**Figure S1**
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-Pk9. The binding profile of Rad21-Pk9 along fission yeast chromosome 2, relative to a whole genome DNA sample, is shown. The y-axis scale is log2. 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 log2 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.
| Peak No | Midpoint/bp | Avg height | Width/bp |
|---------|-------------|------------|----------|
| 1       | 7251        | 0,74       | 13000    |
| 2       | 28251       | 0,76       | 6000     |
| 3       | 57751       | 0,62       | 3500     |
| 4       | 79751       | 1,11       | 9000     |
| 5       | 137876      | 0,57       | 5750     |
| 6       | 154376      | 0,85       | 6250     |
| 7       | 177501      | 1,19       | 4000     |
| 8       | 189751      | 0,63       | 5000     |
| 9       | 204001      | 0,84       | 5000     |
| 10      | 213501      | 0,30       | 6000     |
| 11      | 237126      | 0,42       | 6250     |
| 12      | 246126      | 0,78       | 1750     |
| 13      | 256751      | 0,95       | 5500     |
| 14      | 270501      | 0,46       | 5500     |
| 15      | 286876      | 0,51       | 4750     |
| 16      | 317876      | 0,80       | 7250     |
| 17      | 343751      | 0,93       | 6500     |
| 18      | 356376      | 0,66       | 6750     |
| 19      | 390376      | 0,63       | 10250    |
| 20      | 428626      | 0,53       | 4250     |
| 21      | 438626      | 0,78       | 4250     |
| 22      | 450501      | 0,57       | 5500     |
| 23      | 466876      | 0,70       | 11750    |
| 24      | 494376      | 0,30       | 750      |
| 25      | 523251      | 0,96       | 7500     |
| 26      | 541876      | 0,28       | 3250     |
| 27      | 564001      | 0,75       | 3500     |
| 28      | 572751      | 0,39       | 4000     |
| 29      | 588001      | 0,71       | 5000     |
| 30      | 636626      | 0,48       | 3250     |
| 31      | 639501      | 0,43       | 1000     |
| 32      | 652126      | 0,49       | 2750     |
| 33      | 657751      | 0,50       | 3500     |
| 34      | 676751      | 0,97       | 7000     |
| 35      | 719876      | 0,68       | 6250     |
| 36      | 729501      | 1,06       | 6000     |
| 37      | 784876      | 0,98       | 8250     |
| 38      | 793501      | 0,32       | 3500     |
| 39      | 830751      | 0,81       | 11000    |
| 40      | 850001      | 0,93       | 7500     |
| 41      | 865126      | 0,28       | 1750     |
| 42      | 875376      | 0,61       | 9750     |
| 43      | 888751      | 0,29       | 4000     |

| Peak No | Midpoint/bp | Avg height | Width/bp |
|---------|-------------|------------|----------|
| 115     | 2320501     | 0,21       | 2000     |
| 116     | 2363626     | 0,41       | 9250     |
| 117     | 2375251     | 0,91       | 4500     |
| 118     | 2391251     | 0,41       | 4000     |
| 119     | 2404626     | 0,89       | 8250     |
| 120     | 2418501     | 0,61       | 9000     |
| 121     | 2440751     | 0,55       | 5000     |
| 122     | 2447126     | 0,36       | 2250     |
| 123     | 2457376     | 0,98       | 12250    |
