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Supplemental information

Karyotype engineering reveals spatio-temporal control of replication firing and gene contacts

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Title: Karyotype engineering reveals spatio-temporal control of replication firing and gene contacts

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Supplemental Figures and Tables:

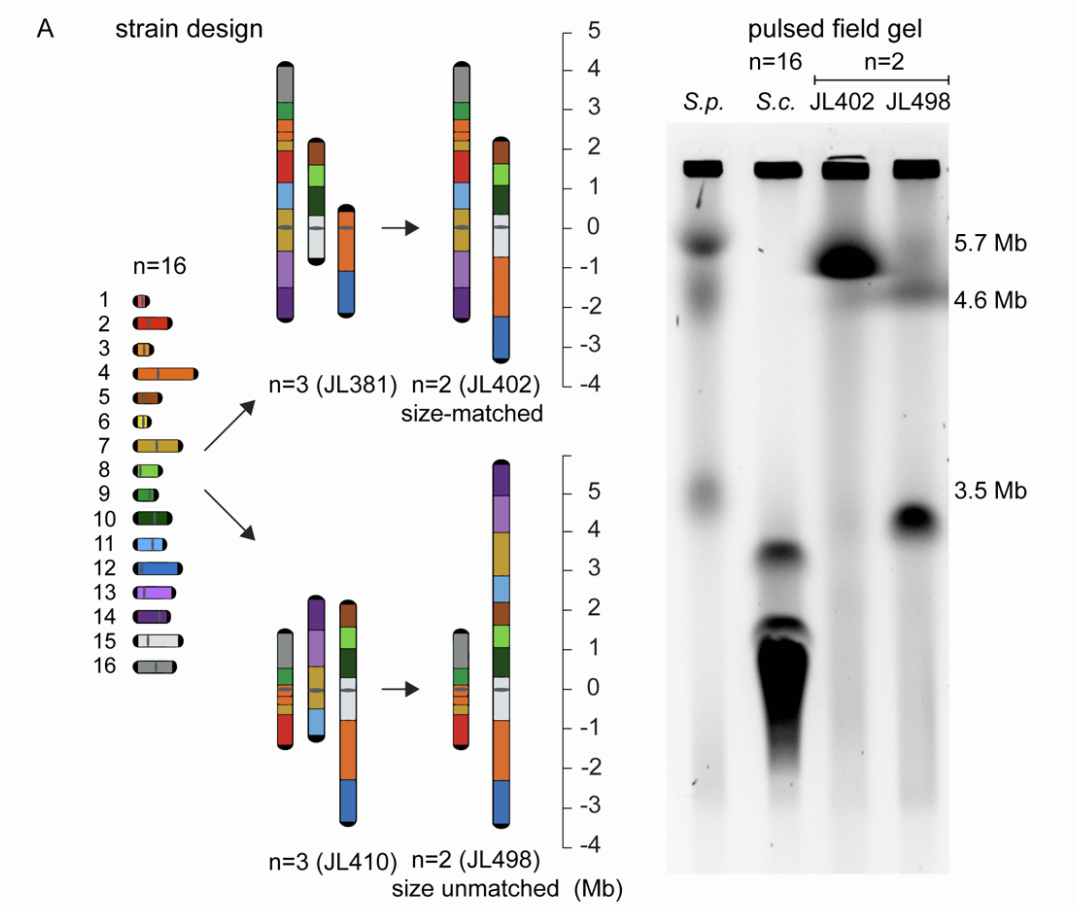
Supplemental Figures 1-7, related to Main Figure 1. Nuclear organization and function of mega-sized chromosomes in *S. cerevisiae*.

Supplemental Figures 8-9, related to Main Figure 2. Contact analysis of repeat-enriched (flocculin) genes in megachromosomes.

Supplemental Figures 10-15, related to Main Figure 3. S-phase progression and DNA replication of megachromosomes.

Supplemental Figures 16-18, related to Main Figure 4. Structural reorganization of megachromosomes during cell division.

Supplemental Table 6, related to Main Figures 1, 2 and 4. Hi-C libraries.


A
strain design
n=16
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
n=3 (JL381)
n=2 (JL402)
size-matched
n=3 (JL410)
n=2 (JL498)
size unmatched
5
4
3
2
1
0
-1
-2
-3
-4
(Mb)
pulsed field gel
n=16
n=2
S.p.
S.c.
JL402
JL498
5.7 Mb
4.6 Mb
3.5 Mb

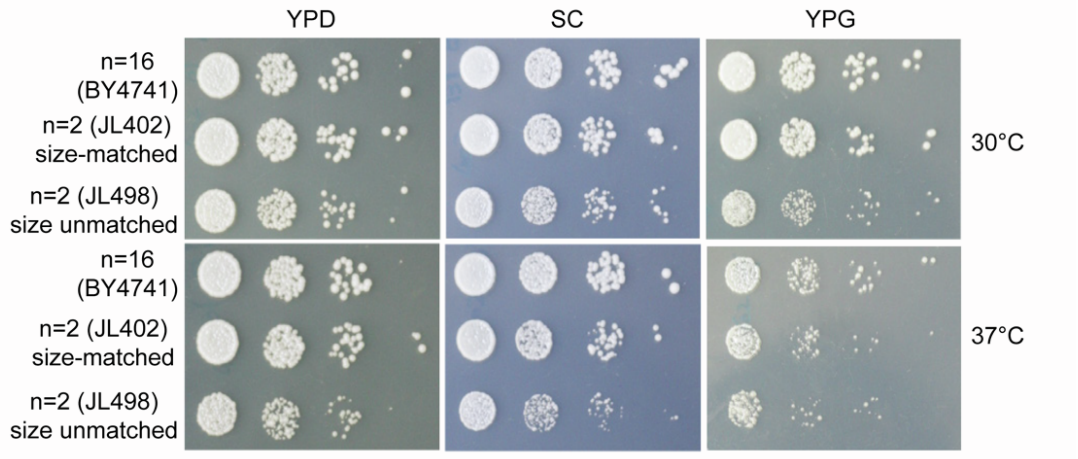

B
growth assays
YPD
SC
YPG
n=16
(BY4741)
n=2 (JL402)
size-matched
n=2 (JL498)
size unmatched
30°C
37°C

Figure S1, related to Main Figure 1. Design of size-matched and unmatched megachromosomes.

(**A**) Design of the megachromosomes. The diagram on the left illustrates the arrangement of the 16 native chromosomes into n=2 (JL402 size-matched and JL498 size unmatched) and the corresponding n=3 intermediate strains used in this study. The 16 native chromosomes are uniquely colored and ordered numerically, while the fused chromosomes are alphabetically ordered (A, B and C). Length of chromosome arms is indicated as a function of distance from the centromere position (Mb). On the right, pulsed-field gel electrophoresis with *S. pombe* (*S.p.*) chromosomal DNA as a ladder followed by *S.c.*, *S. cerevisiae*, n=2 JL402 size-matched and n=2 JL498 unmatched (for n = 3 strains refer to Luo et al.[17]). (**B**) Growth assay: serial dilutions of n=16 (BY4741) and n=2 (JL402 and JL498) on YPD (Yeast Extract–Peptone–2%Dextrose), SC (synthetic complete) and YPG (Yeast Extract–Peptone–3% Glycerol) medium at 30˚C and 37˚C.

