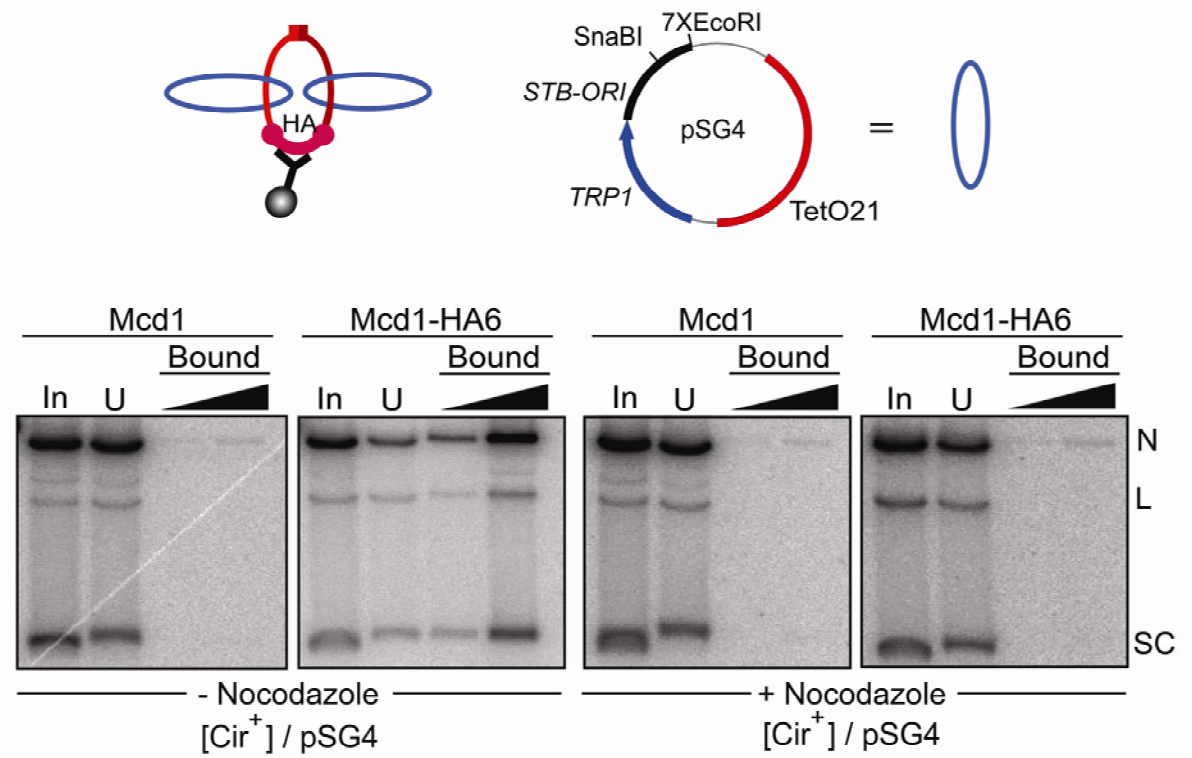

Figure S1. Spindle dependence of cohesin association with the *STB* reporter plasmid pSG4. As was established by the data in Figure 1, plasmid pSG4 associated with cohesin-HA6 could be immunoprecipitated from cell extracts with the HA-antibody. Cleared lysates from pGS4 containing metaphase [cir$^+$] cells obtained by *cdc20* arrest or nocodazole treatment were immunoprecipitated with the HA-antibody, and adsorbed on Protein A beads. Aliquots of DNA extracted from the different fractions were analyzed as in Fig. 1C. In = input; U = unbound or flow-through. The amount of 'bound' DNA loaded in the right lane was 5 times that in the left one. This was also the case for Figures S2 and S3. SC = supercoiled plasmid; L = linear plasmid; N = nicked plasmid. Note that pSG4 was not pulled down by the HA-antibody when Mcd1 did not harbor the HA-tag or when cells were treated with nocodazole.

