Supplementary Materials
Molecular Biology of the Cell
Bailey *et al*.

Supplemental Information

Loops and the activity of loop extrusion factors constrain chromatin dynamics

Mary Lou P. Bailey[1,2]*, Ivan Surovtsev[3,4]*#, Jessica F. Williams[4]*, Hao Yan[2,3]*, Tianyu Yuan[2,3], Kevin Li[4], Katherine Duseau[4], Simon G. J. Mochrie[1,2,3]# and Megan C. King[2,4,5]#

[1] *Department of Applied Physics, Yale University, New Haven, Connecticut 06511, USA*
[2] *Integrated Graduate Program in Physics Engineering Biology, Yale University, New Haven, Connecticut 06511, USA*
[3] *Department of Physics, Yale University, New Haven, Connecticut 06511, USA*
[4] *Department of Cell Biology, Yale School of Medicine, New Haven, Connecticut 06520, USA*
[5] *Department of Molecular, Cell and Developmental Biology, Yale University, New Haven, Connecticut 06511, USA*

* equal contributions
# correspondence to *simon.mochrie@yale.edu*, *megan.king@yale.edu*, or *ivan.surovtsev@yale.edu*

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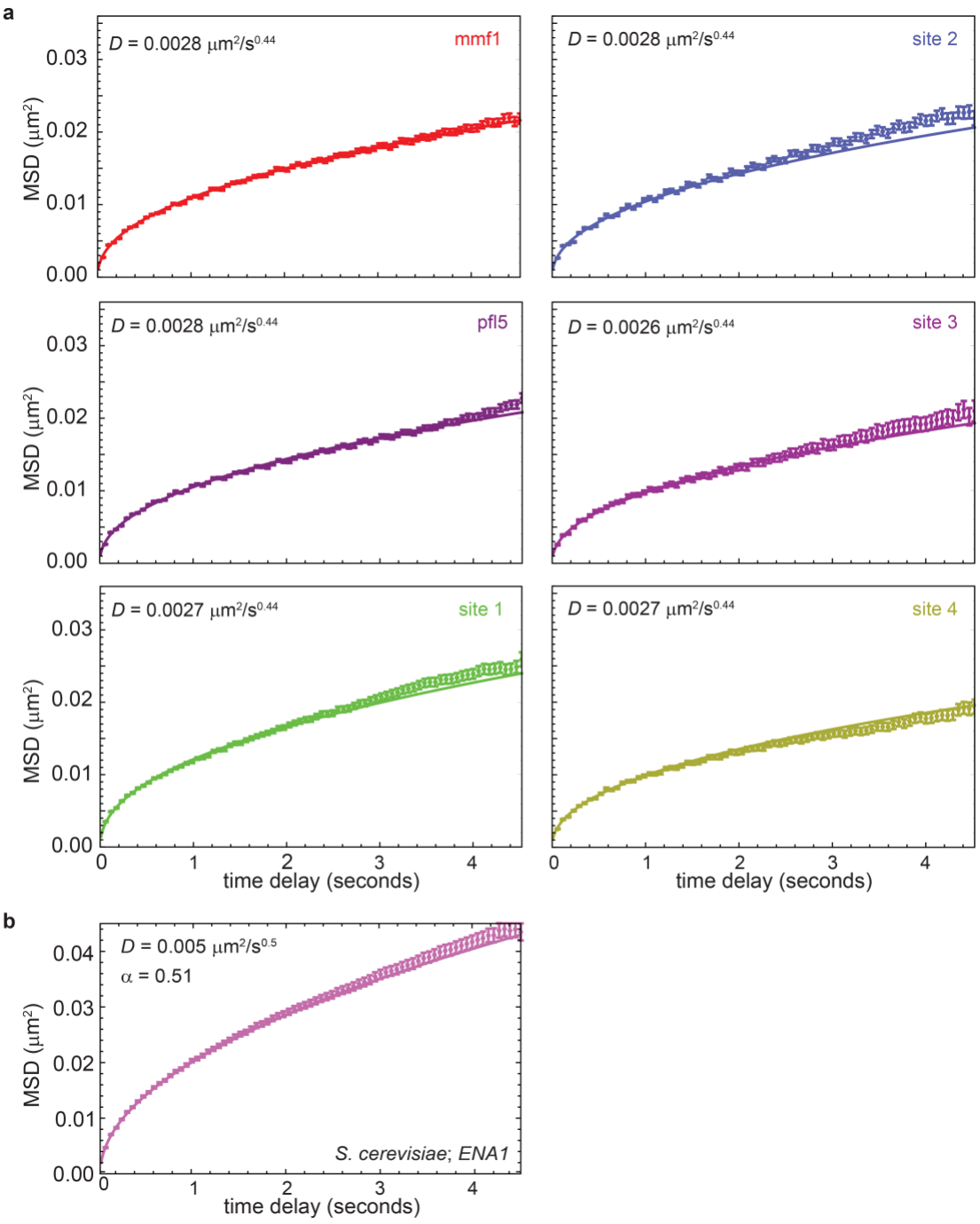

