCAGGGTCGACTGGTTGGTCTG TGCACCATTCC	PCR amplification of <i>NDT80</i> upstream homology region (for Gibson cloning)	Construction of LAKL 220 and LAKL 227 verification
ndt80 1R NAT	TAACCCGGGGATCCGTA CTGGTTGCAGCCTTCG		
ndt80 NAT F	AGGCTGCAACCAGTACGGATCCCCGGGTTAATTAAG	PCR amplification of <i>natMX6</i> resistance cassette from pFa6a-natMX6 plasmid (for Gibson cloning)	
ndt80 NAT R	TCCGTTCTTCGCTACGAATTCGAGCTCGTTTAAACTG		
ndt80 2F NAT	AACGAGCTCGAATTCGTAGCGAAGAACGGAGCTG	PCR amplification of <i>NDT80</i> downstream homology region (for Gibson cloning)	
ndt80 2R NAT	TCGAGCTCGGTACCCGGGGATCCTCTAGAGGTCGACAGCGGAAGCA CGAAGTTAC		
ndt80 1F PHLEO	CAAGCTTG CATGCCTGCAGGGATATCTGGTTGGTCTGTGCACCATTCC	PCR amplification of <i>NDT80</i> upstream homology region (for Gibson cloning)	
ndt80 1R PHLEO	AGCTAAACAGTACTGGTTGCAGCCTTCG		
ndt80 PHLEO F	GCAACCAGTACTGTTTAGCTTGCCTCGTC	PCR amplification of <i>bleMX6</i> resistance cassette from pUG66 plasmid (for Gibson cloning)	
ndt80 PHLEO R	TCTTCGCTACTCGACAGCAGTATAGCGAC		
ndt80 2F PHLEO	CTGCTGTCGAGTAGCGAAGAACGGAGCTG	PCR amplification of <i>PCH2</i> downstream homology region (for Gibson cloning)	
ndt80 2R PHLEO	TACCCGGGGATCCTCTAGAGGATATCAGCGGAAGCACGAAGTTAC		
ndt80 A ext	CGGTCTCGATAGTCCTTGCC	External primers to check the construction	
ndt80 D ext	TGGACCAAGCTTGAAGGCAA		
ndt80 F gene	TGGATGACTGTCCTGTGGAT	primers flanking <i>NDT80</i> gene to check for deletion	
ndt80 R gene	TAAAAGCTGGGCTCGTCGTA		
zip3_tag_fl ag_up	CTAAAGACAATACCAGTTTTGTCCACGGAACAAATTTAGAAGGATCA GGTCCACCACCATCATCACGGA	PCR amplification of <i>6xHIS-FLAG(3x)-TAA(stop)-kanMX6</i>	Construction of LAKL05 6 by 3
zip3_tag_fl ag_low	CGAAGAGATTTTTTTGTTTTGTCTCTTTATTCTTTTTCTTTCTTTTT ACTATAGGGAGACCGGCAGAT		

