Cohesin association along fission yeast chromosome 2. (a) Association profile of cohesin along chromosome 2. cdc25-22 cells were arrested in G2 by shifting to the restrictive temperature of 36.5° C for six hours in minimal EMM2 medium and processed for ChIP against the epitope-tagged cohesin subunit Rad21-Pk<sub>9</sub>. The binding profile of Rad21-Pk<sub>9</sub> along fission yeast chromosome 2, relative to a whole genome DNA sample, is shown. The yaxis scale is log<sub>2</sub>. Every bar represents the average of 11 oligonucleotide probes within 250 bp windows. Peaks in solid red were assigned according to the following criteria: Signal intensities were smoothed using a sliding 2.25 kb window. Local maxima were identified, and those with a raw signal intensity above 0.3 were chosen as a peak. Peaks extend from their maximum to both sides until the raw signal log, ratio reaches a value below 0. The peak position was defined as the midpoint within the peak. Using these criteria 228 peaks were identified along chromosome 2. The illustrated cohesin binding map shows all open reading frames (ORFs) as grey bars above and below the midline, transcribed from left to right and right to left, respectively. tRNA genes, and genes encoding protein components of the large (rpl) and small (rps) ribosomal subunit are highlighted in red. The coordinates are of the NCBI fission yeast genome release NC003423.1 (25 Jan 2005). (b) List of the cohesin peaks identified in (a). The position, average height and width of each of the 228 peaks is indicated.









Chromosome 2 (1600000-1700000)







Chromosome 2 (2000000-2100000)









Chromosome 2 (3200000-3300000)





Chromosome 2 (4000000-4100000)



## (b)

Peak No	Midpoint/bp	Avg height	Width/bp	Peak No	Midpoint/bp	Avg height
1	7251	0,74	13000	115	2320501	0,21
2	28251	0,76	6000	116	2363626	0,41
3	57751	0,62	3500	117	2375251	0,91
4	79751	1,11	9000	118	2391251	0,41
5	137876	0,57	5750	119	2404626	0,89
6	154376	0,85	6250	120	2418501	0,61
7	177501	1,19	4000	121	2440751	0,55
8	189751	0,63	5000	122	2447126	0,36
9	204001	0,84	5000	123	2457376	0,98
10	213501	0,30	6000	124	2474501	0,54
11	237126	0,42	6250	125	2514126	0,88
12	246126	0,78	1750	126	2536126	0,63
13	256751	0,95	5500	127	2548001	0,74
14	270501	0,46	5500	128	2565501	0,96
15	286876	0,51	4750	129	2572501	0,64
16	317876	0,80	7250	130	2578126	0,65
17	343751	0,93	6500	131	2594251	0,89
18	356376	0,66	6750	132	2604126	0,72
19	390376	0,63	10250	133	2613001	0,43
20	428626	0,53	4250	134	2639126	0,33
21	438626	0,78	4250	135	2657126	0,39
22	450501	0,57	5500	136	2674876	0,80
23	466876	0,70	11750	137	2714876	0,65
24	494376	0,30	750	138	2741501	0,54
25	523251	0,96	7500	139	2748751	0,50
26	541876	0,28	3250	140	2783126	0,63
27	564001	0,75	3500	141	2795001	1,00
28	572751	0,39	4000	142	2805376	0,59
29	588001	0,71	5000	143	2834251	0,91
30	636626	0,48	3250	144	2850876	0,55
31	639501	0,43	1000	145	2856751	0,40
32	652126	0,49	2750	146	2865626	0,64
33	657751	0,50	3500	147	2896751	1,20
34	676751	0,97	7000	148	2938376	0,89
35	719876	0,68	6250	149	2949501	0,27
36	729501	1,06	6000	150	2962501	0,35
37	784876	0,98	8250	151	2982251	1,05
38	793501	0,32	3500	152	3000751	0,78
39	830751	0,81	11000	153	3023751	0,53
40	850001	0,93	7500	154	3048376	0,78
41	865126	0,28	1750	155	3060126	0,49
42	875376	0,61	9750	156	3092001	0,69
43	888751	0,29	4000	157	3102751	0,51