Fig. S1. ***Individual MSD plots for fission yeast and budding yeast.*** **(a)** Chromatin diffusivity is nearly identical across six different genomic locations as shown by the mean squared displacement (MSD) of each genetic locus as a function of the time window of observation, along with its calculated diffusion coefficient, *D*. **(b)** For comparison to previous chromatin dynamics measurements, we performed our visualization/tracking/diffusive analysis regime on *S. cerevisiae* cells integrated with a *lacO* array at the *ENA1* locus, resulting in a comparable diffusion coefficient as reported previously. Error bars indicate standard errors of the mean.

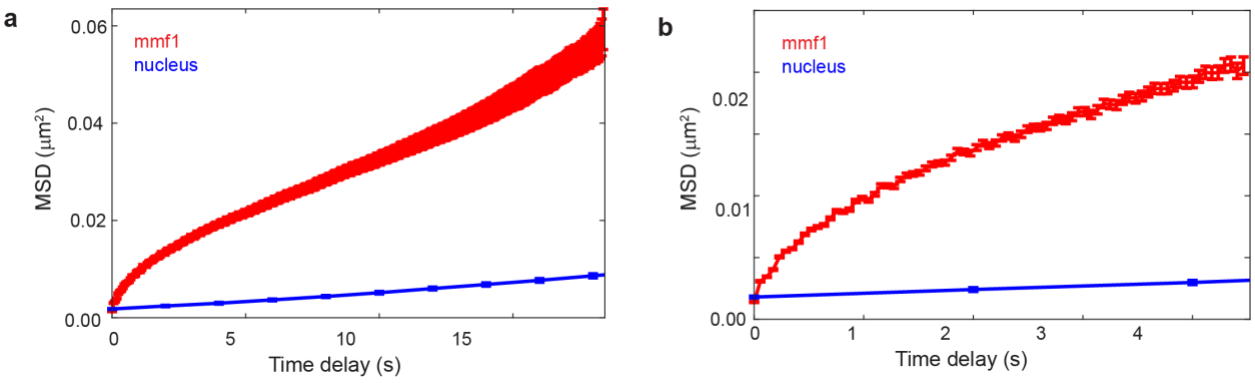

Fig. S2. ***Microtubule dynamics-driven chromatin motion cannot be explained by whole-nucleus motion.*** **(a)** and **(b)** MSD of *lacO* array near *mmf1* relative to MSD of the whole-nucleus motion at different timescales.

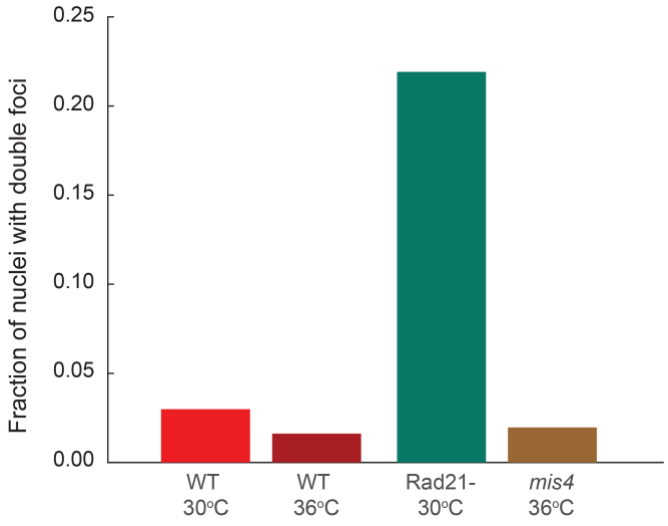

Fig. S3. ***Cohesin depletion but not inactivation of cohesin loading leads to loss of cohesion.*** Shown is a fraction of nuclei (of all nuclei with spots) with two spatially separated *lacO*/GFP-LacI foci as observed in single-plane time-lapse movies (details are in the Materials and Methods) for cells with *lacO* array near *mmf1* locus in wild type cells (MKSP2039) at 30C and 36C, and in cells depleted of Rad21 (MKSP3660, at 30C) or with inactivated temperature-sensitive Mis4 (MKSP2801, at 36C).