		cassette from pVB94 plasmid	way PCR and verification
CST9_1	TGAAAAGAAACAGCACGCCG	PCR amplification of <i>CST9/ZIP3</i> C-terminal domain homology region	
CST9_2	AAACCTGGTATTGTCTTTAG		
CST9_3	AAAAACAAAAAATCTCTTCG	PCR amplification of <i>CST9/ZIP3</i> downstream domain homology region	
CST9_4	TCCCTACAGTGCCATCCAGC		
CST9_A	AATTAATGCCGTTGCCAGG	Primers to check the construction	
CST9_B	CATCCTTGATGCTCTTGCA		
CST9_C	AAACGAGCTCTCGAGAACCC		
CST9_D	ACCAATCAGATTGTCCGATT		
red1 1	CCACTAGCAGAGGATACCAAG	PCR amplification of <i>RED1</i> C-terminal domain homology region	Construction of LAKL23 6 by 3 way (with kan resistance) / 4 way (with hph resistance) PCR and verification
n red1 2	ACGAAACTCCTTGTTCCTTATC		
n red1 C (kan only)	GATAAGGAACAAGGAGTTTCGTTCCCACCACCATCATCATCAC	PCR amplification of <i>6xHIS-FLAG(3x)-TAA(stop)-kanMX6</i> cassette from pVB94 plasmid	
n red1 D kan (kan only)	GCACTATTTGCGAGCCATAAGGCGTTAGTATCGAATCGAC		
n red1 C (hph only)	GATAAGGAACAAGGAGTTTCGTTCCCACCACCATCATCATCAC	PCR amplification of <i>6xHIS-FLAG(3x)-TAA(stop)</i> cassette from pVB94 plasmid	
TEF prom anti (hph only)	AGGGTATTCTGGGCCTCCAT		
TEF prom sens (hph only)	ATGGAGGCCCAGAATACCCT	PCR amplification of <i>hphMX6</i> cassette from pFa6-hphNT1 plasmid	
n red1 D hph (hph only)	GCACTATTTGCGAGCCATAAGAATTCGAGCTCGTTTAAAC		
n red1 3	TTATGGCTGCGAAATAGTGC	PCR amplification of <i>RED1</i> downstream domain homology region	
red1 4	GGTTCCTGATACAGATTTACAG		
red1 A	GAAAAAGGTTGCAGCCAAGG	Verification of the construction	
red1 D	TCAACACGGACTTGAGTGAC		
n hop1 1	CAAAGTACTCTAAAGCAGTG	PCR amplification of <i>HOP1</i> downstream domain homology region	Construction of LAKL23 5 by 3 way (with kan resistance) / 4 way (with hph resistance) PCR and verification
n hop1 2	ACTGTGATAGTTGATCACTC		
n hop1 3 kan (kan only)	GAGTGATCAACTATCACAGTGGCGTTAGTATCGAATCGAC	PCR amplification of <i>kanMX6-TTA(stop)-FLAG(3x)-6xHIS</i> cassette from pVB94 plasmid	
n hop1 4 (kan only)	AGACATTAAGAAGTGTGGTCCCACCACCATCATCATCAC		
n hop1 3 hph (hph only)	GAGTGATCAACTATCACAGTGAATTCGAGCTCGTTTAAAC	PCR amplification of <i>hphMX6</i> cassette from pFa6-hphNT1 plasmid	
TEF prom sens (hph only)	ATGGAGGCCCAGAATACCCT		
TEF prom anti (hph only)	AGGGTATTCTGGGCCTCCAT	PCR amplification of <i>TTA(stop)-FLAG(3x)-6xHIS</i> cassette from pVB94 plasmid	
n hop1 4 (hph only)	AGACATTAAGAAGTGTGGTCCCACCACCATCATCATCAC		
n hop1 7	CCAAACACTTCTTAATGTCT	PCR amplification of <i>HOP1</i> C-terminal domain homology region	
hop1 8	ATTGATATCGATCACAAGGG		
hop1 A	ATCCTCCAGATTCGAAGTCG	Verification of the construction	
hop1 D	TGGTACCTTTGTCATCGACG		
new rec8 5	AAGTGATTTCAAGTTCCTC	PCR amplification of <i>REC8</i> downstream domain homology region	Construction of LAKL22 4 by 3
n rec8 B	AATGAGCTGTACTCCTGC		

