Supplementary Information:

Supplementary Figures

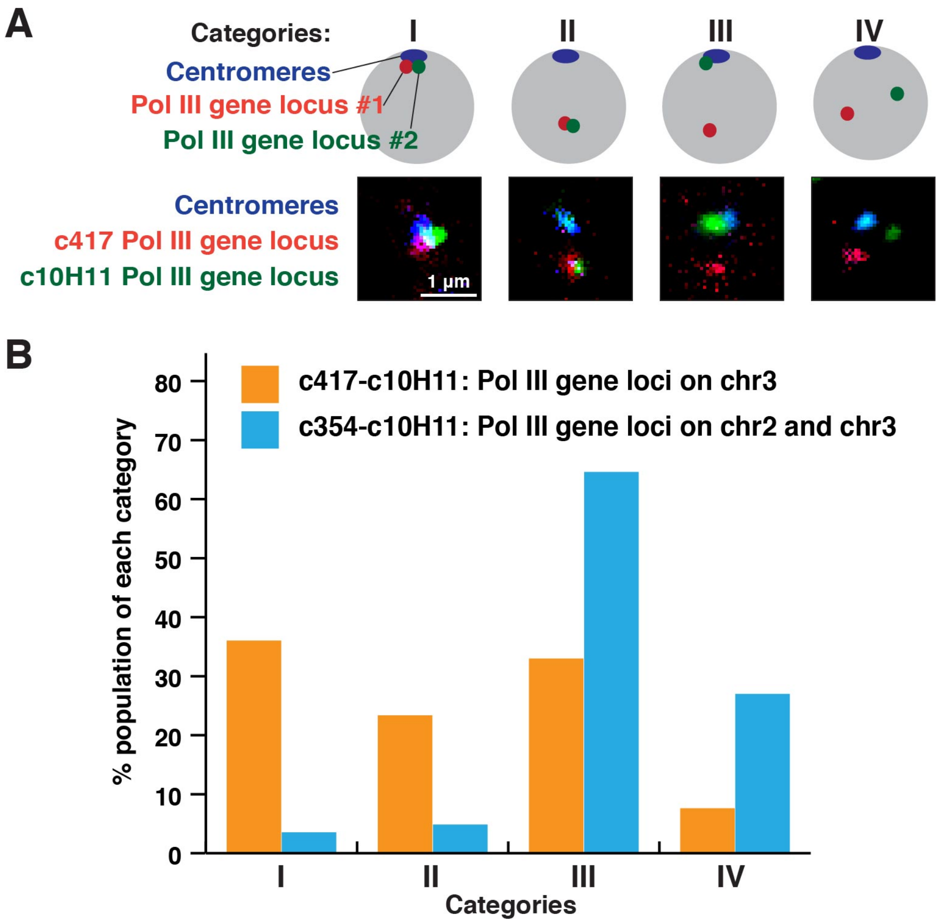


Figure S1. Intra-chromosomal association between Pol III gene loci at/near and away from centromeres

(A) Three-color FISH visualizing centromeres and the two Pol III gene loci (c417 and c10H11) present on the chromosome 3. Relative positioning of these loci was classified into the following four categories: Category I, proximal localization (< 0.3 μm) of the Pol III gene loci near centromeres (< 0.3 μm); II, proximal localization (< 0.3 μm) of the Pol III gene loci away from centromeres (> 0.3 μm); III, one of the Pol III gene loci locates near centromeres (< 0.3 μm); IV all the loci are separated from one another (> 0.3 μm). Representative images are shown at the bottom.

(B) FISH analysis on relative positioning of centromeres and the indicated Pol III gene loci.