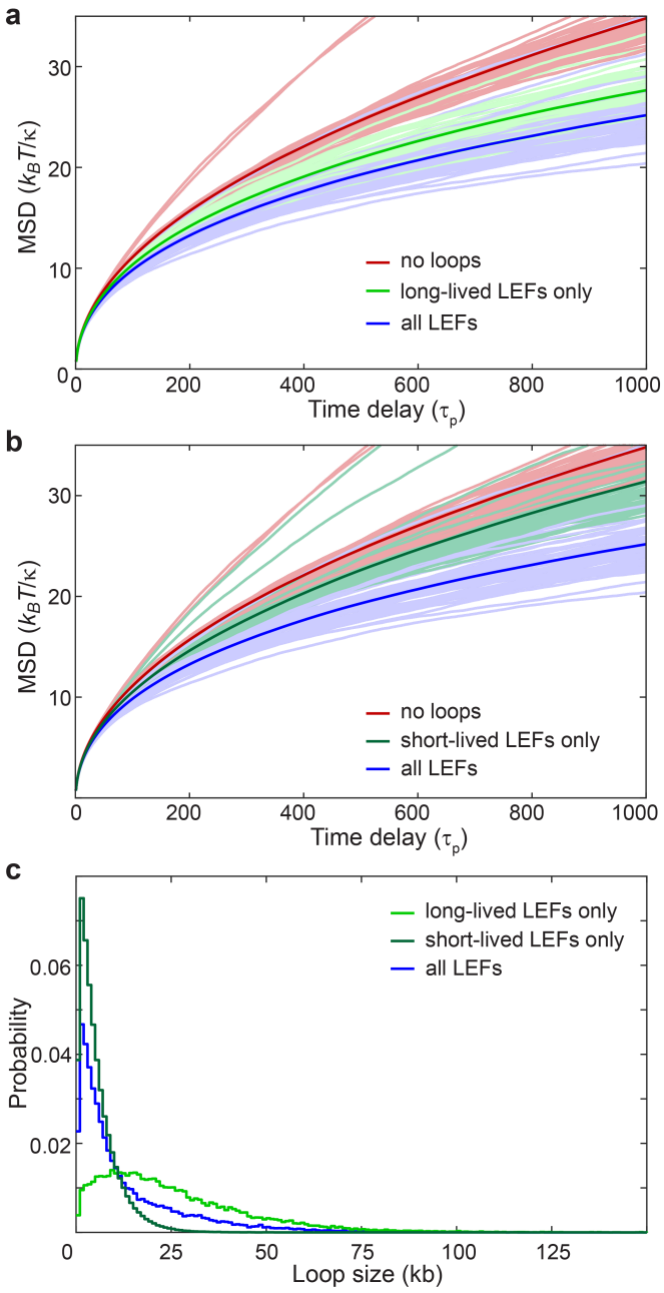

Fig. S4. ***Impact of LEFs on chromatin mobility depends on LEF lifetime(s).*** As in Fig. 5a but showing MSDs for individual beads split into two panels for clarity. **(a)** MSD results of the Rouse-type polymer simulations combined with loop-extrusion simulations that considered two types of LEF complexes (in equal amounts) with different lifetimes. Blue – simulations with both types of LEFs present, green – only the long-lived LEFs, red – no LEFs. Thin lines represent MSDs for each individual bead, thick lines represent the average over all beads and all simulations. **(b)** As for (a) but for simulations with only short-lived LEFs (dark green) compared to simulations with both LEFs (blue) and no LEFs (red). **(c)** Loop size distribution for simulations of fission yeast chromatin subjected to the "2 LEFs" model with both LEFs present (blue), only the long-lived LEFs present (green), and only the short-lived LEFs present (dark green).