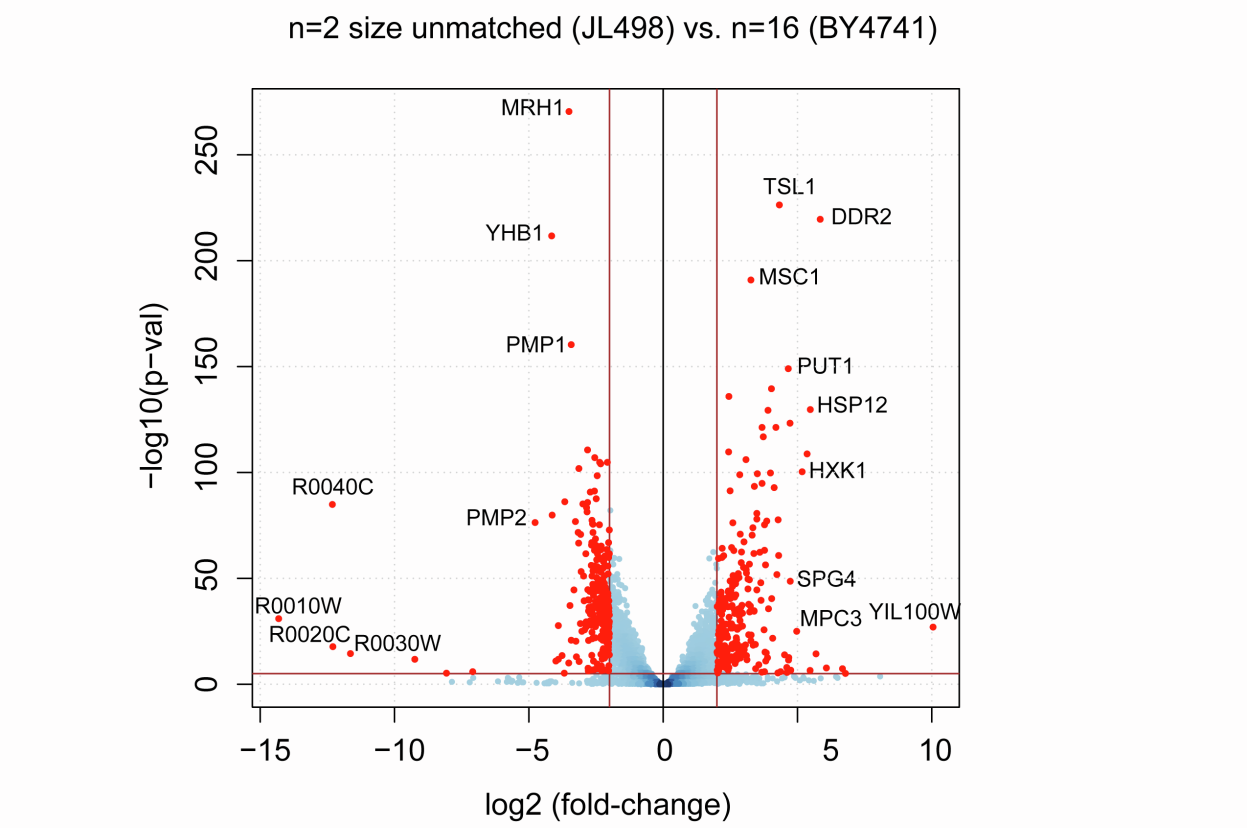
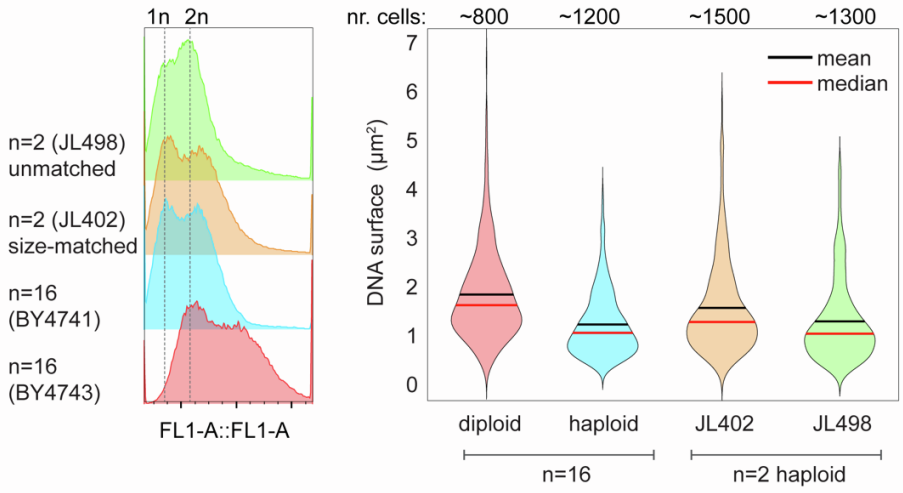


Figure S2, related to Main Figure 1. Transcriptomics of n=2 with unmatched megachromosomes.

Volcano plot of RNA-seq data that compares the transcriptomes of n=16 and n=2 JL498 (for n = 2 JL402 strain refer to Luo et al.[30]). Red dots indicate genes whose expression was significantly different in the n = 2 strain compared to the n = 16 strain (log2 fold-change > 2 or < -2 and *P*-value < $1E10^{-5}$).



DNA surface (µm²)	n=16 haploid (BY4741)		n=16 diploid (BY4743)		n=2 size-matched haploid (JL402)			n=2 unmatched haploid (JL498)		
	R1	R2	R1	R2	R1	R2	R3	R1	R2	R3
MED (µm²)	1.11	1.15	1.68	1.68	1.34	1.46	1.46	1.10	1.27	1.17
MX (µm²)	1.28	1.35	1.90	1.99	1.62	1.69	1.73	1.35	1.56	1.43
DNA surface increase relative to n=16 (BY4741)	%		50%	47%	26%	25%	28%	0%	15%	6%
	p-val		4.36^{-28}	9.09^{-22}	2.56^{-05}	6.91^{-08}	1.27^{-13}	0.65	0.013	0.15

MED (µm²) = median MX (µm²) = mean R(n°) = independent replicates

Summary measurements Supplemental Table 2a

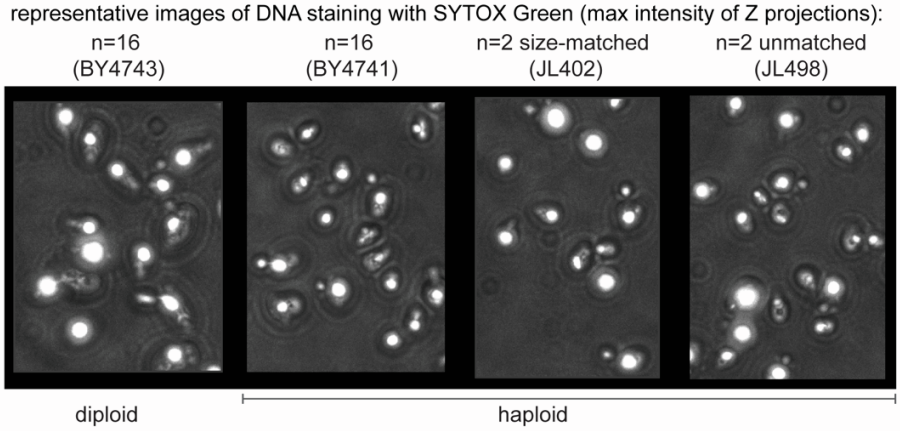


Figure S3, related to Main Figure 1. DNA content and surface in yeasts with n=2 megachromosomes.

Flow cytometry histograms of DNA content and microscopy on SYTOX Green-stained cells of n=16 (BY4743 and BY4741) and n=2 (JL402 and JL498) strains. Violin plots display mean and median values of the DNA surface (µm^2) in haploid (n=16 and n=2) and diploid (n=16 BY4743, positive control for surface increase) strains. Summary table of DNA surface (µm^2) measurements for each independent replicate (R#) showing: their relative (%) increments as compared to n=16 haploid and *P*-values obtained from K-S test. Bottom panels: representative images of GFP excited cells used above.