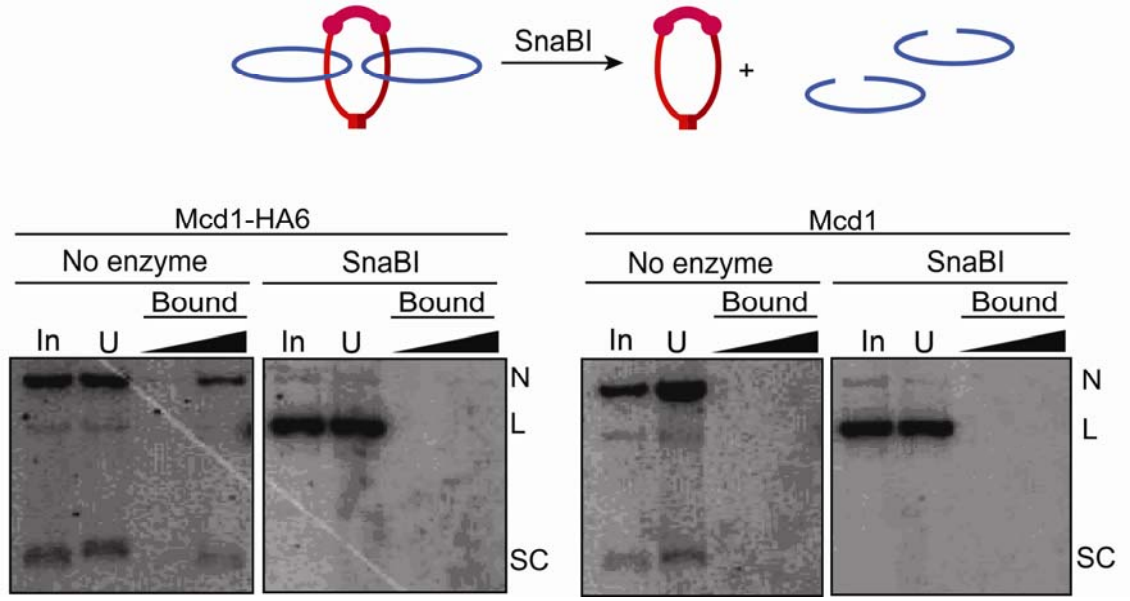

Figure S2. The *STB* reporter plasmid pGS4 is dissociated from cohesin upon linearizing the DNA. The data shown are from an assay analogous to that depicted in Figure 2B. SnaBI digestion, performed using a profligate excess of the enzyme (three times the amount used in a standard assay), in the cleared lysate from metaphase cells went to near completion in this assay. Immunoprecipitation using HA-antibody was conducted following DNA linearization.

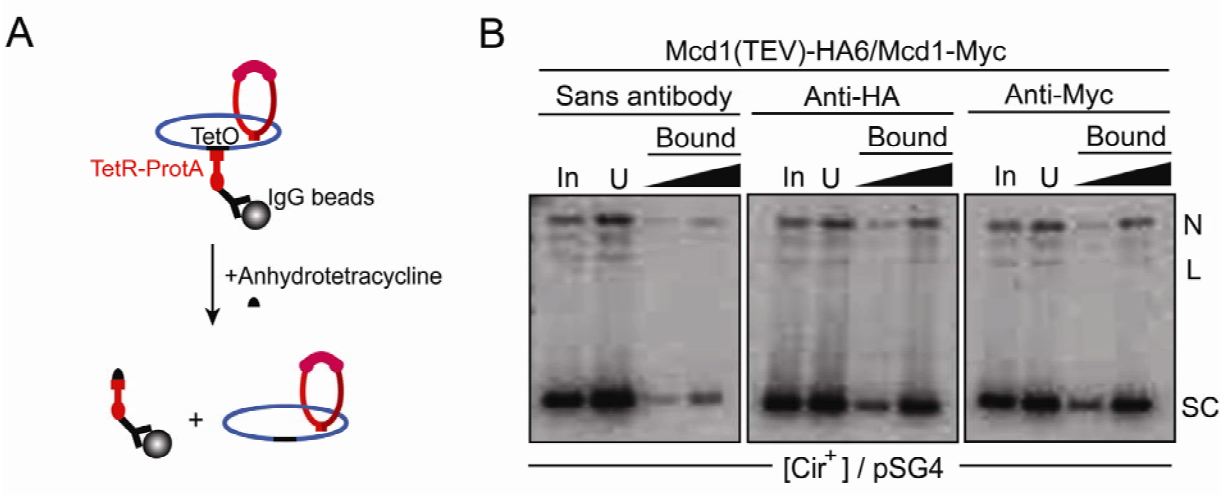

Figure S3. Initial trapping of pSG4 on IgG beads followed by release and subsequent immunoprecipitation by HA- or Myc-antibody. **A**. pSG4 from metaphase cells expressing cohesin-HA6 and cohesin-Myc13 was first adsorbed on IgG beads. The interaction between the TetO sequences on pSG4 and IgG was mediated through the hydbrid repressor Protein A-TetR. The bound DNA was then released by the action of anhydrotetracycline. **B**. The supernatant was treated with the HA- or Myc-antibody, and the immunoprecipitates collected on Protein A beads.