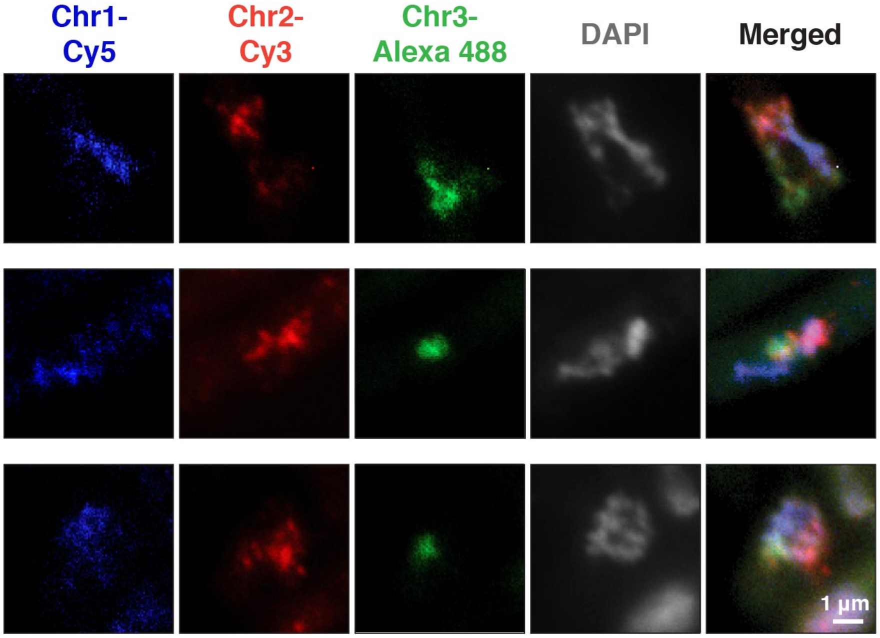
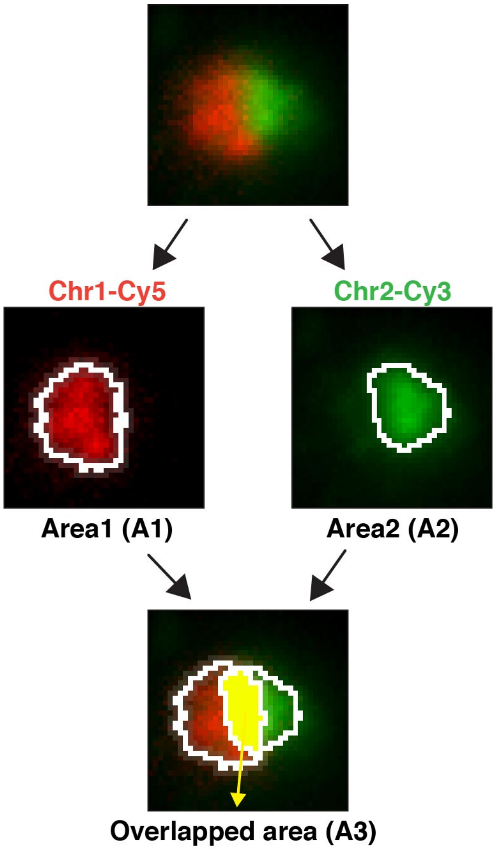


Figure S2. Painting FISH for mitotic chromosomes

(A) PFGE-derived FISH probes were used for chromosome painting. The *cdc25-22* mutant was cultured at the restrictive temperature (36°C) for 4 hours to arrest the cell cycle at the G2/M boundary and subsequently cultured at 25°C for 4 hours in medium containing 25 µg/ml CBZ, preparing prophase-arrested cells with heavily condensed chromosomes. Note that these chromosomes are much more compacted than those in mitotic cells without the treatment.



$$\% \text{ overlap} = \frac{A3}{A1+A2-A3} \times 100$$

Figure S3. Quantification of an overlap between different chromosomes

FISH images were captured by a fluorescence microscope and analyzed by ImageJ software. A threshold that distinguishes chromosome-occupied areas (white lines) from background signals was determined by the Otsu method (1). As a result, the chromosome-occupied areas (A1 and A2) reflecting chromosome territories were estimated as numbers of square pixels. Areas A1 and A2 estimated by ImageJ and their overlapped area (A3) were used to calculate a percentage of the overlapped area in relation to the region covered by A1 and A2.