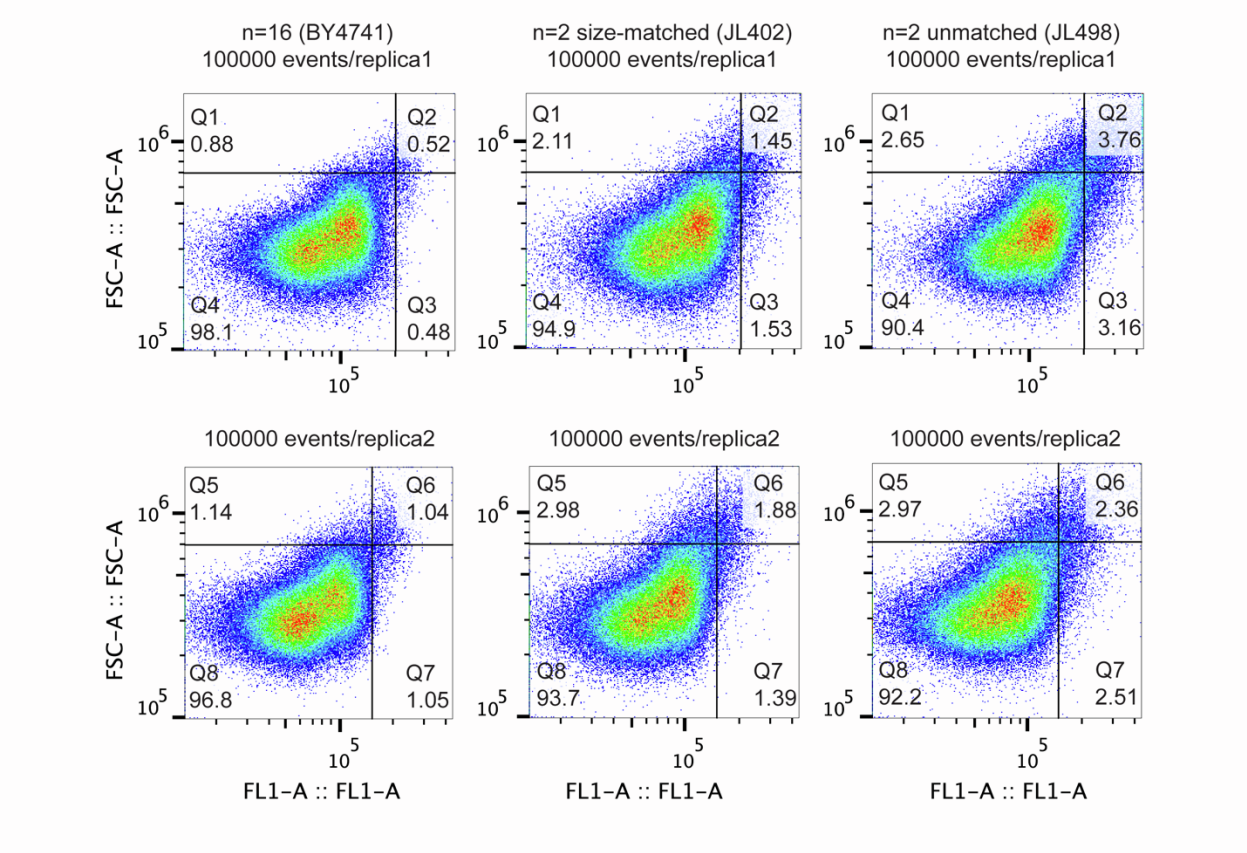
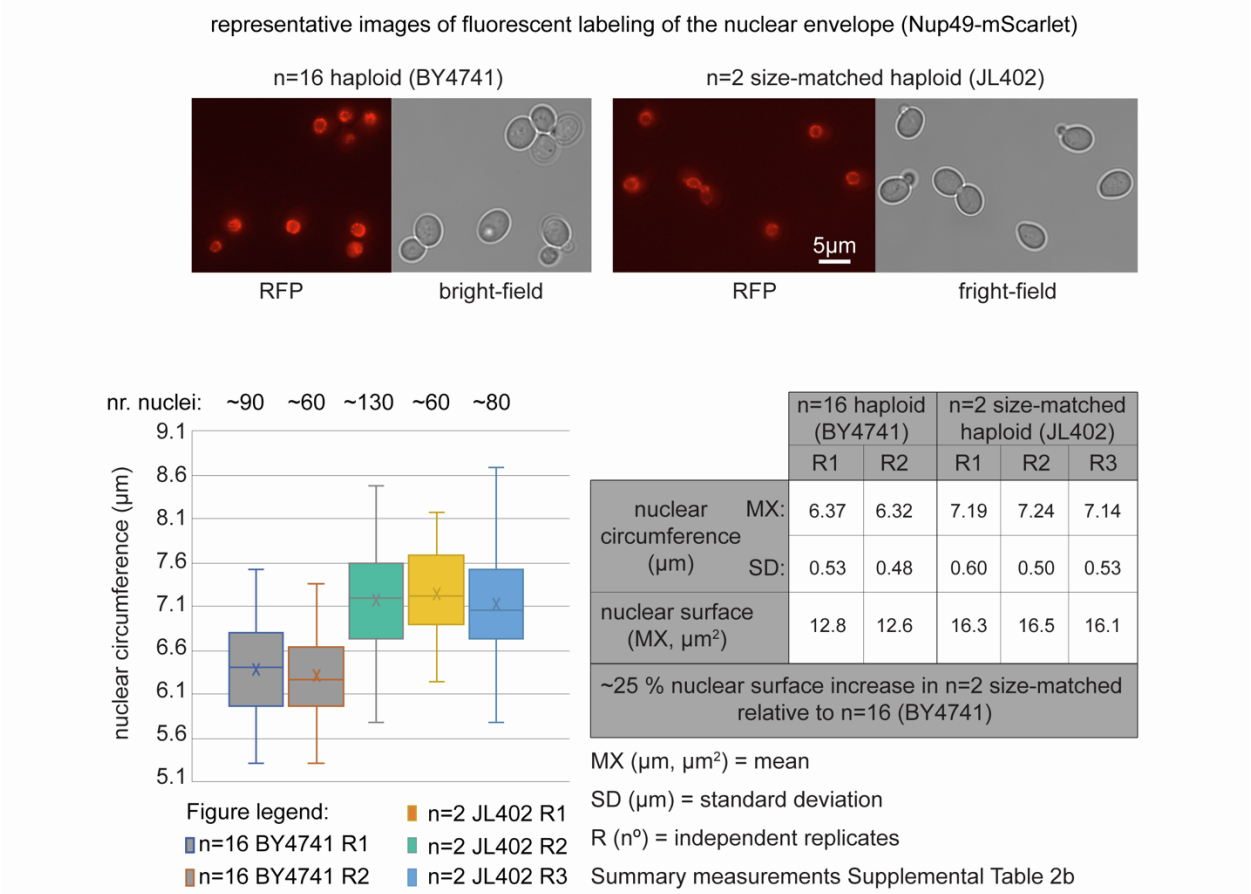


Figure S4, related to Main Figure 1. N=2 yeasts display a mild excess in DNA content. Flow cytometry of haploid cell populations (100,000 events). *x*- and *y*-axis indicate cell size (FSC) and DNA content (FL1) respectively. Note that both n=2 strains display a slight increase in number of cells with an excess of DNA content and/or size relative to n=16.



	n=16 haploid (BY4741)		n=2 size-matched haploid (JL402)		
	R1	R2	R1	R2	R3
nuclear circumference (µm) MX:	6.37	6.32	7.19	7.24	7.14
nuclear circumference (µm) SD:	0.53	0.48	0.60	0.50	0.53
nuclear surface (MX, µm²)	12.8	12.6	16.3	16.5	16.1
~25 % nuclear surface increase in n=2 size-matched relative to n=16 (BY4741)					

MX (µm, µm²) = mean

SD (µm) = standard deviation

R (nº) = independent replicates

Summary measurements Supplemental Table 2b

Figure S5, related to Main Figure 1. Nuclear size increases in n=2 with size-matched megachromosomes.

Nuclear size in haploid cells of n=16 (BY4741) and n=2 size-matched (JL402) with Nup49 (nuclear pore protein) fluorescently tagged with mScarlet. Top panels: representative microscopy images. Bottom panels: mean (MX) and standard deviation (SD) of nuclear circumference (µm) and surface (µm^2).

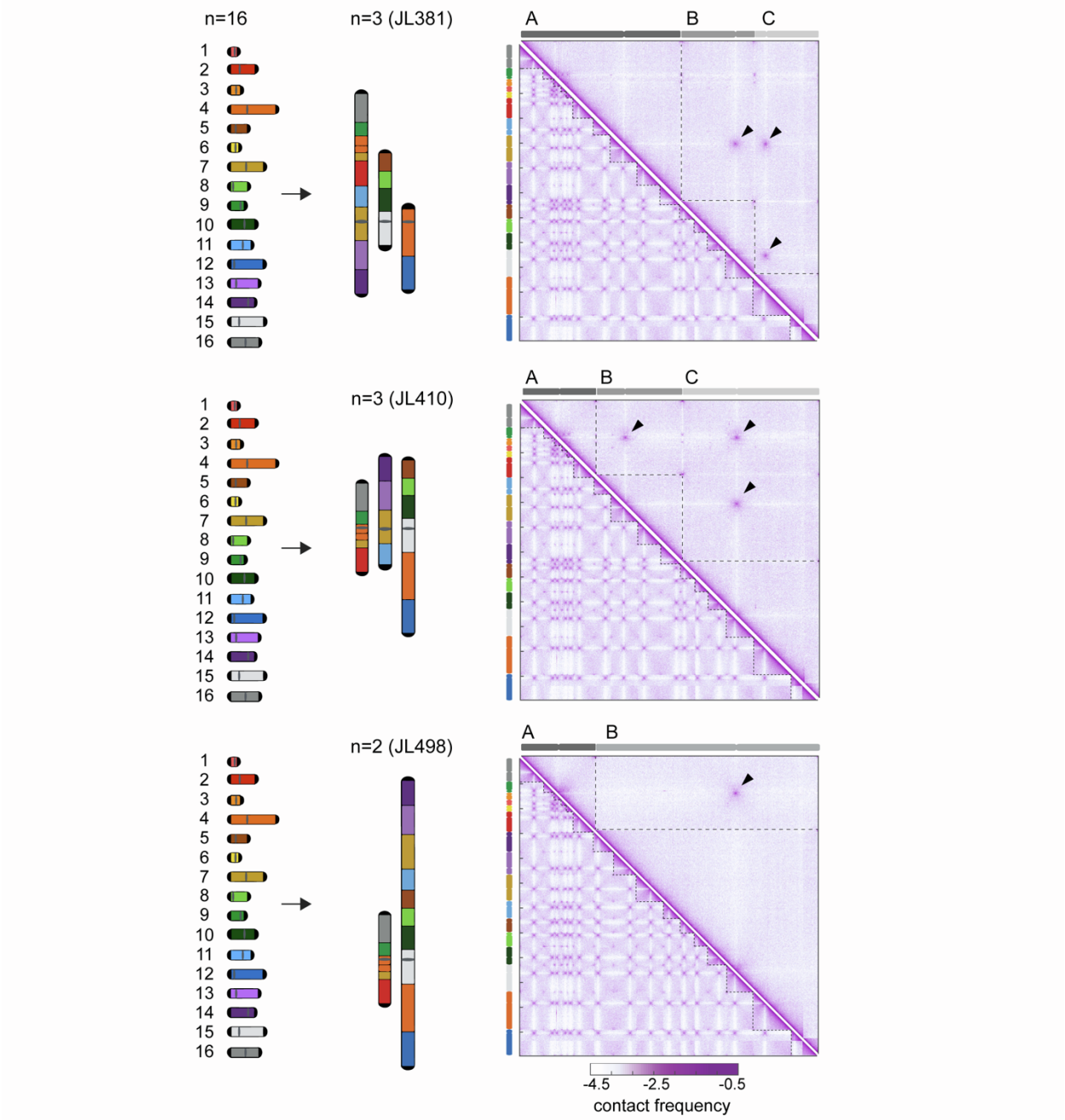


Figure S6, related to Main Figure 1. 3D organization (Hi-C) of megachromosomes.
Contact maps of n=16, n=3 and n=2 (JL498, unmatched size). Diagrams on the left illustrate the design of each fusion while panels on the right show the corresponding Hi-C contact maps. Top left maps were generated by aligning n=3 and n=2 reads to reference sequences containing either 3 or 2 megachromosomes (A, B and/or C, atop the map). Bottom left maps show all 16 native chromosomes in n=16. Chromosomes on the Hi-C maps (5 kb-binned) appear underlined by dotted lines. Black arrowheads point at inter-pericentromeric contacts. Violet to white color scale reflects high to low contact frequencies (log10).