Width/bp

2000

9250 4500

4000 8250

9000

5000

2250

12250

4500

10250

3250

5500

8000 1500

5750

8500

8250

5500

2750

3250

11750

11250

7500

1500

5250

9000

9250

7000

4250

500

5250

6500

8250

3000 4000

7500

6000

3500

14750

6250 5500

4000

	001751	1 00	7500	150	0100051	0.77	1000
44	921/51	1,00	/500	158	3132251	0,//	4000
45	935626	0,55	5750	159	3142251	0,83	9000
46	942501	0,74	7000	160	3154376	0,23	4750
47	958126	0,51	2750	161	3165376	0,73	6250
48	978251	0,26	2500	162	3178376	0,63	8750
49	989376	0,28	2250	163	3231251	0,89	15500
50	1007126	0,86	11250	164	3257751	0,29	2500
51	1041251	0,59	13500	165	3266251	0,62	4500
52	1062751	0,65	4000	166	3277876	0,98	6250
53	1079751	0,76	6000	167	3299751	0,23	2500
54	1089126	0,44	8750	168	3312376	0,79	6750
55	1104376	0,48	5250	169	3323001	0,87	8000
56	1140376	0,44	4750	170	3350626	0,62	5250
57	1154876	0,95	8250	171	3366126	0,88	6250
58	1169626	0,51	4250	172	3400251	0,93	6000
59	1193501	0,52	5500	173	3441001	1,00	8000
60	1199251	0,35	4000	174	3462876	1,05	6250
61	1213251	0,70	6000	175	3488501	0,20	1500
62	1229376	0,30	1250	176	3500751	0,76	12500
63	1236876	0,61	5250	177	3525876	0,78	8250
64	1262501	0,81	7000	178	3537876	0,31	4750
65	1283126		250	179	3551001	0,61	6500
66	1290876	0,81	6250	180	3570251	0,93	4000
67	1349501	0,65	4500	181	3579501	0,35	5000
68	1378501	0,48	7000	182	3593501	0,71	6000
69	1407876	0,80	3750	183	3599001	0,10	500
70	1433376	0,94	4250	184	3604751	0,91	5500
71	1494251	0,61	8000	185	3613501	0,31	2500
72	1508251	0,76	7500	186	3616626	0,20	2250
73	1515876	0,64	2750	187	3652001	0,84	9500
74	1527751	0,38	2500	188	3671376	0,67	5750
75	1535376	0,37	2750	189	3694626	0,71	11250
76	1558126	1,24	13250	190	3703876	0,61	4750
77	1571876	0,71	12750	191	3720876	0,80	12750
78	1600001	1,17	23000	192	3748376	0,49	4750
79	1633626	0,73	7250	193	3766751	0,38	1500
80	1654626	0,74	8750	194	3773376	0,85	750
81	1668501	0.79	5000	195	3779001	0.99	7500
82	1678126	0,61	5750	196	3802376	0,49	3750
83	1692251	0,40	1000	197	3829501	0,91	8500
84	1698876	0.94	7750	198	3867126	0.97	6250
85	1733751	1 03	4000	199	3879126	0.58	6250
86	1741876	0.52	5250	200	3890751	0.53	6000
87	1759751	0.40	2500	200	3897876	0.53	1250
88	1787126	0.61	4750	202	3908876	0.93	9750
89	1792376	0.64	4750	202	3916876	0.40	750
	11/4010	0,04	1,50	205	5710070	0,70	, 50

90	1805876	0,90	7750	204	3933376	0,34	2250
91	1842501	0,97	6000	205	3947876	0,40	2750
92	1869876	0,23	1250	206	3960501	0,83	16500
93	1887626	0,65	6750	207	3998876	0,99	6250
94	1933251	1,16	4500	208	4026376	1,10	6750
95	1946126	0,63	5750	209	4044001	1,06	5500
96	1976126	0,86	5250	210	4062501	0,68	9000
97	1987626	0,43	3250	211	4072501	0,79	6500
98	1991251	0,40	1000	212	4110751	0,87	9000
99	2011501	0,58	8000	213	4132751	0,95	8000
100	2019876	0,49	2250	214	4168501	0,73	2000
101	2035376	0,50	3250	215	4177001	0,61	10500
102	2041001	0,60	1000	216	4191626	0,56	6250
103	2078501	1,13	71500	217	4219251	0,70	6500
104	2127001	0,86	13500	218	4234626	0,43	6250
105	2141126	0,53	2250	219	4257001	0,87	8000
106	2149626	1,00	3750	220	4279501	0,97	8500
107	2174251	0,87	6500	221	4300126	0,40	4250
108	2188626	0,42	9250	222	4312001	0,85	5000
109	2210126	0,61	5250	223	4334376	1,01	10250
110	2222876	0,51	5750	224	4388251	0,57	10500
111	2250626	0,90	13250	225	4416126	0,80	15750
112	2268751	0,63	4000	226	4427751	0,39	2000
113	2292876	0,64	7750	227	4435376	0,63	10750
114	2303751	0,80	13000	228	4455251	0,81	25000

