Supporting Information

Plasmids employed in this study

Two reporter plasmids, p*STB-CEN4* and p*STB-CEN4*' (see Figure 1 of manuscript) were constructed for this study as follows.

p*STB-CEN4* (pCH2): The precursor plasmid of pCH2 was pSG5-1 (1), whose main backbone fragment lacking an approximately 400 bp non-essential region was amplified by PCR using a pair of primers, each of which harbored the BglII recognition sequence. Digestion of this fragment by BglII and self ligation gave rise to pCH1-1. The *CEN4* sequence flanked by two head-to-tail copies of the R recombinase target sites was amplified as a roughly 600 bp DNA that contained a BglII site near each terminus. This fragment was cloned, after BglII digestion, into the BglII site of pCH1-1 to obtain plasmid pCH2-1. pCH2-1 was digested with SalI, and self-ligated to generate pCH2, which did not contain non-yeast sequences. This plasmid was recovered in yeast by transformation, and its authenticity was verified by Southern analysis.

p*STB-CEN4'* (pCH3): To construct pCH3, the *CEN4* DNA was amplified by PCR into an approximately 200 bp fragment carrying a BglII site at its left and right ends. Following BglII digestion, this fragment was cloned into the BglII site of pCH1-1 (see above) to generate pCH3-1. pCH3, the self-ligation product of SalI digested pCH3-1, was introduced into yeast by transformation, and its authenticity ascertained as described for pCH2.

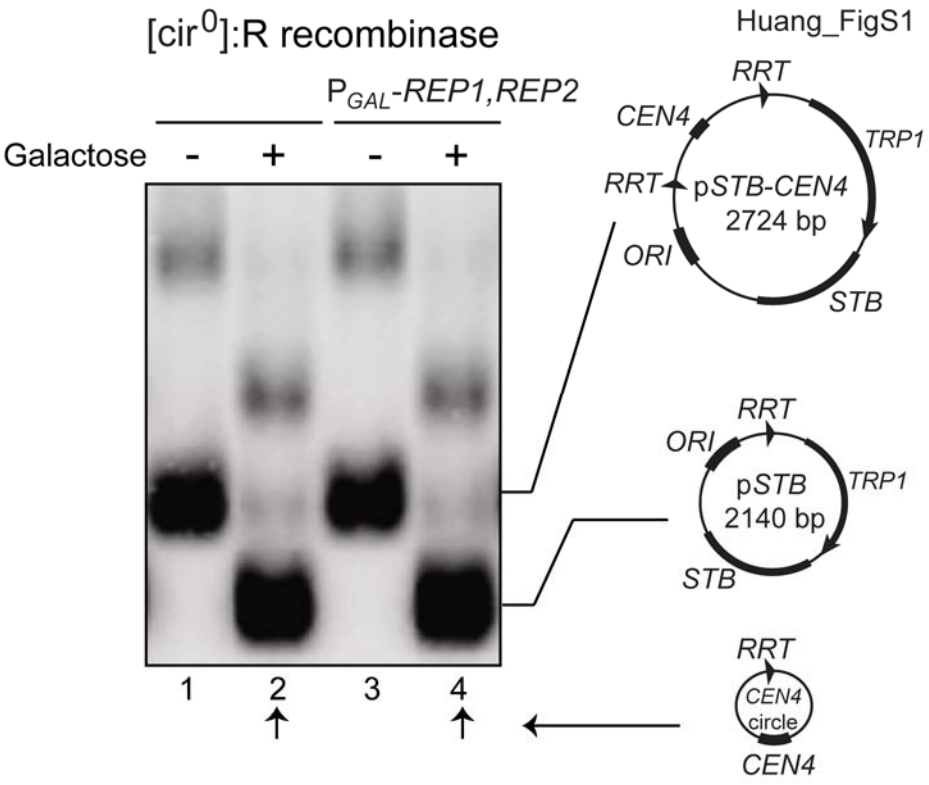

Figure S1. Resolution of *STB* and *CEN* into two separate circles from a parent reporter plasmid harboring both these loci. The reporter plasmid p*STB-CEN4* was resident in two matched host strains, both [cir^0] and harboring a pair of galactose inducible copies of the R recombinase gene. One of the two strains was also galactose inducible for *REP1* and *REP2*. Raffinose grown cells were arrested in G1 using α factor (lanes 1 and 3), and were then shifted to galactose for 2 hr (lanes 2 and 4) in the presence of α factor. Recombination by the induced R recombinase was nearly quantitative under these conditions, yielding p*STB* and the *CEN*4 circle. DNA prepared from cells before and after recombinase induction was subjected to Southern analysis. The parent plasmid and p*STB* formed from it were revealed by using a radio-labeled hybridization probe specific to the *TRP1* marker harbored by them. The 584 bp *CEN4* circle, which cannot hybridize to this probe, migrated below the bottom of the gel section depicted here.