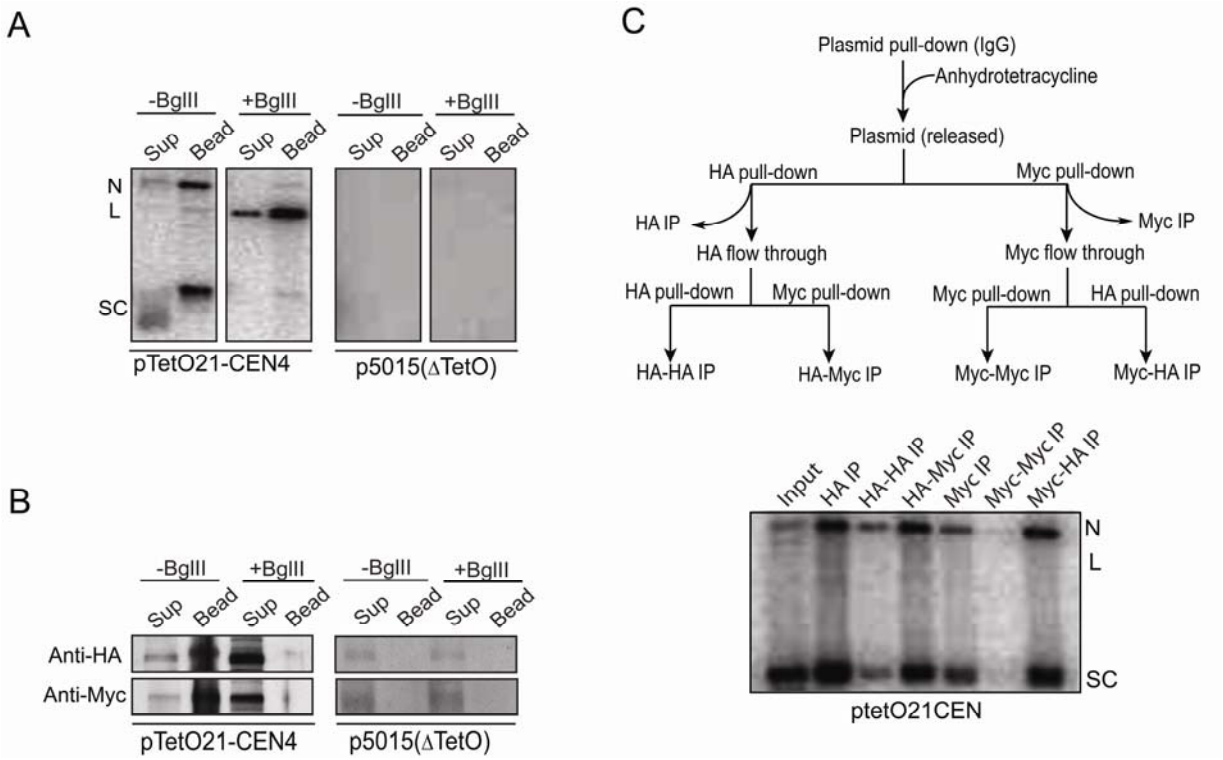

Figure S4. Outcomes of two-step immunoprecipitations performed on a circular minichromosome first adsorbed on IgG beads via Protein A-TetR-TetO interaction and then released by treatment with anhydrotetracycline. These assays were performed on the minichromosome pTetO21-CEN4 described by Ivanov et al (1) as a reference for similar assays performed on pSG4 (see Figure 4). **A, B**. Cleared lysates were incubated with IgG beads to bait plasmid DNA bound by Protein A-Tet repressor. Southern or western analysis was performed to examine the association of plasmid DNA or cohesin, respectively, with the beads in the absence of, or following, BglII digestion. **C**. The scheme for plasmid pull-down by IgG, release by anhydrotetracycline and subsequent two-step immunoprecipitation of cohesin-associated plasmid DNA is shown at the top; the experimental results are displayed below. Note that the HA-antibody immunodepleted cohesin(Mcd1-HA6) but not cohesin(Mcd1-Myc13). The Myc-antibody yielded the reciprocal exclusion. This outcome was similar to that observed for the *STB*

reporter plasmid pSG4 (Figure 4). These findings are consistent with cohesion being mediated by a single monomeric ring of cohesin. Plasmid pairing by a cohesin bracelet or via embrace by more than one cohesin monomer should have resulted in cross-immunodepletion by the HA- or Myc-antibody.