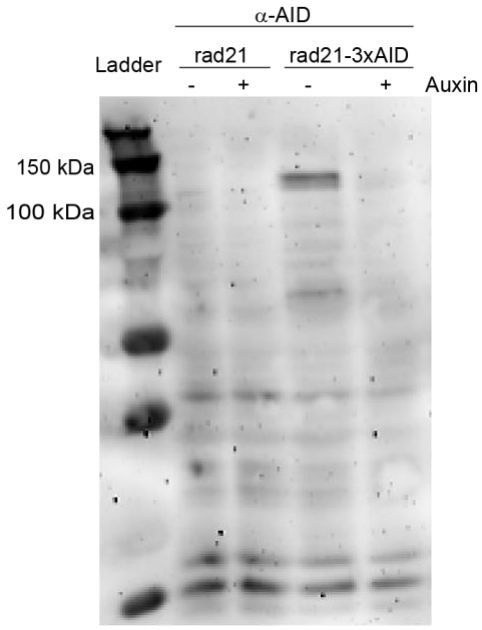

Fig. S5. ***Rad21-AID can be quickly depleted from cells upon addition of the auxin analogue, 5-IAA.*** Western blots of lysates of WT cells or cells with Rad21 tagged with auxin-inducible degron before or after 5-IAA treatment for 20 min.

Supplemental Table S1: *MSD Fitting Results*

	Varied α, σ^2, D						Fixed α = 0.44, varied σ^2, D				
Strain	**α**	**Confidence Interval (CI) on α fit**	**σ^2, μm^2**	**CI on σ^2, μm^2**	***D*, $\mu m^2/s$**	**CI on *D*, $\mu m^2/s$**	**α**	**σ^2, μm^2**	**CI on σ^2, μm^2**	***D*, $\mu m^2/s$**	**CI on *D*, $\mu m^2/s$**
mmf1 WT	0.43	0.3695 - 0.4955	0.0031	0.0027 - 0.0035	0.0028	0.0025 - 0.0032	**0.44**	0.0031	0.0030 - 0.0032	0.0028	0.0027 - 0.0029
pfl5 WT	0.42	0.3613 - 0.4883	0.003	0.0026 - 0.0034	0.0028	0.0025 - 0.0031	**0.44**	0.0031	0.0030 - 0.0032	0.0027	0.0026 - 0.0028
site 1	0.53	0.4910 - 0.5776	0.0033	0.0031 - 0.0035	0.0027	0.0025 - 0.0029	**0.44**	0.0027	0.0026 - 0.0029	0.0031	0.0030 - 0.0031
site2	0.41	0.3092 - 0.5172	0.0032	0.0025 - 0.0039	0.0028	0.0023 - 0.0033	**0.44**	0.0034	0.0032 - 0.0036	0.0027	0.0026 - 0.0028
site 3	0.43	0.3662 - 0.4901	0.003	0.0026 - 0.0034	0.0026	0.0023 - 0.0028	**0.44**	0.0031	0.0029 - 0.0032	0.0025	0.0025 - 0.0026
site 4	0.41	0.3540 - 0.4641	0.0028	0.0024 - 0.0031	0.0027	0.0024 - 0.0030	**0.44**	0.0029	0.0028 - 0.0030	0.0025	0.0025 - 0.0026
mmf1 cut14 36C	0.54	0.5081 - 0.5764	0.0029	0.0027 - 0.0032	0.0032	0.0031 - 0.0034	**0.44**	0.0022	0.0021 - 0.0023	0.0037	0.0037 - 0.0038
mmf1 mis4 36C	0.47	0.3828 - 0.5503	0.003	0.0023 - 0.0038	0.004	0.0035 - 0.0045	**0.44**	0.0028	0.0026 - 0.0031	0.0042	0.0040 - 0.0043
pfl5 cut14 36C	0.43	0.3918 - 0.4750	0.0025	0.0022 - 0.0029	0.0035	0.0033 - 0.0038	**0.44**	0.0026	0.0025 - 0.0027	0.0035	0.0035 - 0.0036