n rec8 C (kan only)	GCAGGAGTACAGCTCATTGGCGTTAGTATCGAATCGAC	PCR amplification of <i>kanMX6-TTA(stop)-FLAG(3x)-6xHIS</i> cassette from pVB94 plasmid	way (with kan resistan ce) / 4 way (with hph resistan ce) PCR and verificat ion
n rec8 D (kan only)	GGTGATGACATTATAATTTGCGTTTCCCACCACCATCATCATCAC		
n rec8 C hph (hph only)	GCAGGAGTACAGCTCATTGAATTCGAGCTCGTTTAAAC		
TEF prom sens (hph only)	ATGGAGGCCAAGAATACCCT		
TEF prom anti (hph only)	AGGGTATTCTGGGCTCCAT	PCR amplification of <i>TTA(stop)-FLAG(3x)-6xHIS</i> cassette from pVB94 plasmid	
n rec8 D (hph only)	GGTGATGACATTATAATTTGCGTTTCCCACCACCATCATCATCAC		
n rec8 E	AACGCAAATTATAATGTCATCACC	PCR amplification of <i>REC8</i> C-terminal domain homology region	
new rec8 8	AGGACCATGTTGATATCTTG		
rec8 A	CCCACCATTGTTCAACTTGG	Verification of the construction	
rec8 D	TTTGGGAGGACAGTAACCAG		
Phleo B	AGAACTCCAGCTTGAGATCC	Internal <i>bleMX6</i> cassette primers to verify cassette insertion	constru ct verificat ion
Phleo C	ACAGAACGAATTGCTTGACAG		
NAT B	GTGTCGTCAAGAGTGGTACC	Internal <i>natMX6</i> cassette primers to verify cassette insertion	
NAT C	AGGCGCTCTACATGAGCATG		
hphB	CCAATGTCAAGCACTTCCG	Internal <i>hphMX6</i> cassette primers to verify cassette insertion	
hphC	ATTTGATGATGCAGCTTGG		
kanB	CTGCAGCGAGGAGCCGTAAT	Internal <i>kanMX6</i> cassette primers to verify cassette insertion	
kanC	TGATTTTGATGACGAGCGTAAT		
spo11 forward	TTTGACTACACTACCGACGCC	primers flanking <i>SPO11</i> gene to check its absence in the mutant strain LS8	
spo11 reverse	CTGGGTTCAATTCCCAGCTC		

### Southern blot probes table

Probe name	Chromosome (coordinates)	Primer sequence used for PCR amplification (5'-3')
C-right	Chr C (1174277-1174792)	Fwd:TACCACAACCACCCAAGGCA Rev: TTACGTGTGTGCAACGGAC
C-left	Chr C (50107-51106)	Fwd: GAGCTGTCTACGCTGTCCC Rev:TCTGCTCGAGTGGGTTTCGA
D-left	Chr D (19951-20960)	Fwd: CAATGCAGACAGTTCACCGA Rev: TCCGTGATCGGCACACTTAT
GPI18 locus	Chr C (999457-999873)	Fwd: GGCCAATATCTCTCCTTCTG Rev: TCCAACGTCATGTTTCTGAT

## FISH probes table

Probe	Chromosome (Coordinates)	Primer sequence used for PCR amplification (5'-3')	Comments
Lakl0C-left probe	Chr C (397258-403510)	Fwd:TGGACATGATCGAGCACAACC Rev:GGATTCGTATCTCAGAGATCTGG	The four PCR products of ~6 kb long were pooled together to generate the probe
	Chr C (594941-601406)	Fwd: AGCCCATCCTGAAGATTCTGG Rev: AAGTCCTGGAGCGTAAAGTTGC	
	Chr C (794705-800915)	Fwd:TGTTCTGCACAAATGTGTCCGG Rev:ATTCGCCGTCCACTTTGCATTGC	
	Chr C (959258-965179)	Fwd:TGCAGCTGCACAATATCGATCC Rev: AAGGGATACACTGGATGACGC	
Lakl0C-right probe	Chr C (1060055-1066084)	Fwd: AACCAACGGTAGCATGTCTCAGG Rev:GTCTTCTCGTCATATTGTCTCCC	The three PCR products of ~6 kb long were pooled together to generate the probe
	Chr C (1150587-1156814)	Fwd: GGGATATCTGGAGCATGTATGG Rev:TTGTCTCTGACCTGAACAGG	
	Chr C (1220332-1225901)	Fwd: GAAAGCGGGCATTGATCAGG Rev: ACCTTGCTTGCTGATGACTCC	
rDNA probe	Chr H	Fwd: AGAACGACCATTACGCTCTTGG Rev: TGACCATACGCGAACTCAGG	rDNA sequence is highly repetitive hence the exact coordinates are not provided here