| 124     | 2474501     | 0,54       | 4500     |
| 125     | 2514126     | 0,88       | 10250    |
| 126     | 2536126     | 0,63       | 3250     |
| 127     | 2548001     | 0,74       | 5500     |
| 128     | 2565501     | 0,96       | 8000     |
| 129     | 2572501     | 0,64       | 1500     |
| 130     | 2578126     | 0,65       | 5750     |
| 131     | 2594251     | 0,89       | 8500     |
| 132     | 2604126     | 0,72       | 8250     |
| 133     | 2613001     | 0,43       | 5500     |
| 134     | 2639126     | 0,33       | 2750     |
| 135     | 2657126     | 0,39       | 3250     |
| 136     | 2674876     | 0,80       | 11750    |
| 137     | 2714876     | 0,65       | 11250    |
| 138     | 2741501     | 0,54       | 7500     |
| 139     | 2748751     | 0,50       | 1500     |
| 140     | 2783126     | 0,63       | 5250     |
| 141     | 2795001     | 1,00       | 9000     |
| 142     | 2805376     | 0,59       | 9250     |
| 143     | 2834251     | 0,91       | 7000     |
| 144     | 2850876     | 0,55       | 4250     |
| 145     | 2856751     | 0,40       | 500      |
| 146     | 2865626     | 0,64       | 5250     |
| 147     | 2896751     | 1,20       | 6500     |
| 148     | 2938376     | 0,89       | 8250     |
| 149     | 2949501     | 0,27       | 3000     |
| 150     | 2962501     | 0,35       | 4000     |
| 151     | 2982251     | 1,05       | 7500     |
| 152     | 3000751     | 0,78       | 6000     |
| 153     | 3023751     | 0,53       | 3500     |
| 154     | 3048376     | 0,78       | 14750    |
| 155     | 3060126     | 0,49       | 6250     |
| 156     | 3092001     | 0,69       | 5500     |
| 157     | 3102751     | 0,51       | 4000     |
| 44 | 921751  | 1,00 | 7500  |
| 45 | 935626  | 0,55 | 5750  |
| 46 | 942501  | 0,74 | 7000  |
| 47 | 958126  | 0,51 | 2750  |
| 48 | 978251  | 0,26 | 2500  |
| 49 | 989376  | 0,28 | 2250  |
| 50 | 1007126 | 0,86 | 11250 |
| 51 | 1041251 | 0,59 | 13500 |
| 52 | 1062751 | 0,65 | 4000  |
| 53 | 1079751 | 0,76 | 6000  |
| 54 | 1089126 | 0,44 | 8750  |
| 55 | 1104376 | 0,48 | 5250  |
| 56 | 1140376 | 0,44 | 4750  |
| 57 | 1154876 | 0,95 | 8250  |
| 58 | 1169626 | 0,51 | 4250  |
| 59 | 1193501 | 0,52 | 5500  |
| 60 | 1199251 | 0,35 | 4000  |
| 61 | 1213251 | 0,70 | 6000  |
| 62 | 1229376 | 0,30 | 1250  |
| 63 | 1236876 | 0,61 | 5250  |
| 64 | 1262501 | 0,81 | 7000  |
| 65 | 1283126 | 0,24 | 250   |
| 66 | 1290876 | 0,81 | 6250  |
| 67 | 1349501 | 0,65 | 4500  |
| 68 | 1378501 | 0,48 | 7000  |
| 69 | 1407876 | 0,80 | 3750  |
| 70 | 1433376 | 0,94 | 4250  |
| 71 | 1494251 | 0,61 | 8000  |
| 72 | 1508251 | 0,76 | 7500  |
| 73 | 1515876 | 0,64 | 2750  |
| 74 | 1527751 | 0,38 | 2500  |
| 75 | 1535376 | 0,37 | 2750  |
| 76 | 1558126 | 1,24 | 13250 |
| 77 | 1571876 | 0,71 | 12750 |
| 78 | 1600001 | 1,17 | 23000 |
| 79 | 1633626 | 0,73 | 7250  |
| 80 | 1654626 | 0,74 | 8750  |
| 81 | 1668501 | 0,79 | 5000  |
| 82 | 1678126 | 0,61 | 5750  |
| 83 | 1692251 | 0,40 | 1000  |
| 84 | 1698876 | 0,94 | 7750  |
| 85 | 1733751 | 1,03 | 4000  |
| 86 | 1741876 | 0,52 | 5250  |
| 87 | 1759751 | 0,40 | 2500  |
| 88 | 1787126 | 0,61 | 4750  |
| 89 | 1792376 | 0,64 | 4750  |