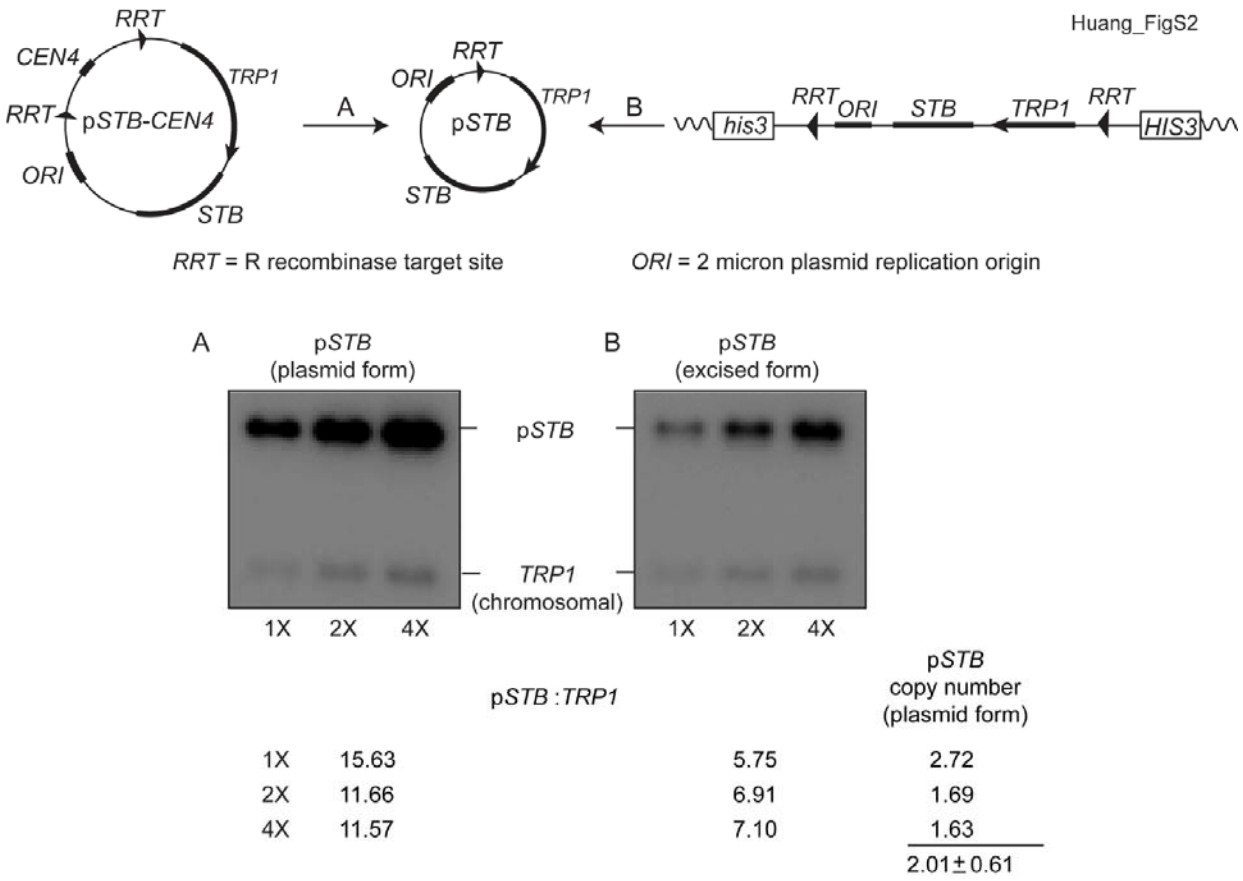

Figure S2. Copy number of the p*STB* plasmid generated by excision of *CEN4*. For estimating the copy number of the p*STB* plasmid generated as described under Figure S1, normalization was performed against a single copy of the linear form of the plasmid integrated at the *HIS3* locus on chromosome XV in the [cir^0] host strain harboring the R recombinase gene (in two copies). The two experimental strains, one containing p*STB-CEN4* (**A**) and the other the integrant (**B**), were arrested in G1, and the R recombinase was induced to generate p*STB*, by deleting *CEN4* in one case and excising the integrant in the other. After isolation of total DNA and EcoRI digestion, Southern analysis was performed using a radio-labeled probe that hybridizes to *TRP1*. Intensities of the p*STB* bands were normalized against the corresponding intensities of the *TRP1* band derived from the chromosome. The appropriate ratios of these values yielded the copy number of

p*STB* generated from p*STB*-*CEN4* as 2.01± 0.61. The DNA isolation procedure enriches plasmid significantly over chromosomes, as indicated by the underrepresentation of the chromosomal *TRP1* band.