pfl5 mis4 36C	0.44	0.3187 - 0.5572	0.003	0.0019 - 0.0040	0.0039	0.0031 - 0.0047	**0.44**	0.003	0.0027 - 0.0033	0.0039	0.0037 - 0.0041
mmf1 36C	0.48	0.4462 - 0.5170	0.0031	0.0029 - 0.0033	0.0027	0.0026 - 0.0028	**0.44**	0.0029	0.0028 - 0.0029	0.0029	0.0028 - 0.0029
pfl5 36C	0.45	0.4154 - 0.4839	0.0026	0.0024 - 0.0028	0.0025	0.0024 - 0.0027	**0.44**	0.0026	0.0025 - 0.0026	0.0026	0.0026 - 0.0027
mmf1 cut14 rad21- 30C	0.36	0.3292 - 0.3932	0.0026	0.0022 - 0.0029	0.0046	0.0043 - 0.0049	**0.44**	0.0033	0.0032 - 0.0035	0.0040	0.0039 - 0.0041
mmf1 cut14 rad21- 36C	0.41	0.3750 – 0.4470	0.0028	0.0025 - 0.0032	0.0043	0.004 – 0.0046	**0.44**	0.0031	0.0031 - 0.0032	0.0041	0.0040 - 0.0041
mmf1 + PFA	near flat MSD	0.0033	0.0029 - 0.0036	5.5×10^{-5}	-2.1×10^{-4}- 3.3×10^{-4}		**0.44**	0.0033	0.0032 - 0.0034	0.00005	
mmf1 + NaN_3	0.46	0.3384 - 0.5864	0.0029	0.0025 - 0.0033	0.0014	0.0011 - 0.0017	**0.44**	0.0028	0.0027 - 0.0029	0.0015	0.0014 - 0.0016
mmf1 + MBC	0.42	0.3327 - 0.4717	0.0027	0.0023 - 0.0032	0.0028	0.0024 - 0.0032	**0.44**	0.0029	0.0028 - 0.0031	0.0026	0.0026 - 0.0027
cen2 + + MBC	0.55	0.1309- 0.9721	0.0042	0.0029- 0.0054	0.0014	6.2×10^{-4}- 0.0023	**0.44**	0.0038	0.0033- 0.0043	0.0017	0.0014- 0.0020
arp8	0.68	0.4822 - 0.8815	0.004	0.0034 - 0.0046	0.0017	0.0014 - 0.0021	**0.44**	0.0031	0.0027 - 0.0034	0.0024	0.0022 - 0.0025
arp8 cut14 30C	0.54	0.4857 - 0.5969	0.003	0.0028 - 0.0033	0.0021	0.0020 - 0.0023	**0.44**	0.0026	0.0024 - 0.0027	0.0025	0.0024 - 0.0025
arp8 cut14 36C	0.68	0.6290 - 0.7331	0.0034	0.0032 - 0.0037	0.0023	0.0022 - 0.0024	**0.44**	0.0023	0.0020 - 0.0025	0.0031	0.0030 - 0.0032
arp9	0.44	0.2652 - 0.6106	0.0037	0.0027 - 0.0047	0.0026	0.0019 - 0.0034	**0.44**	0.0037	0.0034 - 0.0040	0.0026	0.0025 - 0.0028
***S. cerevisiae* ENA**	0.506	0.48 - 0.5326	0.0033	0.0031 0.0036	0.005	0.0048 - 0.0052				0.005	