| 158| 3132251 | 0,77 | 4000  |
| 159| 3142251 | 0,83 | 9000  |
| 160| 3154376 | 0,23 | 4750  |
| 161| 3165376 | 0,73 | 6250  |
| 162| 3178376 | 0,63 | 8750  |
| 163| 3231251 | 0,89 | 15500 |
| 164| 3257751 | 0,29 | 2500  |
| 165| 3266251 | 0,62 | 4500  |
| 166| 3277876 | 0,98 | 6250  |
| 167| 3299751 | 0,23 | 2500  |
| 168| 3312376 | 0,79 | 6750  |
| 169| 3323001 | 0,87 | 8000  |
| 170| 3350626 | 0,62 | 5250  |
| 171| 3366126 | 0,88 | 6250  |
| 172| 3400251 | 0,93 | 6000  |
| 173| 3441001 | 1,00 | 8000  |
| 174| 3462876 | 1,05 | 6250  |
| 175| 3488501 | 0,20 | 1500  |
| 176| 3500751 | 0,76 | 12500 |
| 177| 3525876 | 0,78 | 8250  |
| 178| 3537876 | 0,31 | 4750  |
| 179| 3551001 | 0,61 | 6500  |
| 180| 3570251 | 0,93 | 4000  |
| 181| 3579501 | 0,35 | 5000  |
| 182| 3593501 | 0,71 | 6000  |
| 183| 3599001 | 0,10 | 500   |
| 184| 3604751 | 0,91 | 5500  |
| 185| 3613501 | 0,31 | 2500  |
| 186| 3616626 | 0,20 | 2250  |
| 187| 3652001 | 0,84 | 9500  |
| 188| 3671376 | 0,67 | 5750  |
| 189| 3694626 | 0,71 | 11250 |
| 190| 3703876 | 0,61 | 4750  |
| 191| 3720876 | 0,80 | 12750 |
| 192| 3748376 | 0,49 | 4750  |
| 193| 3766751 | 0,38 | 1500  |
| 194| 3773376 | 0,85 | 750   |
| 195| 3779001 | 0,99 | 7500  |
| 196| 3802376 | 0,49 | 3750  |
| 197| 3829501 | 0,91 | 8500  |
| 198| 3867126 | 0,97 | 6250  |
| 199| 3879126 | 0,58 | 6250  |
| 200| 3890751 | 0,53 | 6000  |
| 201| 3897876 | 0,53 | 1250  |
| 202| 3908876 | 0,93 | 9750  |
| 203| 3916876 | 0,40 | 750   |
|   |       |       |   |   |
|---|-------|-------|---|---|
| 90| 1805876| 0,90| 7750|   |
| 91| 1842501| 0,97| 6000|   |
| 92| 1869876| 0,23| 1250|   |
| 93| 1887626| 0,65| 6750|   |
| 94| 1933251| 1,16| 4500|   |
| 95| 1946126| 0,63| 5750|   |
| 96| 1976126| 0,86| 5250|   |
| 97| 1987626| 0,43| 3250|   |
| 98| 1991251| 0,40| 1000|   |
| 99| 2011501| 0,58| 8000|   |
| 100|2019876| 0,49| 2250|   |
| 101|2035376| 0,50| 3250|   |
| 102|2041001| 0,60| 1000|   |
| 103|2078501| 1,13| 71500|   |
| 104|2127001| 0,86| 13500|   |
| 105|2141126| 0,53| 2250|   |
| 106|2149626| 1,00| 3750|   |
| 107|2174251| 0,87| 6500|   |
| 108|2188626| 0,42| 9250|   |
| 109|2210126| 0,61| 5250|   |
| 110|2228786| 0,51| 5750|   |
| 111|2250626| 0,90| 13250|   |
| 112|2268751| 0,63| 4000|   |
| 113|2292876| 0,64| 7750|   |
| 114|2303751| 0,80| 13000|   |
| 204|3933376| 0,34| 2250|   |
| 205|3947876| 0,40| 2750|   |
| 206|3960501| 0,83| 16500|   |
| 207|3998876| 0,99| 6250|   |
| 208|4026376| 1,10| 6750|   |
| 209|4040001| 1,06| 5500|   |
| 210|4062501| 0,68| 9000|   |
| 211|4072501| 0,79| 6500|   |
| 212|4110751| 0,87| 9000|   |
| 213|4132751| 0,95| 8000|   |
| 214|4168501| 0,73| 2000|   |
| 215|4177001| 0,61| 10500|   |
| 216|4191626| 0,56| 6250|   |
| 217|4219251| 0,70| 6500|   |
| 218|4234626| 0,43| 6250|   |
| 219|4257001| 0,87| 8000|   |
| 220|4279501| 0,97| 8500|   |
| 221|4300126| 0,40| 4250|   |
| 222|4312001| 0,85| 5000|   |
| 223|4334376| 1,01| 10250|   |
| 224|4388251| 0,57| 10500|   |
| 225|4416126| 0,80| 15750|   |
| 226|4427751| 0,39| 2000|   |
| 227|4435376| 0,63| 10750|   |
| 228|4455251| 0,81| 25000|   |
Figure S2
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.
