

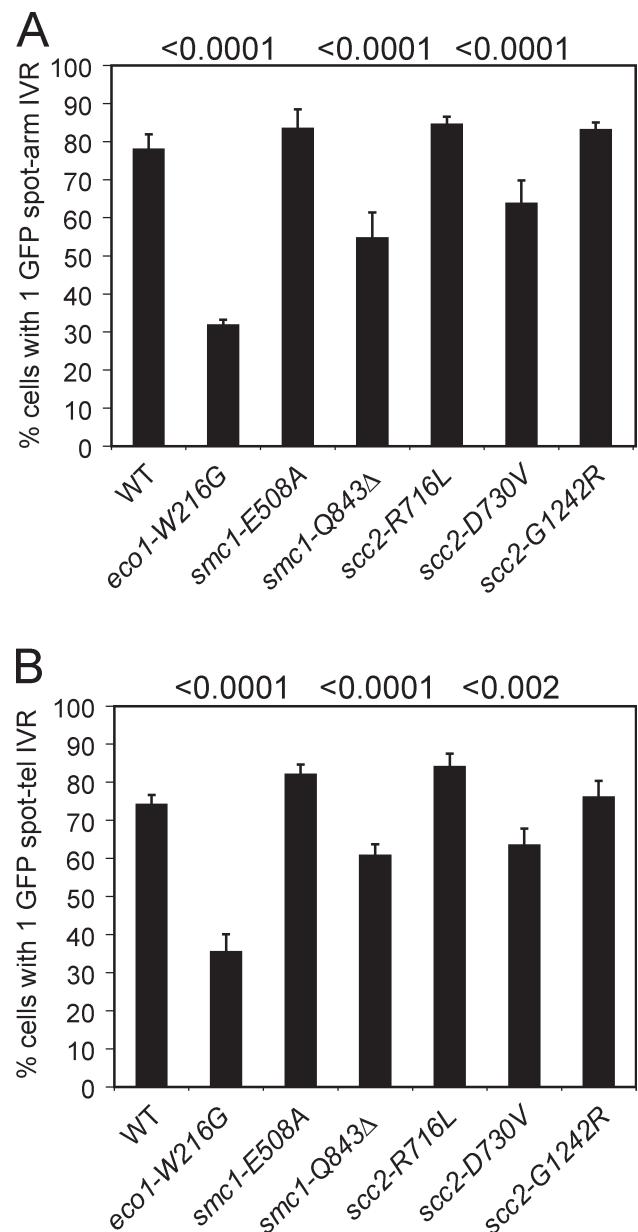
Gard et al., <http://www.jcb.org/cgi/content/full/jcb.200906075/DC1>

Figure S1. Cohesion is disturbed in the cohesinopathy mutants at 37°C. Cultures were grown overnight at 30°C, diluted, and grown at 37°C for 3 h before arrest with nocodazole. Cohesion and telomere tethering were scored and analyzed as described for Fig. 1. (A) Cohesion was scored at a chromosome arm site. (B) Cohesion was scored at a telomeric site. (A and B) Three independent experiments were performed, and ≥300 total cells were scored. Error bars indicate SD. P-values are derived from Fisher's exact test.