Maximal distance analysis between neighboring cohesin peaks. 10,000 random cohesin patterns, distributed among the convergent sites along fission yeast chromosome 2, were created using a bootstrapping approach. The number and widths of the distributed cohesin peaks were those from the observed binding pattern, as assigned in Figure S1. The maximal distance between neighboring cohesin peaks in each of the random distributions was determined and is shown in the histogram below. The red line indicates the maximal distance between two neighboring cohesin-bound convergent sites in the actual observed cohesin pattern.



Histogram of maximal distances from 10,000 simulations

Higher expression levels of convergent gene pairs flanking cohesin binding sites. The distribution of relative mRNA levels from genes flanking cohesin-bound and cohesin-free convergent sites along chromosome 2 is shown. Boxes indicate boundaries of the 25<sup>th</sup> to the 75<sup>th</sup> percentile. The median is shown as a bold black line, whiskers extend to 1.5 times the interquartile range, outliers are marked as circles. A Wilcoxon signed ranks test suggests that genes flanking cohesin-bound convergent sites are more strongly expressed than genes flanking cohesin-free convergent sites (p=0.011).



The cohesin loader Mis4/Ssl3 overlaps with tRNA and ribosomal protein genes. (a) Binding profile of Mis4 and Ssl3 along fission yeast chromosome 2. Chromatin immunoprecipitation was performed against epitope-tagged Mis4-Pk<sub>9</sub> and Ssl3-Pk<sub>9</sub> from exponentially proliferating cells. As a control, cells without epitope-tagged protein were grown under identical conditions and processed in parallel for chromatin immunoprecipitation with an  $\alpha$ -Pk antibody. The map shows an overlay of Mis4 (green), Ssl3 (blue) and the untagged control (purple), as described in Figure S1. Peaks were assigned as detailed in Figure S1, but the threshold for local maxima was a signal intensity of 0.5. The untagged control sample yielded trace amounts of immunoprecipitated DNA, which after amplification led to several strong peaks often in intergenic low complexity regions. Several of these peaks overlapped with Mis4/Ssl3 peaks, which were excluded from the analysis. Peaks in low complexity regions were not observed in chromatin immunoprecipitates of cohesin subunits (compare Figure S1). (b) Table of the 72 specific Mis4 peaks assigned as described in (a) along chromosome 2. The position, average height and width of each of the peaks is indicated. The actual number of Mis4 bindings sites might be higher, as some peaks in low complexity regions that have been removed from the analysis may be significant, while others may have remained below recognition by our peak picking parameters. (c) The distances of the 72 Mis4 binding sites to the nearest tRNA gene (blue) is shown. 16 lie within 5 kb. The distances of the remaining 56 peaks to the nearest ribosomal protein gene (red) are also shown, of which 18 lie again within 5 kb.











Chromosome 2 (1600000–1700000)



Chromosome 2 (2000000-2100000)







Chromosome 2 (2800000-2900000)









# (b)

Peak No	Midpoint/bp	Avg height	Width/bp
1	12251	0,53	3500
2	101876	0,47	3750
3	136001	0,55	3000
4	178751	1,11	2500
5	205376	0,82	1750
6	246126	0,75	2250
7	332876	0,63	4250
8	385501	0,82	4000
9	490001	0,60	3500
10	496876	0,85	2250
11	636001	0,80	4500
12	656376	0,48	3250
13	675126	0,63	2750
14	803751	0,77	2000
15	1001126	0,71	4750
16	1157376	0,50	4750
17	1298626	0,60	3250
18	1462001	0,53	3000
19	1471626	0,83	1750
20	1505376	0,73	3250
21	1516376	0,88	2750
22	1610001	0,63	3000
23	1667001	0,74	3000
24	1686376	0,66	2250
25	1692626	0,86	2250
26	1734501	0,64	2500
27	1751626	1,16	5250
28	1785376	0,90	2750
29	1847001	0,77	2500
30	2005001	1,18	2500
31	2019626	0,63	2250
32	2042001	0,97	5000
33	2065001	0,51	3000
34	2073501	0,90	2500
35	2080876	0,68	2250
36	2091251	0,96	11500