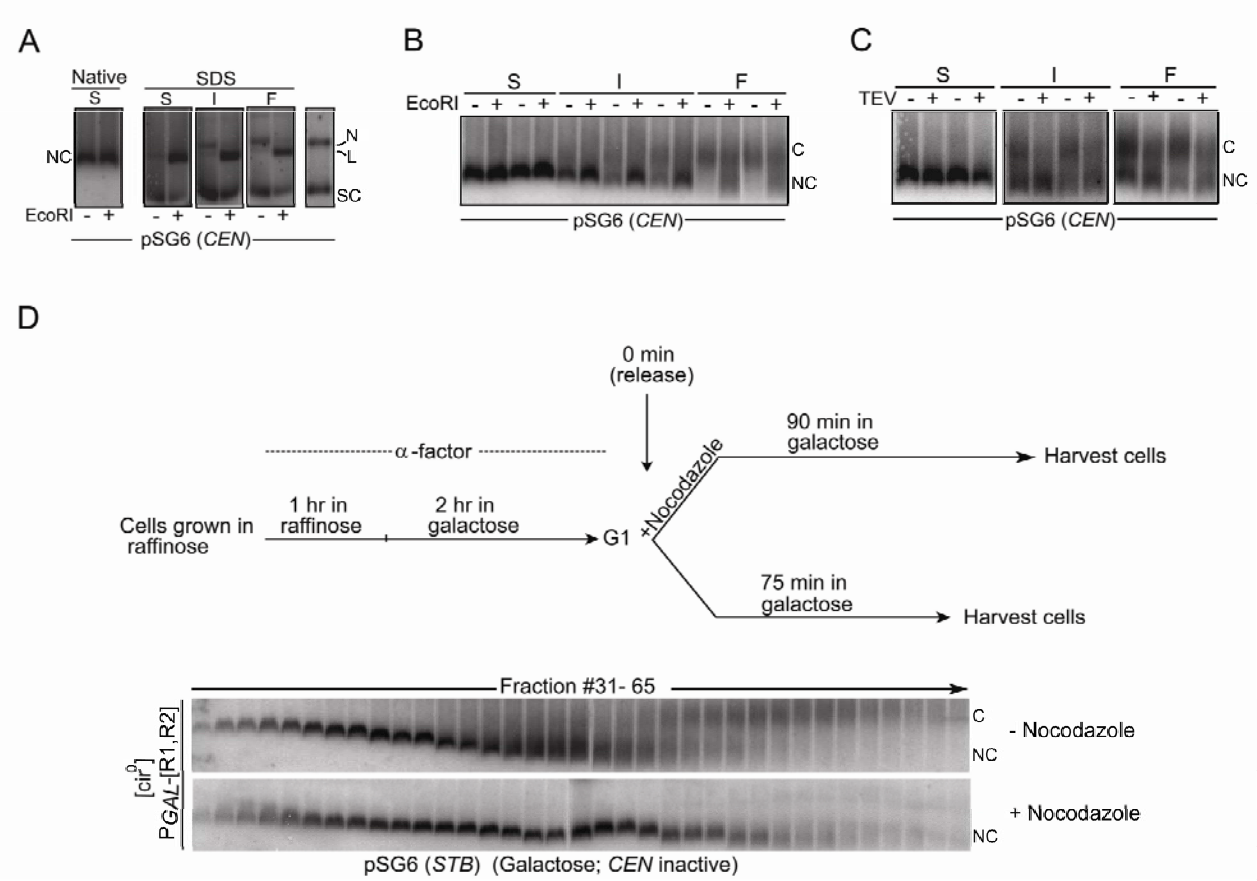

Figure S5. Analysis of cohesion in pSG6(*CEN*) by sucrose gradient sedimentation followed by EcoRI and TEV protease digestion: nocodazole abolishes cohesion in pSG6(*STB*). For details of the sedimentation procedure and subsequent analysis, see Figure 5 and the relevant text under 'Results'. Data shown in A-C represent the third panel from the top in Figure 5B (pSG6: [cir^0]; glucose). **A**. EcoRI digestion did not change the mobility of the slow-sedimenting (S) fractions during agarose gel electrophoresis under native conditions. When slow-, intermediate- (I) and fast-sedimenting (F) fractions were reanalyzed after SDS treatment, the DNA migrated essentially as the monomeric form, supercoiled circles primarily along with a small fraction (≤20%) of nicked circles. Gradient fractions digested with EcoRI at 4°C prior to SDS treatment and agarose electrophoresis revealed the majority of plasmids in the linear form. The extent of

enzyme digestion was 60-70%. DNA extracted from the cleared lysate of metaphase cells by phenol-chloroform treatment and ethanol precipitation was run in the rightmost lane as a control. C = cohesed plasmid; NC = non-cohesed plasmid; SC = supercoiled plasmid; N = nicked plasmid; L = linear plasmid. **B**, **C**. Either EcoRI digestion (**B**) or TEV protease cleavage (**C**) resulted in the conversion of the cohesed form to the non-cohesed form, as revealed by native agarose gel electrophoresis. **D**. G1-arrested cells were conditioned in galactose (as in Figure 5) and released into the cell cycle in galactose medium lacking or containing nocodazole. Metaphase cells from the nocodazole-free medium were harvested at 75 min after release (see also Figure 5A), and from nocodazole containing medium at 90 min. The G2/M arrest induced by nocodazole was ~90%. Cleared cell lysates were subjected to sucrose gradient sedimentation, and fractions were probed for cohesed and non-cohesed forms of pSG6.