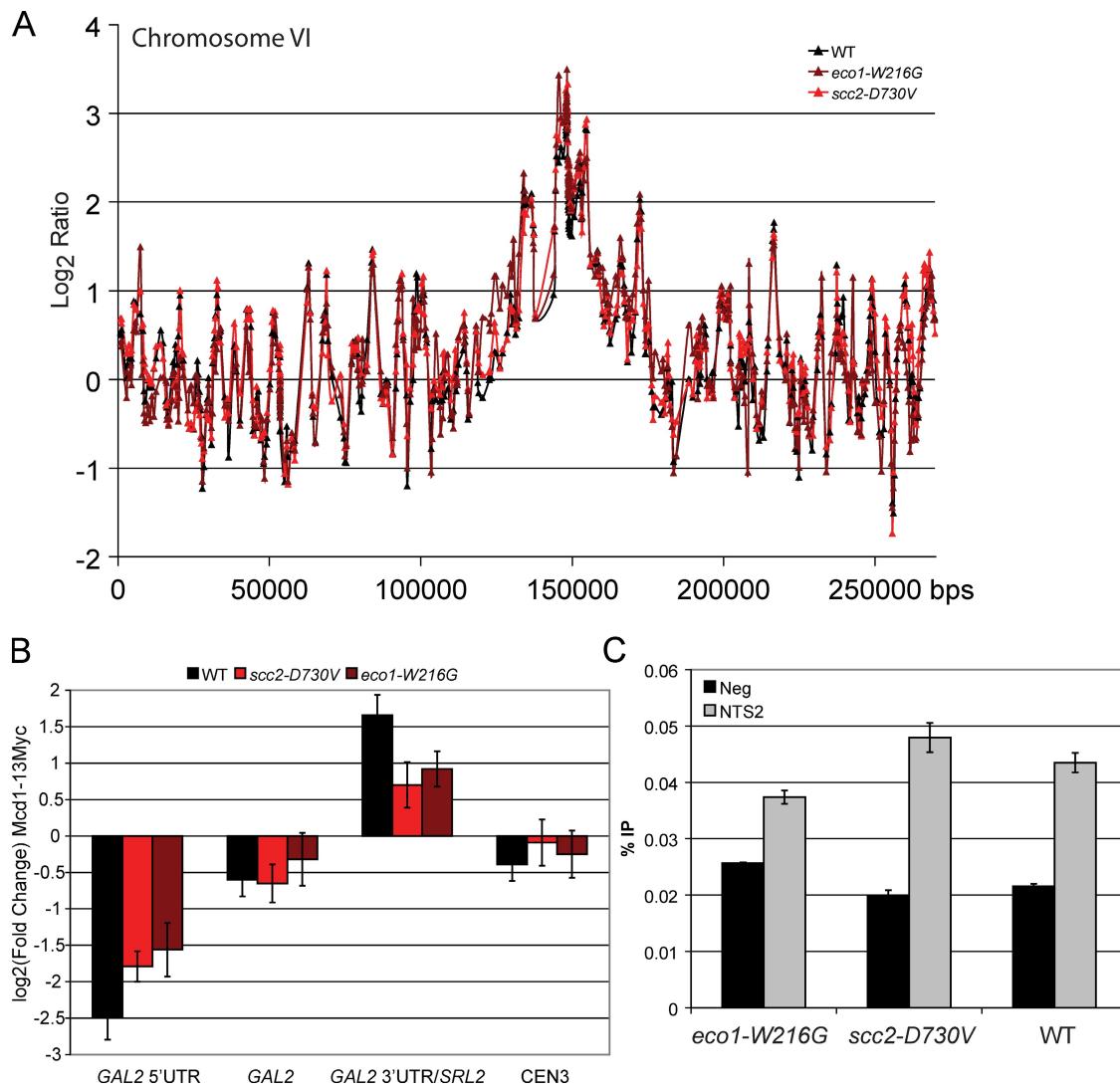


Figure S2. The pattern of cohesin binding in the strong mutants is similar to WT with minor changes at particular loci. Strains with the indicated background and Mcd1-13Myc were grown in YP raffinose, arrested with nocodazole, and 2% galactose was added to half of the culture for 60 min. Cells were cross-linked with formaldehyde and subjected to ChIP. (A) ChIP results for Mcd1-13Myc for chromosome VI from the raffinose samples. The x axis shows base pairs, and the y axis shows the log₂ ratio of ChIP/total chromatin. Each ChIP experiment was performed twice with similar results. The Pearson correlation coefficient for biological replicates for WT is 0.89, whereas the Pearson correlation coefficient for the mean value for WT to scc2-D730V is 0.96 and 0.93 for eco1-W216G, demonstrating that the pattern of binding is very similar in all cases. (B) qPCR was performed for several loci, including the 5'UTR of GAL2, GAL2, 3'UTR of GAL2, CEN3, and FMP32, a site where cohesin binding is not altered in raffinose versus galactose (Bausch et al., 2007). The signal was normalized to the FMP32 signal. The change in binding is shown for each strain from raffinose to galactose. The experiment was performed twice with similar results. (C) For the samples collected in raffinose, the ChIP DNA and total chromatin were used to analyze binding to the rDNA region using qPCR at one site where cohesin has been shown to bind (NTS2) and one site where cohesin does not bind (neg; Laloraya et al., 2000). The signal from a ChIP performed without antibody was subtracted from each value, and the signal was normalized to the signal for PGK1 (a single-copy site in which cohesin is absent). Error bars indicate SD of reactions performed in triplicate.