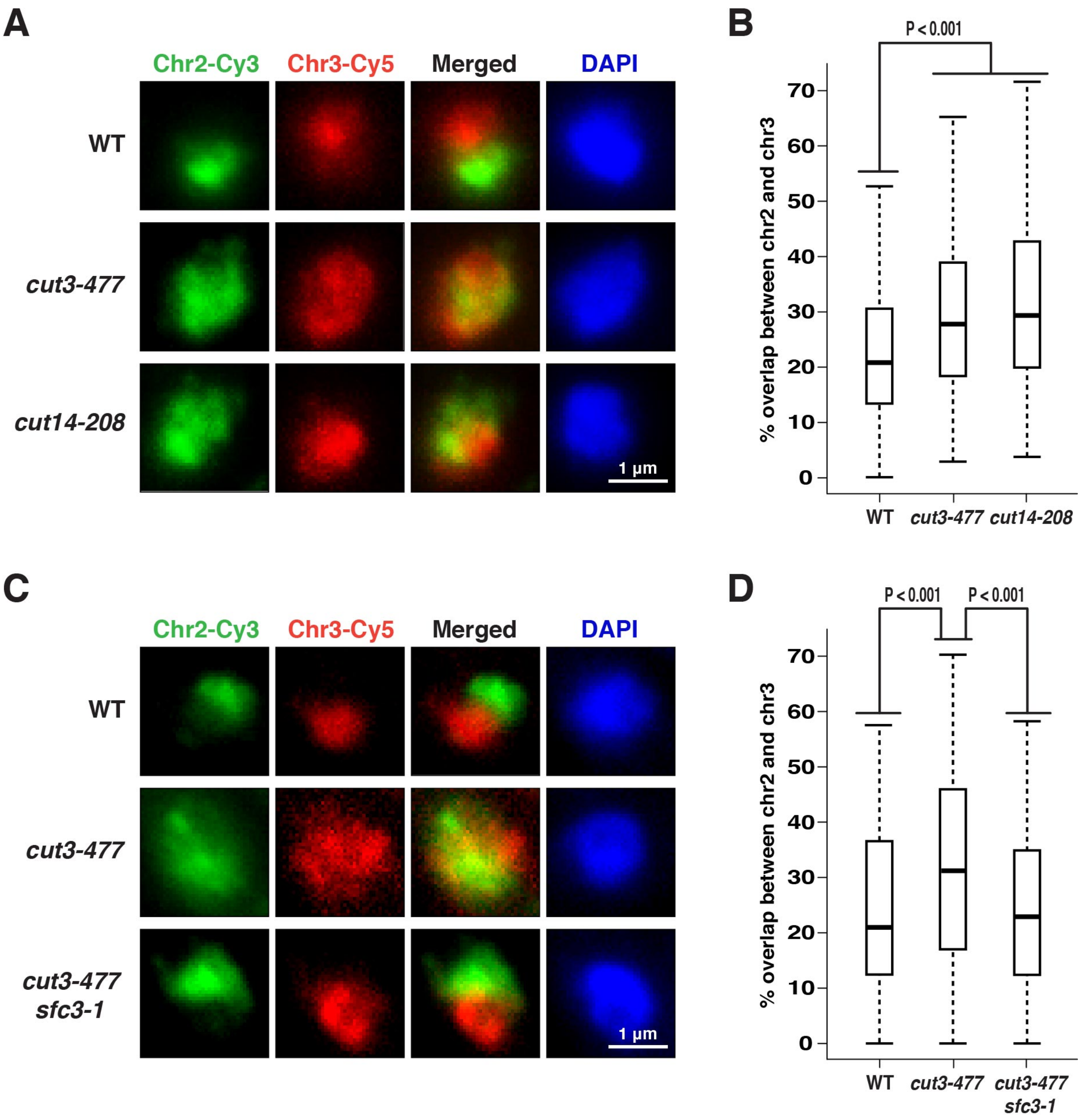


Figure S4. Chromosomes 2 and 3 are significantly overlapped in condensin mutants

(A and B) The wild type and condensin mutants were cultured as described in Fig. 2A. The chromosomes 2 (green) and 3 (red) were co-visualized using PFGE-derived FISH probes (A). The overlap between the chromosomes 2 and 3 was estimated in more than 100 cells as described in Fig. S3, and the data were represented as boxplots (B).

(C and D) The wild-type, *cut3-477*, and *cut3-477 sfc3-1* cells were subjected to the same analysis.