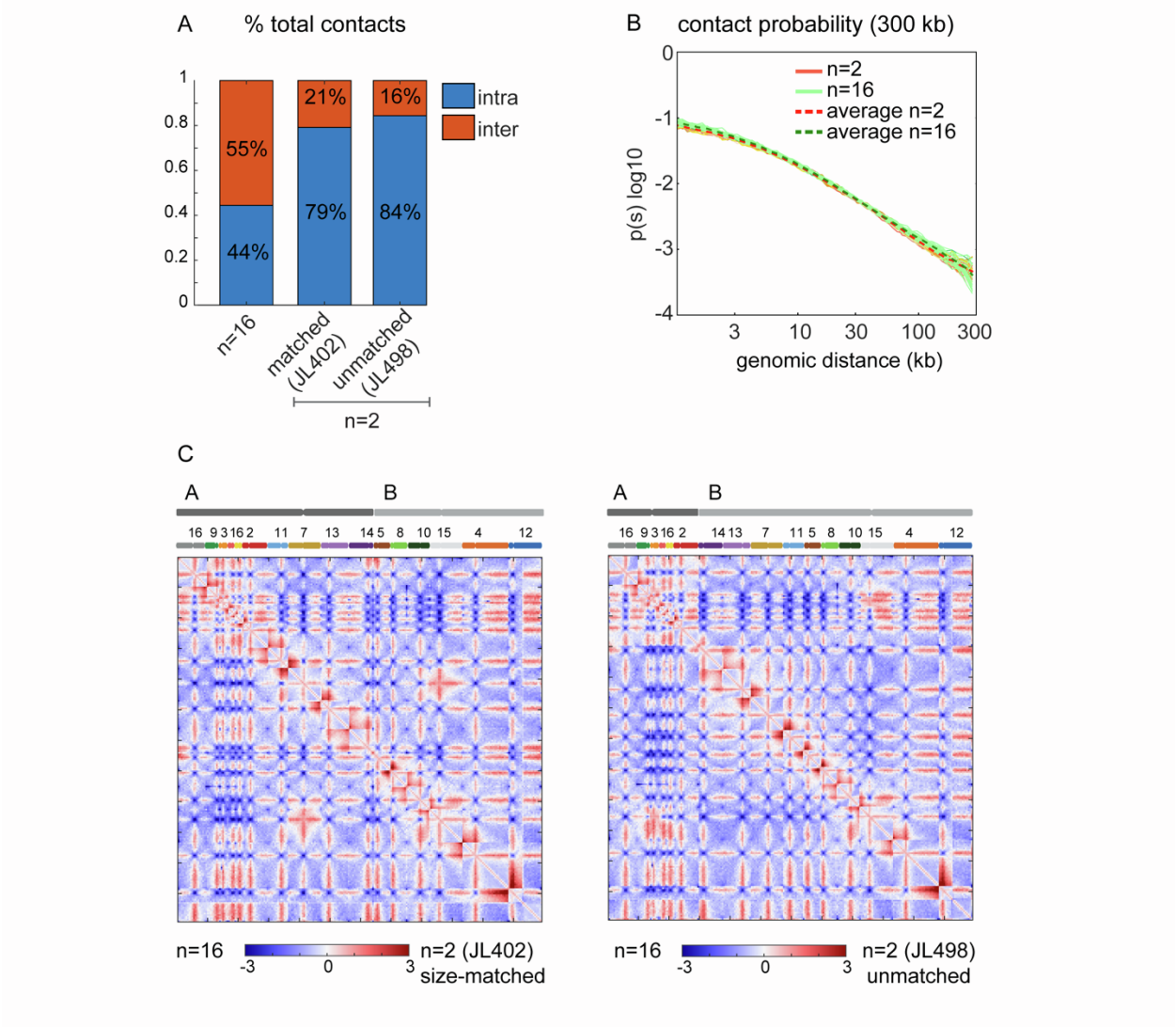
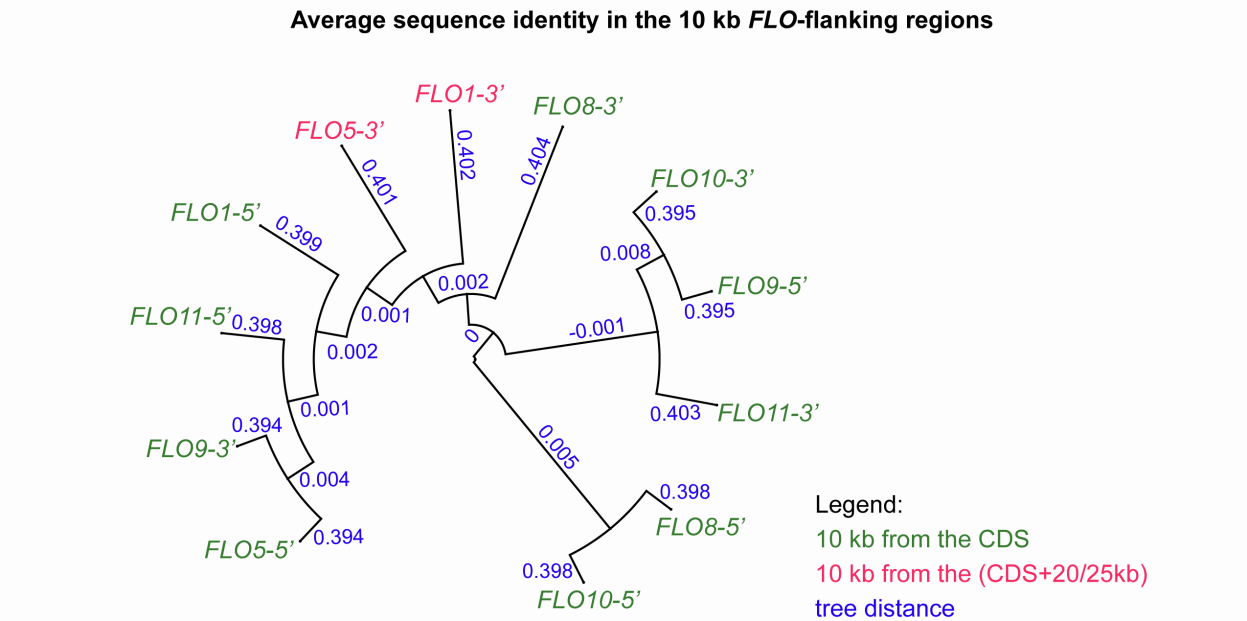


Figure S7, related to Main Figure 1. Contact analysis of megachromosomes.
(**A**) Quantitative bar chart showing the relative (%) of intra- and inter-chromosome contacts in n=16 and n=2 (JL402 and JL498). (**B**) Contact probability, *p*(*s*), spanning 300 kb genomic windows in n=16 and n=2, dash line represents the average decay of the intrachromosomal contact frequency *p* between loci with respect to their genomic distance *s*. (**C**) Log2-ratios between contact maps (50kb-binned). Left map: n=16 vs. n=2 size-matched (JL402). Right map: n=16 vs. n=2 unmatched (JL498). Blue to red color scale reflects the enrichment in contacts in n=2 with respect to n=16 (log2). Chromosomes are annotated atop the maps.