Table S1. Strains used in this study

Parent strain	Strain name	Genotype
Telomeric cohesion		
W303	SLJ1989 (NBY292)	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2
SLJ1989	SG153.6	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 ECO1::eco1-W216G::HYG
SLJ1989	SG151.1	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 SMC1::smc1-E508A::HYG
SLJ1989	SG152.6	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 SMC1::smc1-Q843Δ::HYG
SLJ1989	SG144.1	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 SCC2::scc2-R716L::HYG
SLJ1989	SG145.2	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 SCC2::scc2-D730V::HYG
SLJ1989	SG150.1	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 tellV::LacO-LEU2 SCC2::scc2-G1242R::HYG
Arm cohesion		
W303	SLJ1988 (NBY291)	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3
SLJ1988	SG119.6	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 ECO1::eco1-W216G::HYG
SLJ1988	SG124.1	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 SMC1::smc1-E508A::HYG
SLJ1988	SG107.2	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 SMC1::smc1-Q843Δ::HYG
SLJ1988	SG120.2	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 SCC2::scc2-R716L::HYG
SLJ1988	SG121.6	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 SCC2::scc2-D730V::HYG
SLJ1988	SG123.7	MAT α bar1-ura3 leu2 trp1 lys2 ade2 his3::pCUP1-GFP12-LacI12::HIS3 armIV::LacO-URA3 SCC2::scc2-G1242R::HYG
Rap1-GFP		
W303	JG1575.2	MAT α ura3-1 leu2,3-112 his3-1 ade2-1 can1-100 Δbar1 HTA1-mCherry::URA3 trp1-1::RAP1-GFP::TRP1
W303	TB156.1.4	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δbar1 ECO1::eco1-W216G::HYG HTA1-mCherry::URA3 trp1-1::RAP1-GFP::TRP1
W303	JG1576.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δbar1 SCC2::scc2-D730V::HYG HTA1-mCherry::URA3 trp1-1::RAP1-GFP::TRP1
W303	TB15.1	MAT α ura3-1 leu2,3-112 his3-1 ade2-1 can1-100 Δbar1 trp1-1::RAP1-GFP::TRP1 sir4::kanMX4
Flag tagged		
BY4742	AM405.4	MAT α his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 ECO1-Flag::KAN
BY4742	AM415.1	MAT α his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 ECO1::eco1-W216G-Flag::KAN
BY4742	SG94.5	MAT α his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 SMC1-Flag::KAN
BY4742	SG378.8	MAT α his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 SMC1::smc1-E508A-Flag::KAN
BY4742	AM323.1	MAT α his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SMC1::smc1-Q843Δ-Flag::KAN
BY4742	AM505.3	MAT α his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 SCC2-Flag::KAN
BY4742	SG370.8	MAT α his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SCC2::scc2-R716L-Flag::KAN
BY4742	SG131.8	MAT α his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 SCC2::scc2-D730V-Flag::KAN
BY4742	SG133.3	MAT α his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SCC2::scc2-G1242R-Flag::KAN
GAL2 LacO		
W303	JG1555	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1
W303	JG1556	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1 ECO1::eco1-W216G::HYG
W303	JG1561	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1 SMC1::smc1-E508A::HYG
W303	JG1562	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1 SMC1::smc1-Q843Δ::HYG
W303	JG1557	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1 SCC2::scc2-R716L::HYG
W303	JG1558	MAT α ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LacI-GFP::HIS3 GAL2-LacO::TRP1 SCC2::scc2-D730V::HYG

Table S1. Strains used in this study (Continued)