r	r		
Peak No	Midpoint/bp	Avg height	Width/bp
37	2104126	0,68	10750
38	2121626	0,58	3250
39	2140876	0,61	2750
40	2249501	0,64	2500
41	2278126	0,85	1750
42	2307751	1,03	2000
43	2338751	0,57	2500
44	2375626	0,58	2750
45	2408001	0,56	3000
46	2546876	0,73	8250
47	2738876	0,92	3250
48	2857376	0,53	3750
49	2899126	0,63	2750
50	2979376	1,00	1750
51	3113751	0,86	3500
52	3132376	0,73	2750
53	3204626	0,72	2250
54	3234751	0,45	3500
55	3322126	0,73	6750
56	3351751	0,64	3500
57	3465501	0,59	4000
58	3613251	0,55	3000
59	3777751	0,70	2500
60	3833126	0,98	1750
61	3881376	0,54	2750
62	3898001	0,79	2000
63	3916876	0,79	3750
64	4005251	0,51	4000
65	4047751	0,94	5500
66	4057251	0,58	4500
67	4066501	0,64	2500
68	4197876	1,02	2750
69	4243876	1,11	2750
70	4311126	0,55	2750
71	4345626	0,64	2750
72	4396376	1,13	1750



(c)

Similar Mis4 localization in exponentially growing and in G1 arrested cells. The Mis4 localization pattern along chromosome 2 in exponentially growing cells is compared to the pattern observed in cells arrested in G1 using the *cdc10-129* temperature sensitive mutation. (**a**) Exponentially growing *cdc10-129* cells were shifted to the restrictive temperature of 37°C for 3.5 hours. G1 arrest was confirmed by flow cytometry of DNA content. (**b**) Chromatin immunoprecipitation was performed against epitope-tagged Mis4-Pk<sub>9</sub> from the G1 arrested cells 3.5 hours after temperature shift. The association pattern along chromosome 2 is shown (brown) in comparison to that observed in exponentially growing cells that are predominantly in the G2 phase of the cell cycle (geen, data reproduced from Figure S4).

(a)

















Chromosome 2 (2000000-2100000)









tRNI fkb**ra9**414 abp2 Û. cki1 -5<sup>I</sup> 4000000 Chromosome 2 (4100000-4200000) rhp9 HUMP pgk1 tRNA -5| | | 4100000 I 4180000 | 4110000 Chromosome 2 (4200000-4300000) skh 1 4250000 4270000 4280000 4290000 Chromosome 2 (4300000-4400000) chp2rp132-1 uap2spi1pob1 rpa42 -5<mark>1</mark> 4300000

4360000 4370000



Mis4/Ssl3 binding sites correlate with strongly expressed genes. This graph compares the relative mRNA levels of ribosomal protein genes, Mis4-bound genes other than ribosomal protein genes, and all remaining genes that are not associated with Mis4 along chromosome 2. tRNA genes were excluded from this analysis. Box boundaries mark the 25<sup>th</sup> to 75<sup>th</sup> percentile surrounding the median (bold line). Whiskers extend to 1.5 times the interquartile range. Outliers are indicated as circles. Wilcoxon signed ranks tests suggest that Mis4-bound genes are significantly more strongly expressed than non-bound genes.



The cohesin pattern along chromosome arms remains qualitatively unchanged during cohesin removal in mitosis. Cells were arrested in G2 and synchronously released into mitotic progression. Chromatin immunoprecipitation was performed against the Rad21-Pk<sub>9</sub> cohesin subunit. A 100 kb region on the left arm of chromosome 2 in G2-arrested cells is compared to the patterns at the indicated timepoints after release when the majority of cells were in metaphase and in anaphase, respectively (compare Figure 7).



### Table S1

Yeast strains used in this study.