Figure S3
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 25th to the 75th 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).
Figure S4
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-Pk9 and Ssl3-Pk9 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 α-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.
| 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    |
Figure S5
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-Pk9 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 (green, data reproduced from Figure S4).
**Figure S6**

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 25th to 75th 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.
**Figure S7**
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-Pk9 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 leu1-32 nda3-KM311 cnd2-Pks-kanMX6 | Fig. 3 |
| Y2197  | h o leu1-32 ade6-M216 rad21-HA3-kanMX6 | Fig. 1, A1 |
| Y2447  | h o leu1-32 psc3-Pks-kanMX6 | Fig. 1 |
| Y2460  | h o leu1-32 | Fig. A4 |
| Y2468  | h o leu1-32 mis4-Pks-kanMX6 | Fig. 3, 4, 7 |
| Y2699  | h leu1-32 cdc25-22 rad21-Pks-kanMX6 SV40p-GFP-atb2-LEU2 | Fig. 1, 2, 3, 4, 5, 7, A1, A7 |
| Y2703  | h o nda3-KM311 rad21-Pks-kanMX6 | Fig. 5 |
| Y2828  | h pds5-Pks-kanMX6 | Fig. 1 |
| Y2863  | h leu1-32 cdc10-129 rad21-Pks-kanMX6 | Fig. 2, 7 |
| Y3071  | h ssl3-Pks-kanMX6 | Fig. 3, A4 |
| Y3127  | h ura4-D18 rad21-3eGFP-kanMX6 mis4-Pks-kanMX6 | Fig. 3 |
| Y3169  | h leu1-32 cdc25-22 ssl3-Pks-kanMX6 SV40p-GFP-atb2-LEU2 | Fig. 6 |
| Y3250  | h o leu1-32 cdc25-22 cnd2-Pks-kanMX6 SV40p-GFP-atb2-LEU2 | Fig. 6 |
| Y3272  | h leu1-32 ura4-D18 cdc25-22 rad21-Pks-kanMX6 SV40p-GFP-atb2-LEU2 swi6::ura4 | Fig. A4 |
| Y3298  | h o leu1-32 ura4-D18 his3-D1 lys1 ade6-M210 mis4-Pks-kanMX6 mis6-GFP-LEU2 | Fig. 2 |
| Y3300  | h o leu1-32 ura4-D18 rad21-Pks-kanMX6 mis6-GFP-LEU2 | Fig. 6 |
| Y3303  | h o leu1-32 his3-D1 cut14-Pks-kanMX6 mis6-GFP-LEU2 | Fig. 6 |
| Y3350  | h o leu1-32 mis4-Pks-kanMX6 fhll1-GFP-hygMX6 | Fig. 3 |
| Y3351  | h o leu1-32 mis4-Pks-kanMX6 sf3-GFP-hygMX6 | Fig. 3 |
| Y3359  | h o leu1-32 sf6-Pks-kanMX6 | Fig. 3 |
| Y3384  | h o leu1-32 mis4-GFP-hygMX6 cnd2-Pks-kanMX6 | Fig. 3 |
| Y3385  | h leu1-32 mis4-GFP-hygMX6 ssl3-Pks-kanMX6 | Fig. 3 |
| Y3403  | h o leu1-32 fhll1-Pks-kanMX6 | Fig. 3 |
| Y3586  | h leu1-32 ark1-as3-hygMX6 cdc25-22 rad21-Pk9-kanMX6 SV40p-GFP-atb2-LEU2 | Fig. 7 |
| Y3650  | h leu1-32 ura4-D18 ade6-M210 mis4-Pks-kanMX6 SPBTRNAGLY.05Δ SPBTRNAARG.04Δ | Fig. 4 |
| Y3724  | h o leu1-32 ura4-D18 cdc25-22 rad21-Pks-kanMX6 SPBTRNAGLY.05Δ SPBTRNAARG.04Δ | Fig. 4 |
| Y3863  | h o leu1-32 mis4-Pks-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 *arkl-as3* [41]. A *mis6-GFP-LEU2* strain was a kind gift from Kazu Tomita.