Supplemental Table S2: ***Loop-extrusion-factor (LEF) Simulation and Rouse Simulation Parameters***

	Mouse CTCF Model	**Mouse LEF Only Model**	***S. pombe* LEF Only Model**	***S. pombe* 2 LEFs Model**
LEF stepping rate *v* (bp/s)	60	60	60	60
LEF mean lifetime (s)	2000	2000	500	500, 50
Boundary elements parameter *S*	20	N/A	N/A	N/A
Boundary elements parameter μ	3	N/A	N/A	N/A
Number of LEFs	48	48	30	15, 15
Polymer characteristic time (s)	0.9	0.9	0.00225	0.00225
Rouse spring stiffness (pN·μm^{-1})	0.3	0.3	6	6
Friction coefficient (pN·s·μm^{-1})	1.08	1.08	0.054	0.054

Supplemental Table S3: *Strains used in the study*

Strain	Relevant genotype	Description	Source
S. pombe			
Specific lacO *position*			
MKSP1642	*h+ leu1-32 ura4-D18 his7+:P_{Dis1}-gfp-lacI-NLS sad1::sad1-dsRed-leu+ cen2:ura4-$lacO_n$*	WT strain with *lacO* array integrated near *cen2* (Chr2 centromere) locus and with expression of LacI-GFP (with nucleus localization signal) under control of the constitutive promoter. *Fig. 2.*	*this study derived from* PX342 and PN10127
MKSP1661	*h- leu1-32 ura4-D18 his7+:P_{Dis1}-gfp-lacI-NLS cut11::cut11-mCherry-natR pfl5: ura4-$lacO_n$*	WT strain with *lacO* array (5.6 kb) integrated near *pfl5* locus (at *ChrII: 4403547-4403553*). *Fig. 1b, 3b, Fig. S1a*.	[1]
MKSP1794	*leu1-32 ura4-D18 mis4::mis4-242^{ts} his7+:P_{Dis1}-gfp-lacI-NLS cut11::cut11-mCherry-natR pfl5:ura4-$lacO_n$*	Temperature-sensitive mutant of Mis4 cohesin loading factor with *lacO* array (5.6 kb) integrated near *pfl5* locus. *Fig. 3b.*	*this study derived from* MY3655 and *MKSP1340*
MKSP2039	*h+ leu1-32 ura4-D18 his7+:P_{Dis1}-gfp-lacI-NLS mmf1:ura4-$lacO_n$*	WT strain with *lacO* array (10.3 kb) integrated near *mmf1* locus (at *ChrII:3442981*). *Fig. 1b-c, 2, 3a, 5, 7, Fig. S1a, S2*	*this study derived from MKSP1381*
MKSP2583	*h- leu1-32 ura4-D18 arp8::hygR his7+:P_{Dis1}-gfp-lacI-NLS mmf1:ura4-$lacO_n$ urg1:: loxP-kanR-loxM3*	*arp8* (INO80 complex subunit) deletion mutant with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 7.*	*this study derived from* MKSP2190
MKSP2693	*leu1-32 ura4-D18 cut14::cut14-208^{ts} cut11::cut11-mCherry-natR his7+:P_{Dis1}-gfp-lacI-NLS pfl5:ura4-$lacO_n$*	Temperature-sensitive mutant of Cut14 condensin subunit with *lacO* array (5.6 kb) integrated near *pfl5* locus. *Fig. 3b.*	*this study derived from* MY1983
MKSP2760	*leu1-32 ura4-D18 cut14::cut14-208^{ts} his7+:P_{Dis1}-gfp-lacI-NLS mmf1:ura4-$lacO_n$*	Temperature-sensitive mutant of Cut14 condensin subunit with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 3a, 5b.*	*this study derived from* MY1997
MKSP2801	*leu1-32 ura4-D18 mis4::mis4-242^{ts} his7+:P_{Dis1}-gfp-lacI-NLS cut11::cut11-mCherry-natR mmf1:ura4-$lacO_n$*	Temperature-sensitive mutant of Mis4 cohesin loading factor with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 3a.*	*this study derived from* MY3655 and *MKSP1340*
MKSP3021	*leu1-32 ura4-D18 arp9::kanR his7+:P_{Dis1}-gfp-lacI-NLS mmf1:ura4-$lacO_n$*	*arp9* (SWI/RSC complexes subunit) deletion with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 7a.*	*this study derived from* MKSP2352
MKSP3053	*leu1-32 ura4-D18 arp8::kanR cut14::cut14-208^{ts} his7+:P_{Dis1}-gfp-lacI-NLS mmf1:ura4-$lacO_n$*	Double mutant with *arp8* deletion and temperature-sensitive mutant of Cut14 condensin subunit with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 7b.*	*this study derived from* MKSP2187