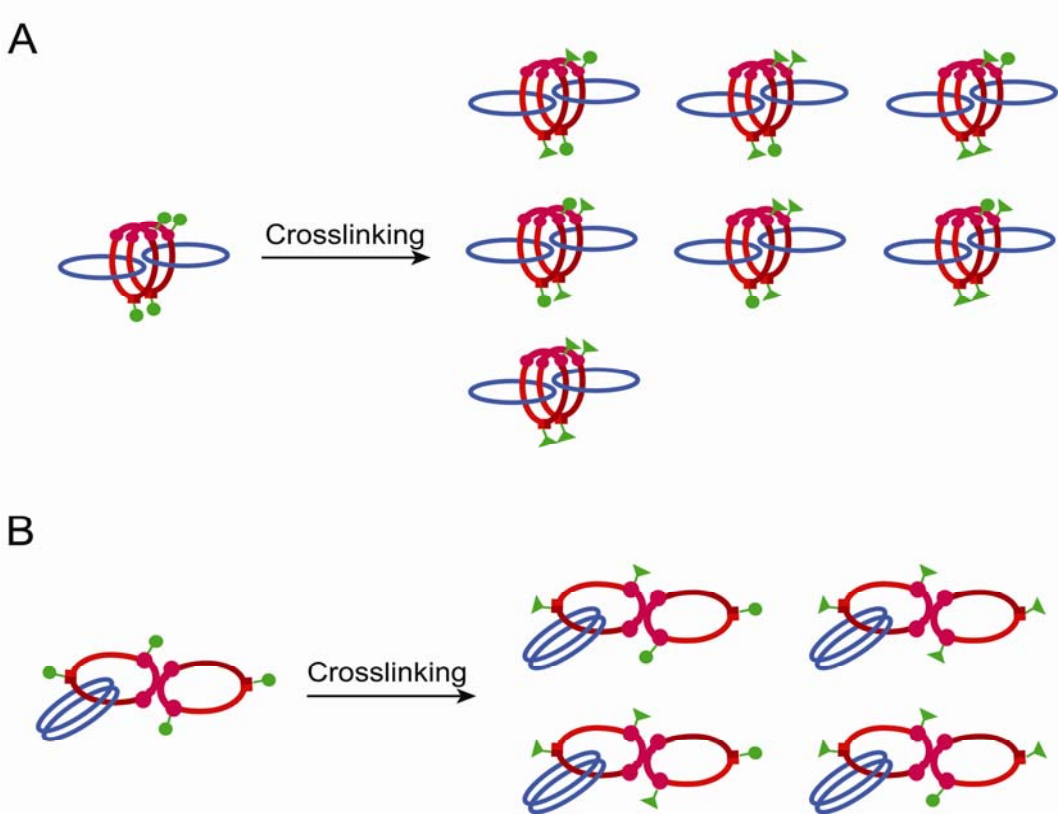

Figure S6. Ruling out two or more copies of a monomeric cohesin ring around minichromosome sisters. In the study by Haering et al. (2), formation of a covalently closed ring of a cohesin monomer required crosslinking two pairs of cysteines (green circles) to form chemical bridges (green triangles) between them. The probability of crosslinking a cysteine pair 'p' was ~55% (see Figure 6). **A**. For the case of two monomeric cohesin rings formed around sister minichromosomes, the probability of their SDS-resistant entrapment by crosslinking $P = 1-[1-p^2]^2 = 51\%$. Two crosslinks formed in either cohesin ring or both rings will entrap DNA sisters. **B**. For the cohesin hand-cuff, physical or topological, with DNA sisters confined to one ring alone, $P = p^2 = 30\%$. The observed value for $P = 30\% = p^2$ is consistent with a monomeric cohesin ring or only one of the two hand-cuff rings entrapping both DNA sisters. It is inconsistent with two or more monomeric cohesin rings surrounding the DNA sisters.

Table S1. The yeast strains used for this study and their genotypes are listed.

Strain	Genotype	Reference
MAY5402	*MAT***a** *his3 ura3 ade2 trp1 leu2 bar1 cdc20-1* [Cir^{+}]	[3]Hildebrandt and Hoyt, 2001
K11282	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3 ura3 scc1::SCC1-6HA::HIS3, ProtA-TetR::LEU2* [Cir^{+}]	[1]Ivanov and Nasmyth, 2005
K11580	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3 112 his3 ura3 scc1::SCC1(TEV3)-6HA::HIS3 ProtA-TetR::LEU2* [Cir^{+}]	[1]Ivanov and Nasmyth, 2005