Supplementary Reference

1. Otsu N (1979) A threshold selection method from gray-level histograms. *IEEE Trans. Sys., Man., Cyber,* **9**, 62-66.

Table S1. PCR primers used to generate FISH probes against chromosome 1

Primer	Sequence (5’ to 3’)	Genomic position
Chr1_1_Fw	CTTCATAATCATTAATAAACCAACGG	62043
Chr1_1_Rv	ACTCATGCAAGTGGTCCAGATAACC	67007
Chr1_2_Fw	CGACCGACCAACGAATTGGCTGAATTG	151777
Chr1_2_Rv	GTATTCCACATGGCGTCTGTAACGCTG	156867
Chr1_3_Fw	CATCATCATTTTCGGTGTCTGCTGC	256964
Chr1_3_Rv	GAATTAAATGCCTCTAATAGAGCGTGC	262037
Chr1_4_Fw	ATTCAACGAGGTGTCAGTAAAGCAGGC	349595
Chr1_4_Rv	AGATAGATGATGAGCTCCTAGGCGTAG	354662
Chr1_5_Fw	GAATTCATCACTTCCAGAAGATGATG	457481
Chr1_5_Rv	TCGACGCAACCGCTGGTATGATACG	462842
Chr1_6_Fw	TCACCACCAATGAATCTGGCGAAAGGC	550751
Chr1_6_Rv	ACTGGAATAAATATATTCGGAAGGCTTGG	555701
Chr1_7_Fw	TGCGGTGCAATCATAAAGATATTGTGC	643701
Chr1_7_Rv	TGTTTGACGATGGATCTTCTGAAGG	648711
Chr1_8_Fw	ATCAATGAGTTCCGCCTCGTTATCAGC	744070
Chr1_8_Rv	ACTTGATAGTGGAGACGTTAAGGCGAG	749088
Chr1_9_Fw	CGATTCTAAGGTTGATTCCGCTTTGG	849963
Chr1_9_Rv	TCAGACCTTTAGGTGAAATAGCTCTC	854920
Chr1_10_Fw	CCCACCATTCATGCCATCTATTATCGG	957687
Chr1_10_Rv	ATCCCTTTAGCGGGTTTGATGATCTTG	962708
Chr1_11_Fw	GCTGATGAGGAGCAAGTTGATCACAG	1042326
Chr1_11_Rv	CATAGCATCACGAAGCTTTACAGTACC	1047478
Chr1_12_Fw	TCCGAACTAGACACTTTAAGCGCAGCG	1151190
Chr1_12_Rv	TACCCGTAATTAACAGATTTAGCAAGTC	1156234
Chr1_13_Fw	AAGGTTAGAAAGAATAGGTTATTGGC	1238519
Chr1_13_Rv	TATCATCATATCTGATATGGTAGCG	1243601
Chr1_14_Fw	AAACTGCAATGTGGAGTTGGTGTTGGC	1348463
Chr1_14_Rv	CAGAGGTTAGCTCGCCTTTAGCAGAAC	1353463
Chr1_15_Fw	TCTTCAGTCTTGGGTAATAAACGTTGC	1458281
Chr1_15_Rv	CAATATTCGAGTCGCAGAGTTAACTGG	1463457
Chr1_16_Fw	TCCGCTGTCTGCATCTTGTGTACCTAG	1557297
Chr1_16_Rv	AACCTATCGATGGCGGAATATTGTACG	1562235
Chr1_17_Fw	ATGGAATAGCTCCAGTACTGCTAATGG	1650562
Chr1_17_Rv	ATAATCCACGGCTCTTATGTCAACTGG	1655572
Chr1_18_Fw	CAGTAATATCTCTTGAATGCGGAGGGG	1754198
Chr1_18_Rv	GTTTTGGCGGTATCATCATACGCTGAG	1759175