Parent strain	Strain name	Genotype
W303	JG1560	MAT α <i>ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 Lac-GFP::HIS3 GAL2-LacO::TRP1 SCC2::scc2-G1242R::HYG</i>
GAL2-13myc		
BY4742	SG262.3	MAT α <i>his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 GAL2-13MYC::KAN</i>
BY4742	SG264.1	MAT α <i>his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 ECO1::eco1-W216G::HYG GAL2-13MYC::KAN</i>
BY4742	SG263.2	MAT α <i>his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 SCC2::scc2-D730V::HYG GAL2-13MYC::KAN</i>
GAL2 mRNA induction		
BY4742	JG1650	MAT α <i>his3Δ1 leu2Δ0 LYS2 met15Δ0 ura3Δ0 sir2::kanMX4</i>
GAL2 mRNA induction/growth		
BY4742		MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0</i>
BY4742	SG246.1	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SCC2::scc2-D730V::HYG</i>
BY4742	SG247.1	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 ECO1::eco1-W216G::HYG</i>
Growth		
BY4742	SG244.1	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SMC1::smc1-E508A::HYG</i>
BY4742	SG245.2	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SMC1::smc1-Q843Δ::HYG</i>
BY4742	SG241.1	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SCC2::scc2-R716L::HYG</i>
BY4742	SG243.1	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SCC2::scc2-G1242R::HYG</i>
Mcd1-13myc ChIP		
W303	AM127.4	MAT α <i>ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δbar1 MCD1-13MYC::KAN</i>
W303	SG216.1	MAT α <i>ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δbar1 ECO1::eco1-W216G::HYG MCD1-13MYC::KAN</i>
W303	SG215.1	MAT α <i>ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δbar1 SCC2::scc2-D730V::HYG MCD1-13MYC::KAN</i>
Chromosome XII LacO		
W303	Y2869	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1563	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 ECO1::eco1-W216G::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1564	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 SMC1::smc1-E508A::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1565	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 SMC1::smc1-Q843Δ::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1566	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 SCC2::scc2-R716L::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1567	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 SCC2::scc2-D730V::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
W303	JG1569	MAT α , <i>ade2-1 trp1-1 can1-100 leu2,3-112 his3-11,15 ura3-52 SCC2::scc2-G1242R::HYG LacOs::YLRO03c-1 LacOs:: MMP1 GFP-Lacl::HIS3</i>
Centromere clustering		
BY4742	JG1546	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 CSE4::GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1547	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 MET15 ura3Δ0 ECO1::eco1-W216G::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1548	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SMC1::smc1-E508A::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1549	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SMC1::smc1-Q843Δ::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1550	MAT α <i>his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 SCC2::scc2-R716L::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1551	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SCC2::scc2-D730V::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>
BY4742	JG1552	MAT α <i>his3Δ1 leu2Δ0 LYS2 MET15 ura3Δ0 SCC2::scc2-G1242R::HYG CSE4-GFP::HIS3 {pSpc42-RFP [LEU2]}</i>

Table S1. Strains used in this study (Continued)

Parent strain	Strain name	Genotype
Synthetic genetic interactions		
W303	JG680	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1
W303	SG156.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 ECO1::eco1-W216G::HYG
W303	SG154.2	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 SCC2::scc2-D730V::HYG
W303	BXI119	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN::URA3 ycs4-1
W303	BXI139.6	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 ycs4-1 SCC2::scc2-D730V::HYG
W303	BXI140.10	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 ycs4-1 ECO1::eco1-W216G::HYG
Moazed silencing		
W303	DMY2798	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 leu2::mURA3
W303	DMY2804	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS1::mURA3
W303	DMY2800	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS2::mURA3
DMY2798	DMY2827	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 leu2::mURA3 sir2 Δ ::KANR
DMY2804	DMY2835	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS1::mURA3 sir2 Δ ::KANR
DMY2800	DMY2831	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS2::mURA3 sir2 Δ ::KANR
DMY2798	BH102.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 leu2::mURA3 ECO1::eco1-ack::HYG
DMY2804	BH103.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS1::mURA3 ECO1::eco1-ack::HYG
DMY2800	BH104.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS2::mURA3 ECO1::eco1-ack::HYG
DMY2798	BH105.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 leu2::mURA3 ECO1::eco1-W216G::HYG
DMY2804	BH106.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS1::mURA3 ECO1::eco1-W216G::HYG
DMY2800	BH107.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS2::mURA3 ECO1::eco1-W216G::HYG
DMY2798	BH108.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 leu2::mURA3 SCC2::scc2-D730V::HYG
DMY2804	BH109.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS1::mURA3 SCC2::scc2-D730V::HYG
DMY2800	BH110.1	MAT α ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 Δ bar1 RDN1-IGS2::mURA3 SCC2::scc2-D730V::HYG
rDNA recombination		
W303a	DMY3010	MAT α RAD5+ RDN1::ADE2
DMY3010	DMY3011	sir2 Δ ::TRP1
DMY3010	JG1615	SCC2::scc2-D730V::HYG
DMY3010	JG1617	ECO1::eco1-W216G::HYG
DMY3010	JG1618	ECO1::eco1-ack::HYG
Condensin ChIP		
Unknown	Y2315	Smc4-Pk9
Y2315	BXI146.2	Smc4-Pk9 SCC2::scc2-D730V::HYG
Y2315	BXI145.1Q4	Smc4-Pk9 ECO1::eco1-W216G::HYG

References

- Bausch, C., S. Noone, J.M. Henry, K. Gaudenz, B. Sanderson, C. Seidel, and J.L. Gerton. 2007. Transcription alters chromosomal locations of cohesin in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 27:8522–8532. doi:10.1128/MCB.01007-07
- Laloraya, S., V. Guacci, and D. Koshland. 2000. Chromosomal addresses of the cohesin component Mcd1p. *J. Cell Biol.* 151:1047–1056. doi:10.1083/jcb.151.5.1047