Percent Identity Matrix - created by Clustal 2.1

	FLO10-5'	FLO8-5'	FLO11-3'	FLO9-5'	FLO10-3'	FLO8-3'	FLO1-3'	FLO5-3'	FLO1-5'	FLO11-5'	FLO9-3'	FLO5-5'
FLO10-5'	**100.00**	46.05	33.18	32.64	33.35	34.26	33.02	33.12	34.78	33.57	33.59	33.72
FLO8-5'	46.05	**100.00**	32.55	33.72	33.51	33.55	32.24	33.53	35.07	34.11	34.11	34.63
FLO11-3'	33.18	32.55	**100.00**	40.21	41.78	32.70	32.99	32.95	32.65	32.36	32.32	33.29
FLO9-5'	32.64	33.72	40.21	**100.00**	45.23	32.17	32.56	32.60	32.23	32.15	33.04	32.78
FLO10-3'	33.35	33.51	41.78	45.23	**100.00**	33.45	32.54	33.48	33.45	32.91	33.61	33.74
FLO8-3'	34.26	33.55	32.70	32.17	33.45	**100.00**	37.84	36.61	37.09	36.93	37.40	37.14
FLO1-3'	33.02	32.24	32.99	32.56	32.54	37.84	**100.00**	37.70	37.45	37.98	37.91	37.86
FLO5-3'	33.12	33.53	32.95	32.60	33.48	36.61	37.70	**100.00**	38.56	37.72	38.08	38.51
FLO1-5'	34.78	35.07	32.65	32.23	33.45	37.09	37.45	38.56	**100.00**	38.77	40.17	40.16
FLO11-5'	33.57	34.11	32.36	32.15	32.91	36.93	37.98	37.72	38.77	**100.00**	40.67	41.36
FLO9-3'	33.59	34.11	32.32	33.04	33.61	37.40	37.91	38.08	40.17	40.67	**100.00**	45.81
FLO5-5'	33.72	34.63	33.29	32.78	33.74	37.14	37.86	38.51	40.16	41.36	45.81	**100.00**

Figure S8, related to Main Figure 2. Sequence identity in the flocculin flanking genes.
Tree diagram and identity matrix illustrate the relative sequence identity between all 10kb regions adjacent to the CDS (CoDing Sequence) of each *FLO* gene. A ~35% sequence identity was obtained using the multiple alignment option in Clustal Omega.

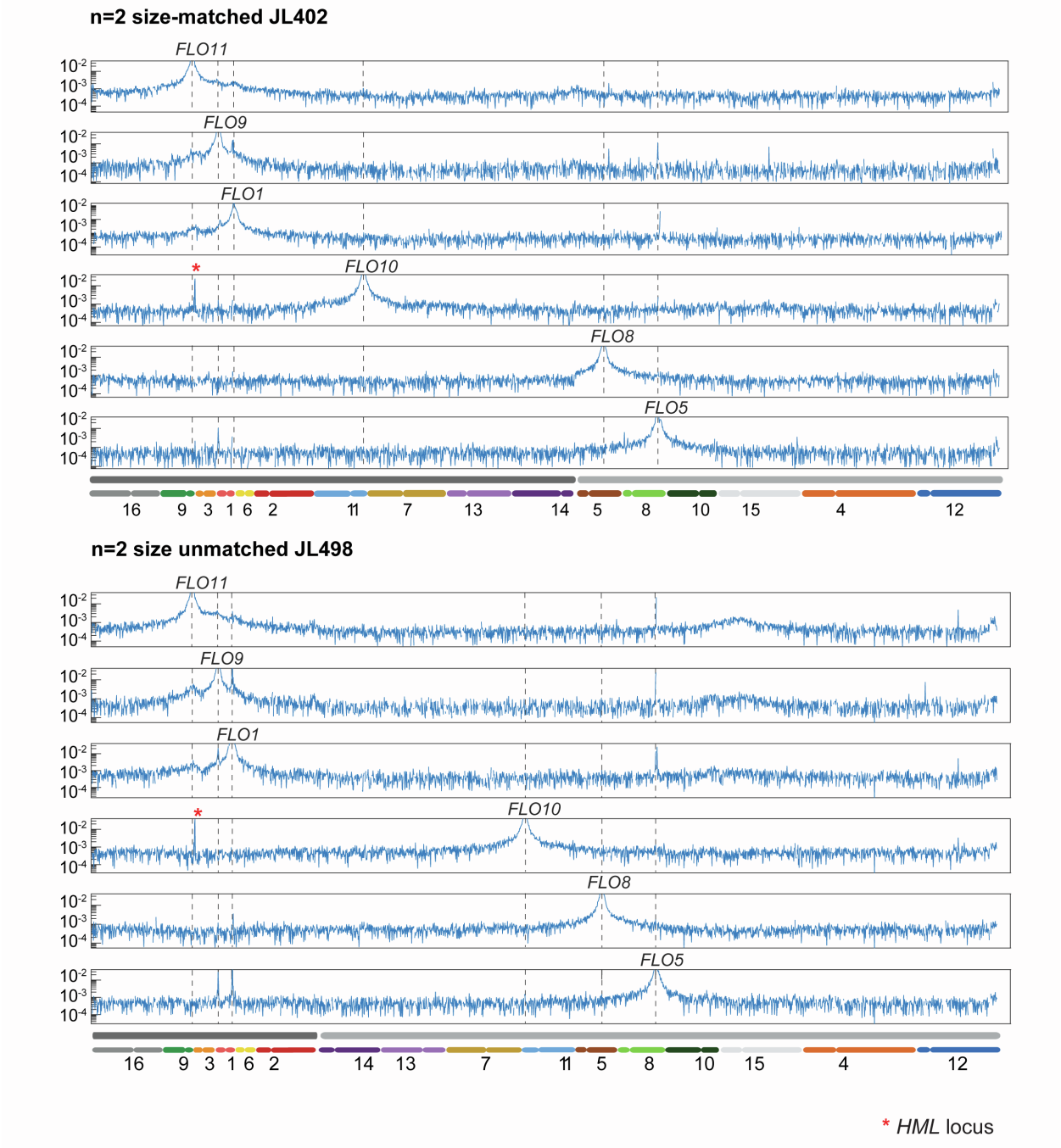


Figure S9, related to Main Figure 2. 4C-like profiles with *FLO* genes as viewpoints.
Each plot shows the contact pattern of a 15 kb window, centered on a single *FLO* locus in n=2 size-matched (JL402 top panels) and n=2 size-unmatched (JL498 bottom panels). Red star indicates *HML* locus.

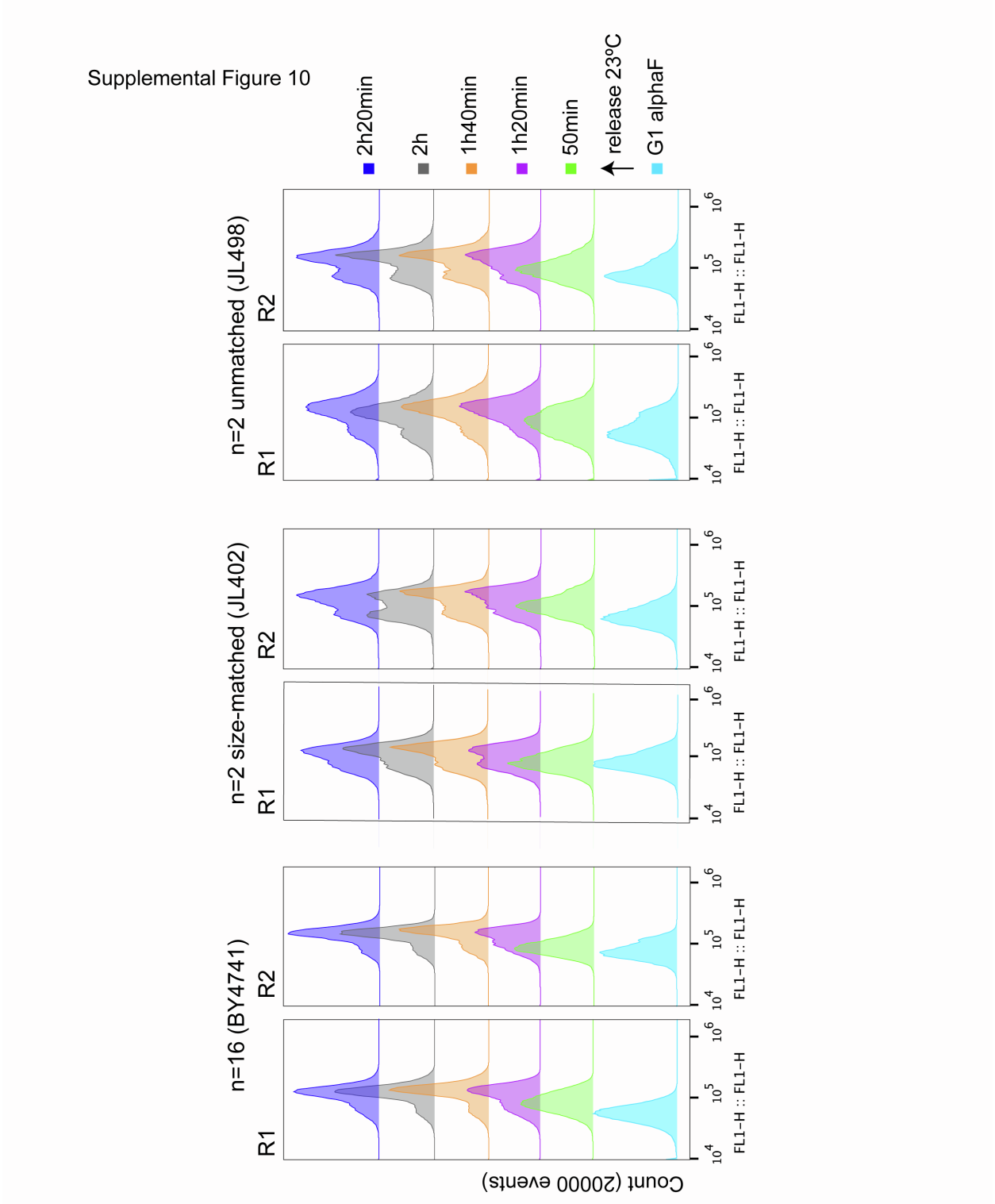


Figure S10, related to Main Figure 3. S-phase progression in n=16 vs. n=2 (JL402 size-matched and JL498 unmatched) strains.