MJY4258	*MAT***α** *ade2 trp1 his3 leu2 ura3 scc1::SCC1-6HA::KAN ProtA-TetR::LEU2* [Cir^{+}]	This study
MJY4293	*MAT***a** *ade2 trp1-1 his3 leu2 ura3 scc1::SCC1-6HA::HIS3 ProtA-TetR::LEU2 cdc20-1* [Cir^{+}]	This study
MJY4294	*MAT***a** *ade2 trp1-1 his3 leu2 ura3 scc1::SCC1 (TEV3)-6HA::HIS3 ProtA-TetR::LEU2 cdc20-1* [Cir^{+}]	This study
MJY4292	*MAT***a** *ade2 trp1-1 his3 leu2 ura3 scc1::SCC1 (TEV3)-6HA::HIS3 ProtA-TetR::LEU2 cdc20-1* [Cir^{0}]	This study
MJY4319	*MAT***a**/α *CDC20/cdc20-1 ProtA-TetR::LEU2 scc1::SCC1-13Myc::KAN/scc1::SCC1(TEV3)-6HA::HIS3 trp1-1/trp1-1* [Cir^{+}, pTetO21CEN4]	This study
MJY4324	*MAT***a**/α *cdc20-1/cdc20-1 ProtA-TetR::LEU2 scc1::SCC1-13Myc::KAN/scc1::SCC1(TEV3)-6HA::HIS3 trp1-1/trp1-1* [Cir^{+}, pSG4]	This study
MJY5626	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3 ura3 scc1::SCC1(TEV3)-6HA::HIS3 ProtA-TetR::LEU2* [Cir^{0}, pSG6]	This study
MJY5627	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3 scc1::SCC1(TEV3)-6HA::HIS3 ProtA-TetR::LEU2 GAL-Rep1+Rep2::URA3* [Cir^{0}, pSG6]	This study

References

1. Ivanov, D. and Nasmyth, K. (2005) A topological interaction between cohesin rings and a circular minichromosome. *Cell*, **122**, 849-860.

2. Haering, C.H., Farcas, A.M., Arumugam, P., Metson, J. and Nasmyth, K. (2008) The cohesin ring concatenates sister DNA molecules. *Nature*, **454**, 297-301.

3. Hildebrandt, E.R. and Hoyt, M.A. (2001) Cell cycle-dependent degradation of the Saccharomyces cerevisiae spindle motor Cin8p requires APC(Cdh1) and a bipartite destruction sequence. *Mol Biol Cell*, **12**, 3402-3416.