Chr1_19_Fw	GCGTAGCTAACGTAGTGGTAAATCC	1858146
Chr1_19_Rv	TCGGCTAGATATCTTTATGACTACG	1863228
Chr1_20_Fw	TGCAGTGATACGCCGAATTCCTTTACC	1963319
Chr1_20_Rv	TGATAGAACGGCTAACATACTCAGAGC	1968561
Chr1_21_Fw	AGCTGATGACTGAACAAGCCTTTGTCG	2055392
Chr1_21_Rv	GAGTACTGCAAACCGTCCATCAAGC	2060709
Chr1_22_Fw	TACCAAGTACGATCTTGTCGCTCACTC	2158102
Chr1_22_Rv	CAGATAGTCCTCGAAGGCACTTTGATC	2163194
Chr1_23_Fw	CAGCTTGCAACCTTCGGTTTTCATTGC	2250701
Chr1_23_Rv	GTTGAGGATCTCATCCTTGATCATACC	2256165
Chr1_24_Fw	GATGAATAGAGCTGAGTATGACCCAGC	2355936
Chr1_24_Rv	TGGATGTTGGTTGTAATCGAACCCTTG	2361092
Chr1_25_Fw	CCAGACAATAATACATGGAAGCAC	2452437
Chr1_25_Rv	AAATAGCGTTGTAGTCATGTAATAACC	2457479
Chr1_26_Fw	ACTTGCCTACTGTTCAGCAATCTCAGC	2545242
Chr1_26_Rv	TGGCGGATTATCAACATAGTTCGCAGC	2550733
Chr1_27_Fw	CACACATTGGCATATGATAAAGGATGG	2655091
Chr1_27_Rv	ACTTTAATGAGCATTCTCGAGCCGTAC	2660199
Chr1_28_Fw	AACTATGCAGAGTTCGGCATAAGCTGG	2751732
Chr1_28_Rv	AAGTTTCCCGATTATGAAATTGTTGATGAT	2756298
Chr1_29_Fw	GTGGTATTGATGACATCTGTTGTATGG	2861790
Chr1_29_Rv	GCTTTGGCCGCTGTTAATGTACTTGC	2867068
Chr1_30_Fw	CTGTTGAAGTAGTTGAACCTGCTGCTG	2945066
Chr1_30_Rv	CTTGGTTATGGTTCATGGCGCTTATGC	2950349
Chr1_31_Fw	GGTACTCTTGCTCAGCTACCCTACTC	3065468
Chr1_31_Rv	AGATCCTTGTTGCTGGTACTTCTGC	3070473
Chr1_32_Fw	AGTGTAGCATTCGTTTCTACGGCCTTG	3151159
Chr1_32_Rv	TGGCTTTGTTGTCATTGCGTACAACTC	3156173
Chr1_33_Fw	ACTGTCTCTCGTAATGAACTAATGACC	3256274
Chr1_33_Rv	TCATACGTTGGGTATCTTATTTGTTGC	3261344
Chr1_34_Fw	TTGGATTGGAGGATTTCGCTAGCCACG	3356535
Chr1_34_Rv	ATGGTTCGTCCTCGTCAGTTATTGTTC	3360619
Chr1_35_Fw	GAGGTTGCCGAGATTCATAGTCTTC	3450767
Chr1_35_Rv	AATGTCCCAATTTCCTACGCCAAATAC	3455899
Chr1_36_Fw	GCGCGCTAGCATCTAAACATTACAGCG	3549178
Chr1_36_Rv	GCTACCGACTCTCTCGATATTTCTCAC	3554204
Chr1_37_Fw	CAGAGTTAAAGTATAAACACTATGCC	3653567
Chr1_37_Rv	CTAGCATCAAAGATTCAGGATTTGC	3658634
Chr1_38_Fw	AGTGCAGTGTTTACCAACAAGCGTACG	3790442
Chr1_38_Rv	TTAGCCTAACCTGTTAGATATGCGGTG	3795574
Chr1_39_Fw	CGAATTGTCGAAGAAATTGTCACTGAC	3858498