Flow cytometry profiles of DNA content of cells synchronized in G1 with α -factor and release in S phase at 23˚C. Two independent replicates are shown (R1 and R2) for each strain.

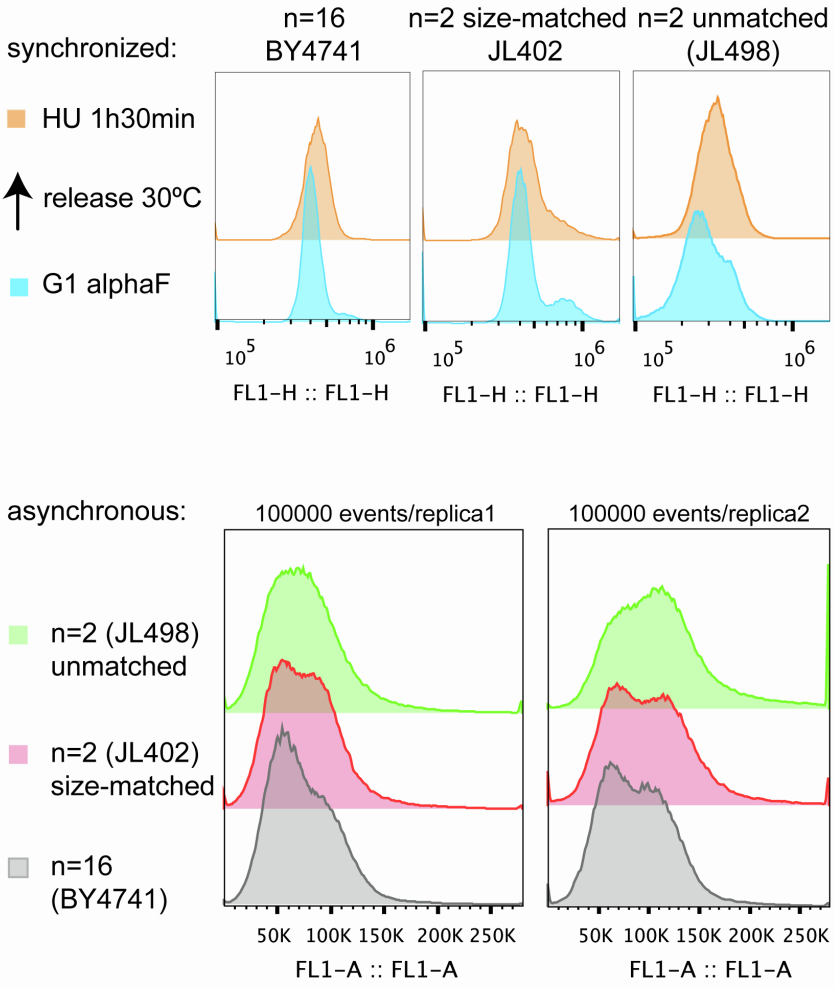


Figure S11, related to Main Figure 3. G1 and S-phase synchronizations of samples used for computing replication firing profiles in n=16 and n=2 (JL402 and JL498).
Top panels: DNA content of cells synchronized in G1 with α -factor, released and arrested in early S phase with HU. Bottom panels: DNA content of asynchronized cell populations.

Figure S12, related to Main Figure 3. Comparison of origin firing profiles in n=2 JL402 vs. n=16 BY4741.

Each profile is the average representation of three independent replicates, showing the sequencing coverage ratio of S phase (HU) synchronized cells normalized on the G1 (α-factor) non-replicating cells. Replication timing profiles (1kb-binned) of n=16 are shown in gray, while those of n=2 are in orange. Inactivated centromeres in n=2 are shown in brackets.

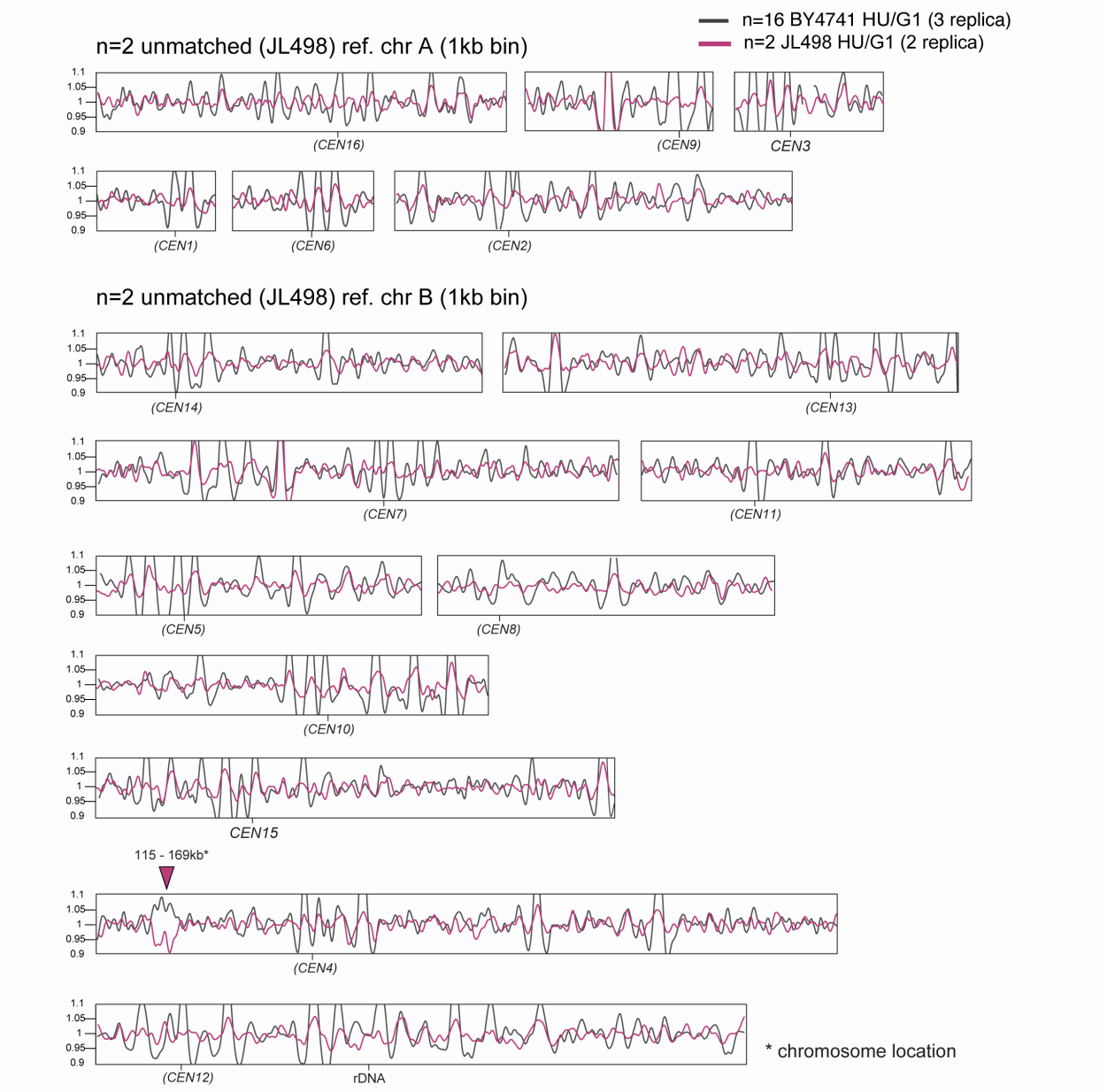


Figure S13, related to Main Figure 3. Comparison of origin firing profiles in n=2 JL498 vs. n=16 BY4741.

Notice that origin firing in n=2 JL498 is overall less distinct compared to n=2 JL402, a result of its inefficient synchronization in G1 (see histograms in B). Arrowheads in C and D point at 3 distinct locations where the replication firing differ between n=2 and n=16. Two of them (on former chr7 and chr12) contain repeated Ty-1 elements (LTR retrotransposon) and appear only in the n=2 size-matched strain. Whereas the 3rd (on former chr4) in coherent in both n=2 strains and is located in a region where none of the manually curated replication studies[55,69,70] identified any origin in ~90kb.