MKSP3660	*leu1-32* *cut14::cut14-208^{ts}* *his7+:P_{Dis1}-gfp-lacI-NLS* *mmf1:ura4-lacO$_n$* *rad21::rad21-XTEN17-3sAID-kanR* *ura4::ura4-P_{adh1}-OsTir1-F74A*	Double mutant bearing temperature-sensitive allele of Cut14 condensin subunit and rad21 cohesin subunit tagged with auxin-inducible degron with *lacO* array (10.3 kb) integrated near *mmf1* locus. *Fig. 5b*	*this study derived from* DY48569
Random lacO *position*			
MKSP3117	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *random locus:ura4-lacO$_n$*	WT strain with *lacO* array (10.3 kb) integrated at random locus of the genome (noted as "site1"), and with LacI-GFP (with nucleus localization signal) under control of the constitutive promoter. *Fig. 1b*, *Fig. S1a.*	*derived from* MKSP1120
MKSP3118	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *random locus:ura4-lacO$_n$*	Same as MKSP3117 but with a different random *lacO* array position (noted as "site2"). *Fig. 1b, Fig. S1a.*	*derived from* MKSP1120
MKSP3119	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *random locus:ura4-lacO$_n$*	Same as MKSP3117 but with a different random *lacO* array position (noted as "site3"). *Fig. 1b, Fig. S1a.*	*derived from* MKSP1120
MKSP3123	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *random locus:ura4-lacO$_n$*	Same as MKSP3117 but with a different random *lacO* array position (noted as "site4"). *Fig. 1b, Fig. S1a.*	*derived from* MKSP1120
Labeled nuclear envelope strain			
MKSP3140	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *cut11::cut11-mCherry-natR* *isd90: ura4-lacO$_n$*	WT strain with *lacO* array (10 kb) integrated near *isd90* locus (at *ChrII: 1950422*). *Fig. S2*.	
Auxiliary strains			
AW563	*h− leu1-32* *urg1:loxP-kanR-loxM3$_6$*	WT strain with recombination-mediated cassette exchange system (*RMCE$_{kanMX6}$*).	[2]
DY48569	*h+ mat1PΔ17* *leu1-32 lys1-131 ade6-M216* *ura4::ura4-P_{adh1}-OsTir1-F74A*	Strain bearing an auxin-inducible degron system	*YGRC* FY39923 [3]
JM210 FN198 MKSP1116	*h- leu1-32 ura4-D18 ade-* *cut11::cut11-mCherry-natMX6*	WT strain with Cut11-mCherry (a nuclear envelope marker) expression controlled by the native promoter.	*Nurse lab*
MKSP1120	*h+ leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS*	WT strain with LacI-GFP (with nucleus localization signal) under control of the constitutive promoter. Used to generate random *lacO* array integrations.	
MKSP1340	*h- leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *cut11::cut11-mCherry-natR* *mmf1:ura4-lacO$_n$* *ChrII:3442981::ura4-lacO$_n$*	WT strain with *lacO* array (10.3 kb) integrated near *mmf1* locus and with LacI-GFP (with nucleus localization signal) under control of the constitutive promoter	*this study similar to* MKSP1381
MKSP1381	*h- leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *ChrII:3442981::ura4-10.3kbLacO* *ChrII:3446249::HOcs-hphMX6* *cut11::cut11-mCherry-natMX6*	Original *lacO* array (10.3 kb) integration near *mmf1* locus. Used to generate all other *mmf1-lacO$_n$* strains in this study	[4]
MKSP1128	*h- leu1-32 ura4-D18* *his7+:P_{Dis1}-gfp-lacI-NLS* *cut11::cut11-mCherry-natMX6*	WT strain with Cut11-mCherry (a nuclear envelope marker) expression controlled by the native promoter and with LacI-GFP (with nucleus localization signal) under control of the constitutive promoter.	
MKSP2187	*h- leu1-32 ura4-D18* *arp8::kanR*	*arp8* deletion.	*this study*
MKSP2190	*h- leu1-32 ura4-D18* *arp8::hygR*	*arp8* deletion.	*this study*

MKSP2352	*h- leu1-32 ura4-D18 arp9::kanR*	*arp9* deletion.	*this study*
MY1983	*h- ura4 cut14::cut14-208^{ts}*	Strain encoding for the temperature sensitive allele of *cut14*.	*YGRC* FY9883
MY1997	*h+ cut14::cut14-208^{ts}*	Strain encoding for the temperature sensitive allele of *cut14*.	*YGRC* FY9897
MY3655	*h- leu1-32 mis4::mis4-242^{ts}*	Strain encoding for the temperature sensitive allele of *mis4*.	*YGRC* FY111578
PN10127	*h- sad1::sad1-dsRed-leu+*	WT strain with Sad1-dsRed (a spindle pole body marker) expression controlled by the native promoter.	
PX342	*h+ leu1-32 ade6-M216 bub1::ura4 cen2-lacO$_n$*	Strain with *cen2* locus (centromere of ChrII) containing *lacO* array insertion.	*YGRC* FY13812 [5]
S. cerevisiae			
PCCPL645	*W303 LacI-GFP::HIS*	W303 strain with *LacI-GFP* (with nucleus localization signal) under control of the constitutive promoter.	[6]
PCCPL835	*W303 LacI-GFP::HIS ENA-lacO$_n$::TRP1*	W303 strain with *lacO* array integrated near *ENA* locus and with *LacI-GFP* (with nucleus localization signal) under control of the constitutive promoter. *Fig. S1b.*	*this study derived from PCCPL 645 as described* [6] and [7]

Table S3 References:

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