Chr1_39_Rv	AGATATTCGTTTGCTGATCGCAATAGC	3863406
Chr1_40_Fw	ATACACATTCTTCCCGAGCAAGACAGC	3954697
Chr1_40_Rv	ATACTGCTAGAAGCAGATGAGCTGAGG	3959962
Chr1_41_Fw	GTCAAAGCCGATATTGATATGCTGTCC	4073855
Chr1_41_Rv	GTAGCACTCTTTCTATGACTACTTCTC	4079116
Chr1_42_Fw	TGATATAGGCATGCGTTCGACTCATGC	4154882
Chr1_42_Rv	TTGGTTGCACCTACAGGAATCATACTC	4160119
Chr1_43_Fw	GCATCACAAGCTTTACGCTTGATG	4259702
Chr1_43_Rv	CCACATTGAAAGTTGTCAGGTATG	4264779
Chr1_44_Fw	AATACCGGCGTGTTAGATTTAGCACCC	4349027
Chr1_44_Rv	TTACCGGAGGAACTTCCTTAATACCTG	4354073
Chr1_45_Fw	AAGGTTTACTGCTTTGTCAAACTTAGC	4446028
Chr1_45_Rv	TTCATAGTTTCGATAGCATCCTGTGC	4451584
Chr1_46_Fw	GATAATTACAACAATCTGTCCGGCCGG	4514696
Chr1_46_Rv	TCTCACTAACCCATTCTTGTAGAGCAG	4519682
Chr1_47_Fw	GATTTGTGTGACGAAGAATCTATGAGG	4660933
Chr1_47_Rv	AAACGAAGGACGAGTCAAGGCATC	4665989
Chr1_48_Fw	TTGCGACATTCAGCGTACGTGCTTCAC	4744677
Chr1_48_Rv	AGGCCGTTGTCAATCTATGGTTCTACC	4749718
Chr1_49_Fw	AGTTGAACGGATACGTTGGATTCG	4853334
Chr1_49_Rv	ACCTTGAATTATATCCCAGTACTGC	4858392
Chr1_50_Fw	CTACGAAGAACGAGGATCGGTTGATTG	4958822
Chr1_50_Rv	TAAGTTAGGTGATTAGAACTTTCAATCGG	4963838
Chr1_51_Fw	TTCGCCTCCAGCCATATAATCTGTCAC	5047459
Chr1_51_Rv	GCTTTGTATTGTCAATTAACAGTCCTG	5052590
Chr1_52_Fw	CCAATATCGCTGGATCGAGGATTGTTC	5140345
Chr1_52_Rv	TTGTTGGTCATGAAGACTGGTTGCTTC	5145057
Chr1_53_Fw	TCAGAAGCATACCTGGTTTGCTGAAGC	5249674
Chr1_53_Rv	AATGGCATGCATCATTCCAAGGCCAAC	5254666
Chr1_54_Fw	GCGTTGCTTAGAGAAGTTCCCAATTGC	5346346
Chr1_54_Rv	AGACTACATCTACTTCTGAAGCTCCAC	5351507
Chr1_55_Fw	GCGACTGCATGCTACGTATTTATC	5451794
Chr1_55_Rv	AAGCTAAAGATCATGTCTACGTTCG	5456929
Chr1_56_Fw	GGCAAGTTATGCAGAACTTATCGCAGC	5510095
Chr1_56_Rv	ACTGCATCCACATAATTCATGGGTTCG	5514678