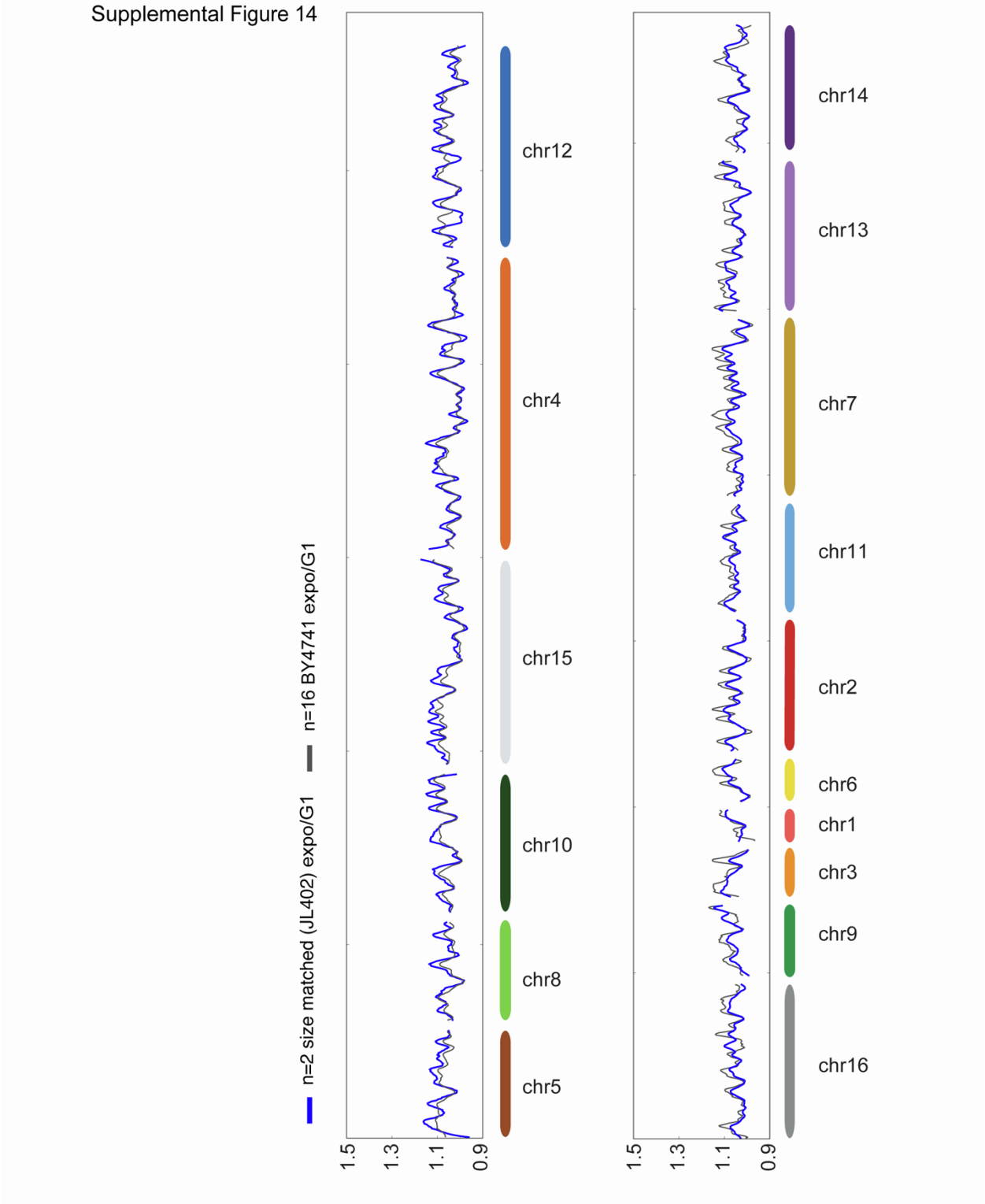


Figure S14, related to Main Figure 3. Comparison of replication profiles of n=2 JL402 vs. n=16 BY4741. DNA replication profiles, binned at 5kb, were computed on exponential growing cells normalized on non-replicating G1 profiles.

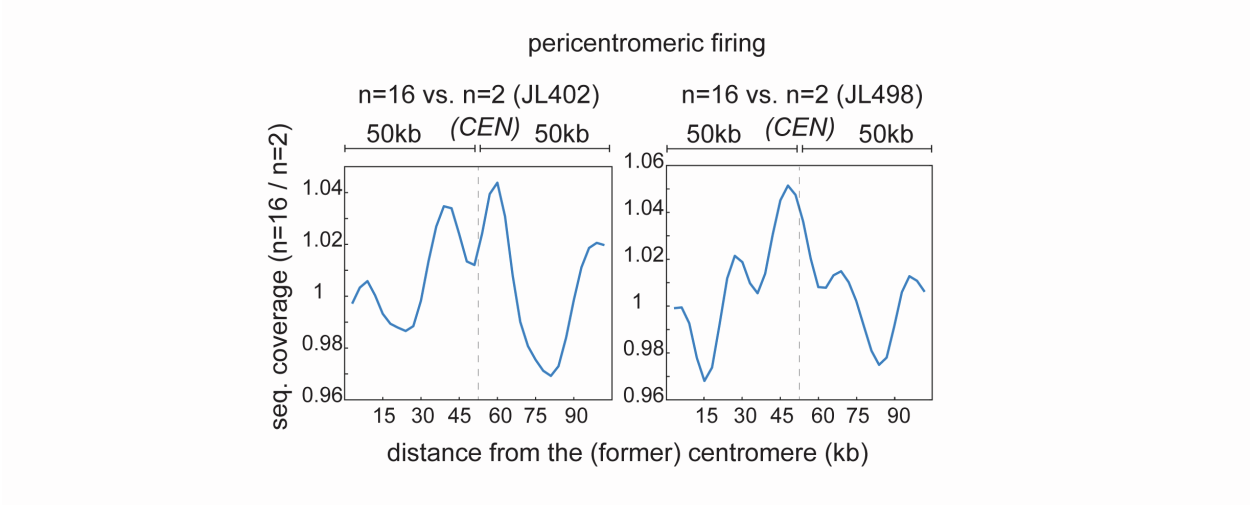


Figure S15, related to Main Figure 3. Inactivated centromeres fire late during S-phase. Pericentromeric firing in n=16 vs. n=2 (JL402 size-matched and JL498 unmatched). Ratio plots show the early firing of pericentromeric regions (~100 kb) in n=16 in respect to n=2, in which centromeres were inactivated. Centromere position is indicated with a dotted line.

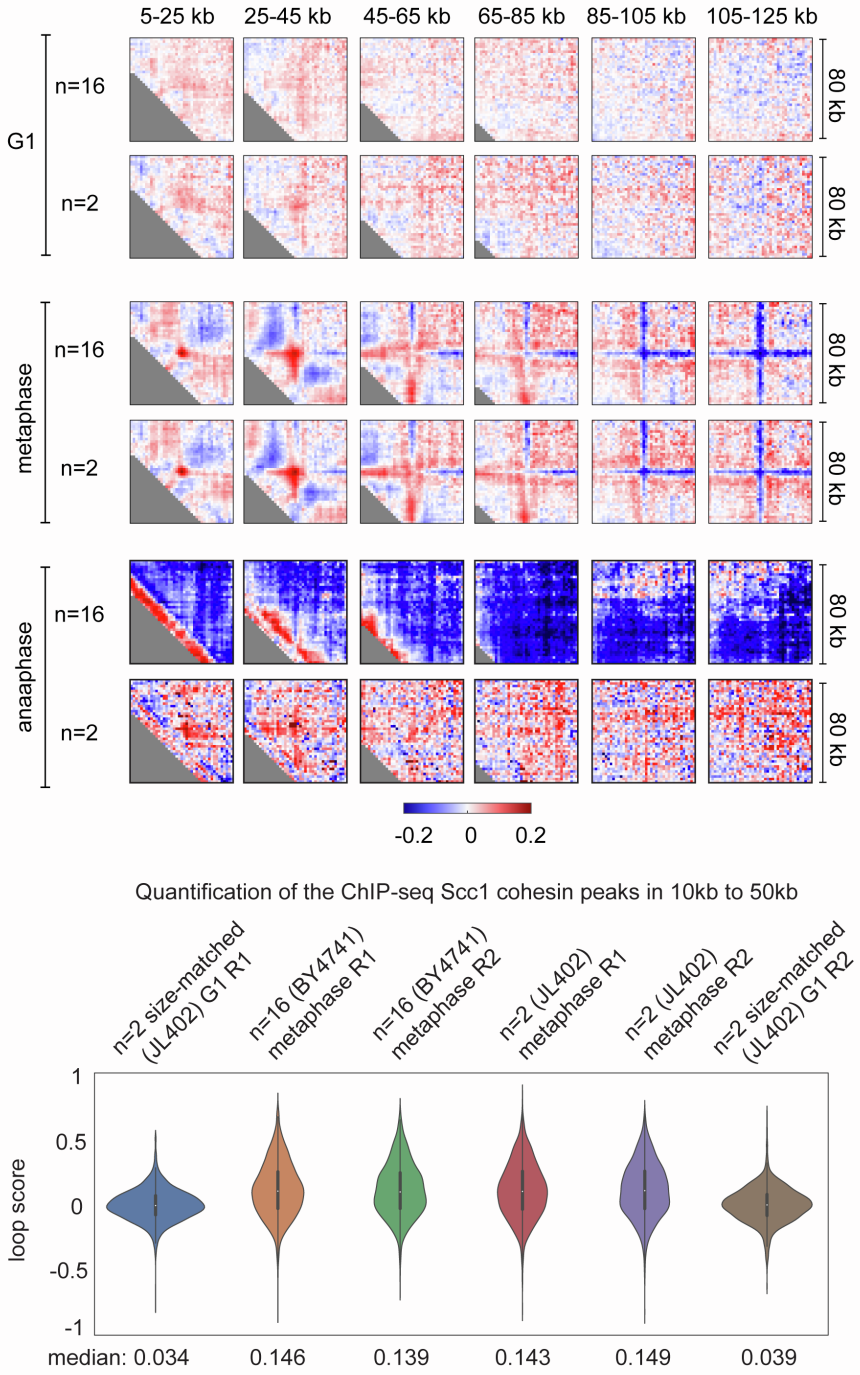


Figure S16, related to Main Figure 4. Cohesin-dependent reorganization of megachromosomes during cell division.