Strain	Genotype	Usage
Y252	h <sup>-</sup> leu1-32 nda3-KM311 cnd2-Pk <sub>9</sub> -kanMX6	Fig. 3
Y2197	$h^{90}$ leu1-32 ade6-M216 rad21-HA <sub>3</sub> -kanMX6	Fig. 1, A1
Y2447	h <sup>90</sup> leu1-32 psc3-Pk <sub>9</sub> -kanMX6	Fig. 1
Y2460	h <sup>90</sup> leu1-32	Fig. A4
Y2468	h <sup>90</sup> leu1-32 mis4-Pk <sub>9</sub> -kanMX6	Fig. 3, 4, 7
		A4
Y2699	h <sup>-</sup> leu1-32 cdc25-22 rad21-Pk <sub>9</sub> -kanMX6 SV40p-GFP-atb2-LEU2	Fig. 1, 2, 3,
		4, 5, 7, A1, A7
Y2703	h <sup>90</sup> nda3-KM311 rad21-Pk <sub>9</sub> -kanMX6	Fig. 5
Y2828	$h^{-}pds5-Pk_{9}-kanMX6$	Fig. 1
Y2863	h <sup>-</sup> leu1-32 cdc10-129 rad21-Pk <sub>9</sub> -kanMX6	Fig. 2, 7
Y3071	$h^+$ ssl3-Pk <sub>9</sub> -kanMX6	Fig. 3, A4
Y3127	h <sup>-</sup> ura4-D18 rad21-3eGFP-kanMX6 mis4-Pk <sub>9</sub> -kanMX6	Fig. 3
Y3169	h <sup>+</sup> leu1-32 cdc25-22 ssl3-Pk <sub>9</sub> -kanMX6 SV40p-GFP-atb2-LEU2	Fig. 6
Y3250	h <sup>90</sup> leu1-32 cdc25-22 cnd2-Pk <sub>9</sub> -kanMX6 SV40p-GFP-atb2-LEU2	Fig. 6
Y3272	h <sup>-</sup> leu1-32 ura4-D18 cdc25-22 rad21-Pk <sub>9</sub> -kanMX6	
	SV40p-GFP-atb2-LEU2 swi6::ura4 <sup>+</sup>	Fig. 2
Y3298	h <sup>90</sup> leu1-32 ura4-D18 his3-D1 lys1 ade6-M210 mis4-Pk <sub>9</sub> -kanMX6	
	mis6-GFP-LEU2	Fig. 6
Y3300	h <sup>90</sup> leu1-32 ura4-D18 rad21-Pkg-kanMX6 mis6-GFP-LEU2	Fig. 5
Y3303	h <sup>90</sup> leu1-32 his3-D1 cut14-Pk <sub>9</sub> -kanMX6 mis6-GFP-LEU2	Fig. 6
Y3350	h <sup>90</sup> leu1-32 mis4-Pk <sub>9</sub> -kanMX6 fhl1-GFP-hygMX6	Fig. 3
Y3351	h <sup>90</sup> leu1-32 mis4-Pk <sub>9</sub> -kanMX6 sfc3-GFP-hygMX6	Fig. 3
Y3359	h <sup>90</sup> leu1-32 sfc6-Pk <sub>9</sub> -kanMX6	Fig. 3
Y3384	h <sup>90</sup> leu1-32 mis4-GFP-hygMX6 cnd2-Pk <sub>9</sub> -kanMX6	Fig. 3
Y3385	h <sup>-</sup> leu1-32 mis4-GFP-hygMX6 ssl3-Pk <sub>9</sub> -kanMX6	Fig. 3
Y3403	h <sup>90</sup> leu1-32 fhl1-Pk <sub>9</sub> -kanMX6	Fig. 3
Y3586	h leu1-32 ark1-as3-hygMX6 cdc25-22 rad21-Pk9-kanMX6	
	SV40p-GFP-atb2-LEU2	Fig. 7
Y3650	h <sup>-</sup> leu1-32 ura4-D18 ade6-M210 mis4-Pkg-kanMX6	
	$SPBTRNAGLY.05\Delta SPBTRNAARG.04\Delta$	Fig. 4
Y3724	h <sup>90</sup> leu1-32 ura4-D18 cdc25-22 rad21-Pk <sub>9</sub> -kanMX6	
	$SPBTRNAGLY.05\Delta SPBTRNAARG.04\Delta$	Fig. 4
Y3863	h <sup>90</sup> leu1-32 mis4-Pk <sub>9</sub> -kanMX6 cdc10-12	Fig. A5

Generation of these strains was based on previously described strains and constructs: *cdc25-22* [54], *nda3-KM311* [55], *cdc10-129* [56], *SV40p-GFP-atb2-LEU2* [57], and *ark1-as3* [41]. A *mis6-GFP-LEU2* strain was a kind gift from Kazu Tomita.