Table S2. PCR primers used to generate FISH probes against chromosome 2

Primer	**Sequence (5' to 3')**	**Genomic position**
Chr2_1_Fw	TTAAGAATAGCGAACATCGCACTTTG	47021
Chr2_1_Rv	GGTGTCGAATAAGTAGAATAGTACTCC	52157
Chr2_2_Fw	TCTTCAGAATCCTTTGCATCTGCTGGC	159133
Chr2_2_Rv	TATTGGTTCACCAGCTTGTACCTAACG	164150
Chr2_3_Fw	AGTTTTGAAAAGTCGAACGACCTCC	237447
Chr2_3_Rv	AATTGGTGATCGTGTTCCTGCTGATC	242635
Chr2_4_Fw	CCATTTCAAGCTTGCGTTCTCCGATTC	340435
Chr2_4_Rv	TGGGAACATATGAGCATGTGAAGTCTC	345431
Chr2_5_Fw	ATTTCCAGTCCGAATTGCTTCCAACTG	440384
Chr2_5_Rv	ACCAACGTTACCGTCTACTACTAGAC	445440
Chr2_6_Fw	CCGTATGATTCTAGCGAAGCATAGGCC	538663
Chr2_6_Rv	GTTCAAGTAATTGAGCCCTCAGCTTGG	543710
Chr2_7_Fw	AAGATCTGTCATAAGACAATCGTTCAG	640452
Chr2_7_Rv	TGCATTGCTATTCAGACTATTGGAAGG	645450
Chr2_8_Fw	GCGCCCTTATAGTTCACCCAATTAGAG	734105
Chr2_8_Rv	TGGGTAGGTGTTCTTCACATTGGAGAG	739188
Chr2_9_Fw	GCAGCCTGGTTCTATTGAATATTTCTCC	851494
Chr2_9_Rv	TTGTAGTCTGCTGTAAAATGGACAAGC	856580
Chr2_10_Fw	CCGTAACTGGACTGCTGGAATTTCTTC	949010
Chr2_10_Rv	TGTGCTTTACGGAGTTGCTAGTACAGC	954163
Chr2_11_Fw	AGTACCTGACTTCATCCTATCGAAGC	1036988
Chr2_11_Rv	TGGTGTAATCGTATTTGCACTCCACC	1042045
Chr2_12_Fw	AGCAAGAATTCCCAGAACGCTTCTCAG	1126673
Chr2_12_Rv	ACTATGAGTCTCGTGGATCAGTTGAAG	1131673
Chr2_13_Fw	TGCGTACCAATACTTGAATGCATCTGC	1246273
Chr2_13_Rv	TTGAATTACGATAGACACGACTCTGC	1251528
Chr2_14_Fw	GTACGAAACAGAGCTACTTGTACTGCG	1323892
Chr2_14_Rv	AGGTTCTGAAGCATCTGATGAGATCTC	1329134
Chr2_15_Fw	TTAACCTGTCTTACAGCTTTTGGCTGG	1444428
Chr2_15_Rv	TATATCTCCAGGCATGAAACATTACC	1449597
Chr2_16_Fw	TGCGAGGTAGCATTGTTGGAGATTGTG	1556115
Chr2_16_Rv	TACCGTCCATGTTTCGATCATTTGGAC	1561130
Chr2_17_Fw	ATTTCACAAGTCGCGGAGTAAGCGAAG	1655329
Chr2_17_Rv	AGAACTGCTAATTCTTCCTGAGAATGG	1660351
Chr2_18_Fw	TACGGCCTTATCTTCTCCAACATCCTC	1756844
Chr2_18_Rv	TGATAATGCAATAGTGCACGCGATGTG	1761842
Chr2_19_Fw	TATGTCTTCTGACTTTCCCTGAAGAGC	1848824
Chr2_19_Rv	CAAGACAGATTCTTCTATTCAAGCTGC	1853853
Chr2_20_Fw	CGAGTGAGTTACAAACACGGTTGGTTG	1945117