Cumulative log-ratio maps of cohesin-dependent contact enrichment as a function of distance from Scc1 *cis* sites during cell cycle progression: G1, metaphase (nocodazole) and anaphase (*cdc15-2* ts). Blue to red color scale represent an enrichment in contacts dependent on Scc1 in respect to random sites. Violin plots quantify contact enrichment in 10-50 kb windows from Scc1 binding sites in n=16 and n=2 size-matched (JL402).

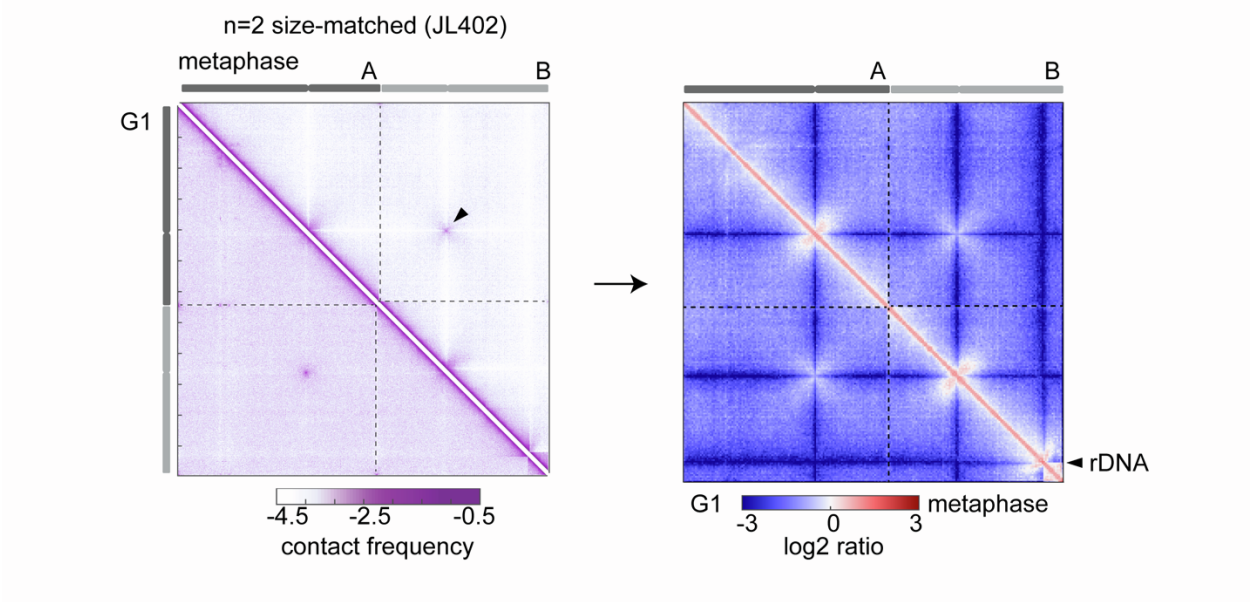


Figure S17, related to Main Figure 4. Contact comparison maps of n=2 (JL402) cells synchronized in G1 and metaphase. Blue to red color scale in the log-ratio map reflects contact enrichment in metaphase compared to G1 (50bk-binned; log2). Black arrowheads indicate inter-centromere contacts.

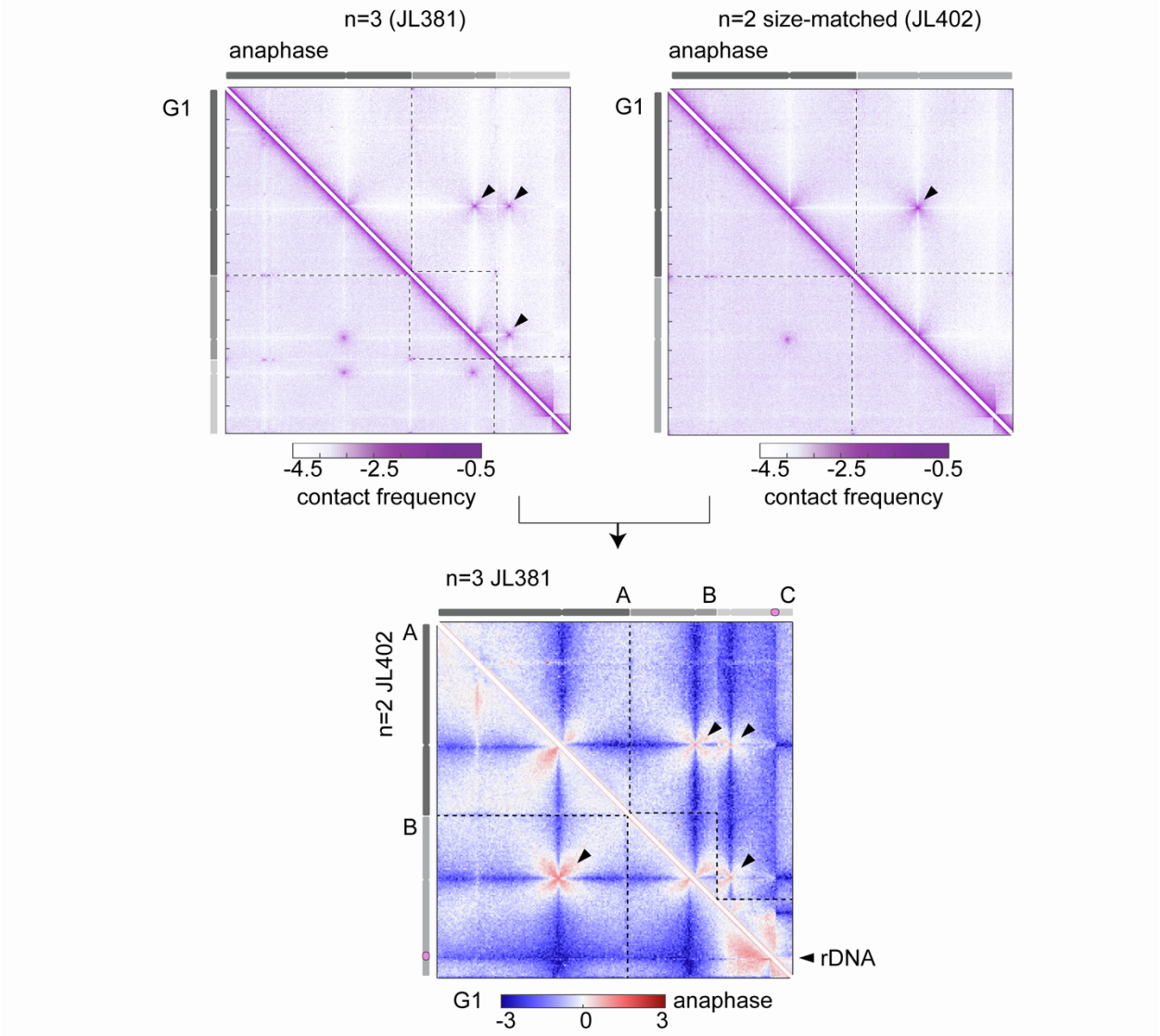


Figure S18, related to Main Figure 4. Contact comparison maps of either n=3 (JL381) or n=2 (JL402) strains synchronized in G1 and anaphase. Blue to red color scale in the log-ratio maps reflects contact enrichment in anaphase compared to G1 (50bk-binned; log2). Black arrowheads indicate inter-centromere contacts.

Table S6, related to Main Figures 1, 2 and 4. Hi-C libraries.

Strain name	Karyotype	Synchronization method	Total paired-end reads	Aligned paired-end reads	Total contacts in map
BY4741 (Lazar-Stefanita et al., 2017)	16	none	43649470	31761165	17649810
BY4741	16	metaphase nocodazole (exp. 1)	31738518	19384914	18264990
BY4741	16	metaphase nocodazole (exp. 2)	27475676	17295275	16137114
JL381	3	none	47645690	36846370	13606005
JL381	3	G1 elutriation	71227924	47667278	14943151
LS381	3	Anaphase *cdc15-2*	32895220	23554200	3173547
JL410	3	none	69886343	55663288	6428681
JL410	3	G1 elutriation	33874272	18479512	13061046
JL402	2	none	31808387	24803480	12993017
JL402	2	G1 elutriation	33942738	26955095	11214102
JL402	2	metaphase nocodazole (exp. 1)	28339485	18963164	17441919
JL402	2	metaphase nocodazole (exp. 2)	26207064	17208531	16102493
JL402	2	Anaphase *cdc15-2* (exp. 1)	49078905	38115942	13116462
JL402	2	Anaphase *cdc15-2* (exp. 2)	30859455	24679199	9090904
JL498	2	none	48557489	38575397	13925428