Chr2_20_Rv	TAGAGATGTTATGGGCAATCCCGTTCC	1950142
Chr2_21_Fw	TCTAAGACACGACGTGTAAGAAATTGG	2057358
Chr2_21_Rv	GACTGATCTTATCAAGCCGCTAAGACC	2062544
Chr2_22_Fw	TTACAGCCATGATTGACTGATGCCACG	2150728
Chr2_22_Rv	TTCATACAAGCTTTGAGCATACGGGTC	2155758
Chr2_23_Fw	CAACCTCATTGCTTTAAGCCAGACTGC	2266120
Chr2_23_Rv	ACTTGCCATCTGATGGTGCTCAAGTTG	2271266
Chr2_24_Fw	ATTCTGACCAGCACAGTGGAATGGAAG	2348264
Chr2_24_Rv	TGCTGAATTTGAGCAACTTACTCCTCC	2353244
Chr2_25_Fw	TCGATGGACCTGGAGCTCTAAATCACG	2446861
Chr2_25_Rv	GTGCTAGAGGACTATGACCTGGTATGC	2451829
Chr2_26_Fw	TCGGATTAAGTGGCTTCAATGCCTCTC	2553046
Chr2_26_Rv	ATATTCATACCGTCGATGGGTTTCAGG	2558026
Chr2_27_Fw	GACAGATCCAAGGTTTCGTAAACGACG	2649154
Chr2_27_Rv	TGTCAAGGTTGGAAAGTGACTCCTTGC	2654200
Chr2_28_Fw	CGGCTTGTTGATTACCGTAATCGGTAC	2748823
Chr2_28_Rv	AGACAGCATCTTTGTACATTTGCTGCC	2753813
Chr2_29_Fw	TCGGAGCTGTAGTGTAGCAAACTTCGC	2835205
Chr2_29_Rv	CGATTTCGCAATGATGGCTTCTCAACC	2840283
Chr2_30_Fw	TTCTGAGCGATACGTTGGTACACGTAG	2925445
Chr2_30_Rv	CGTGACCAGTGGGAATTTGCCTTAAAG	2930507
Chr2_31_Fw	ACTTGTGTAGGCGCAAACTTCTGAGCC	3043933
Chr2_31_Rv	AGTGTATTAGCAGTCGAAGAACTGAGG	3048929
Chr2_32_Fw	AACCGACTCTATAATCGTACGGCTTGC	3139684
Chr2_32_Rv	AGTCTTCAAGGAAGAAACGTCCTCCTC	3144721
Chr2_33_Fw	AAGGCTACGTGAATAGTGAGCGCTTGG	3246384
Chr2_33_Rv	AGTAGGTCATTTCGTAATGGATTCCGC	3251244
Chr2_34_Fw	CAGGTACTTCATTAGTTTGGCGGTACC	3345724
Chr2_34_Rv	AGTAATTAGTTATACATGAAGCTATTATAGACC	3350576
Chr2_35_Fw	GAAATTCGCTGTCGCGTTATCACAAGC	3434859
Chr2_35_Rv	GGTAACTTAACTTCCCTTGCGCGATAC	3439842
Chr2_36_Fw	TGATTGGTATTGCCGTGTGTGAATGCC	3531372
Chr2_36_Rv	AGGTCTTCTTAGGGATAATTGCTTCGG	3536427
Chr2_37_Fw	ACAGTGGTGTAACCCTCCAAATGATGG	3644064
Chr2_37_Rv	TTGTCCGACTTACTTGTCTCACTTAGG	3649022
Chr2_38_Fw	CACCCTGTATGCAATCTATAGTGCCCG	3745207
Chr2_38_Rv	TGAGGCAGTGCATAATAATTGCACTGG	3750237
Chr2_39_Fw	AGGAATACGTTAGCTGCTCGTGGATGC	3841275
Chr2_39_Rv	TCAGACTCAAATGGCAAAGCTGTCAGC	3846294
Chr2_40_Fw	CATCATCAGCTGACCTTTCAACCCTTC	3939641
Chr2_40_Rv	ACACTAGCAAACTGCCACTTTAGAAGC	3945057

Chr2_41_Fw	ATCGAGATCTTGACTACCGAAGGTCCG	4048827
Chr2_41_Rv	GACAAAGCTTCTAACGCCAGAGCCATC	4053887
Chr2_42_Fw	TTTCTACAGAACACCTAAGACGGTCGG	4144593
Chr2_42_Rv	GCAACTTCCATGGCCTCTAGGTTACAC	4149278
Chr2_43_Fw	TTGCACATGTAATGGCGTCTAACGGTC	4243052
Chr2_43_Rv	ACAGTTACCGAACATAACTCAGGCACG	4243394
Chr2_44_Fw	TCCAGTCTACGAATCCAAACTTCTGGG	4248106
Chr2_44_Rv	TTCTAACGAAAGTGCTACCGCAATTGC	4248417
Chr2_45_Fw	GCCTTAGCGTTGATGAATGGACGAGAC	4348915
Chr2_45_Rv	TGAAATTATTCAGGCTTGGTCAACCCG	4353959
Chr2_46_Fw	GCTTCCTGAAACAAAGGGTCGTGATGC	4445847
Chr2_46_Rv	AAATCCAGCTCCAGACATATCAACTCC	4450785

Table S3. Fission yeast strains used in this study

Strain	**Genotype**
SP1173	h^- *leu1-32 ade6-M216 his2 ura4-D18*
SPO25	h^- *leu1-32 ade6-M210 his2 ura4-D18 cut3-477*
SPO279	h^- *leu1-32 ade6-M216 ura4-D18 cut14-208*
SPO22	h^- *leu1-32 ade6-M210 his2 ura4 cut3-477 sfc3-1*
SPO155	h^- *leu1-32 ade6-M216 his2 ura4-D18 cdc25-22*
SPO41	h^+ *leu1-32 ade6-M210 ura4 c162::*$ura4^+$
SPO43	h^+ *leu1-32 ade6-M210 ura4 c162::* $ura4^+$*-tRNA-5S rRNA*