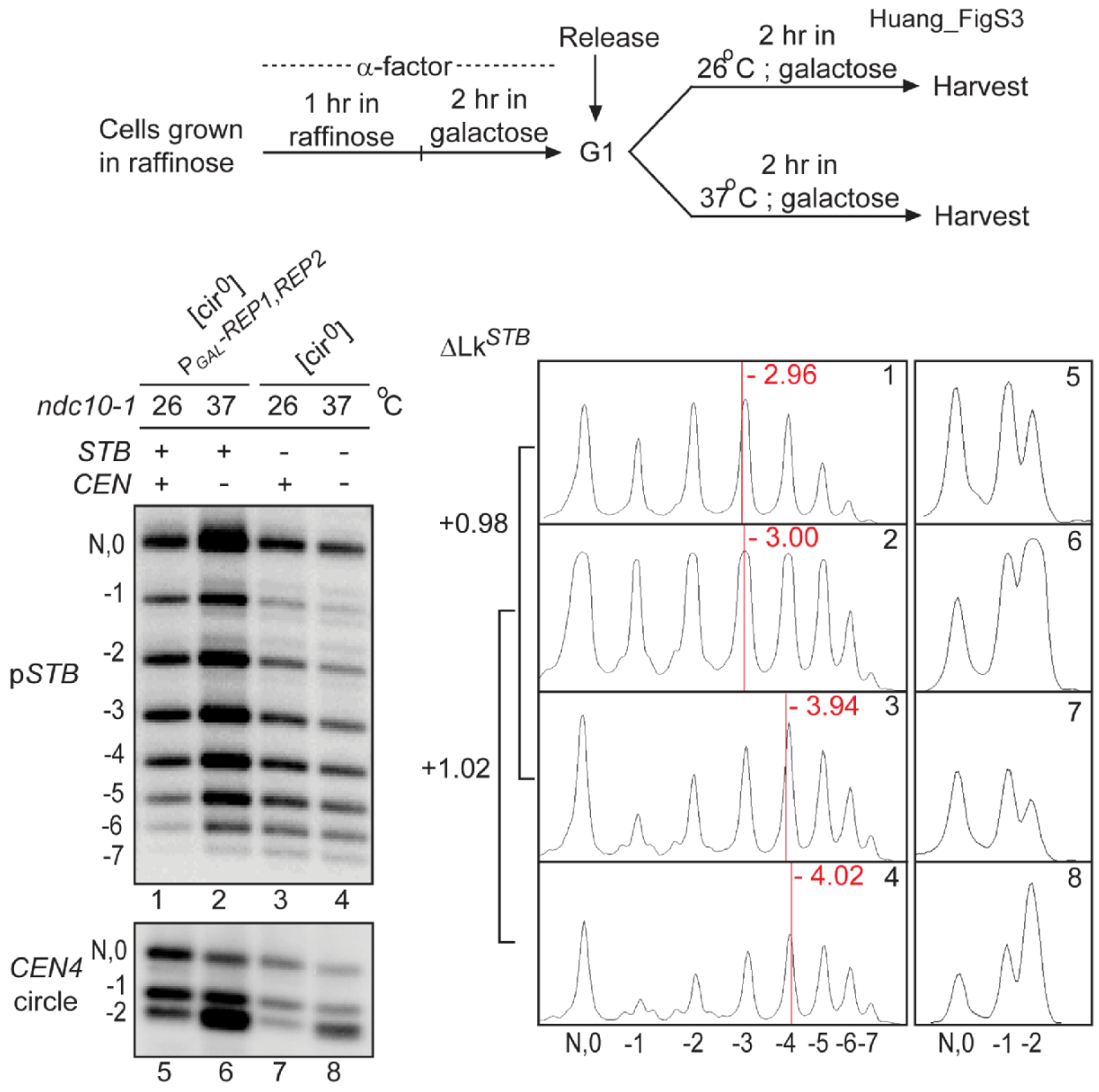

Figure S3. Topological analysis of the *STB* reporter plasmid and the *CEN4* circle after resolution from their parent plasmid by recombination. The p*STB-CEN4* plasmid (see Figure 1 of manuscript and Figure S1) was resident in the indicated [cir^0] *ndc10-1* strains, which also harbored two copies of the galactose inducible R recombinase gene. The plasmid was resolved into p*STB* and the *CEN4* circle in G1 arrested cells by the action of R1 recombinase. Cells were

released from G1 arrest at 26°C or 37°C, permissive or non-permissive, respectively, for Ndc10 function. DNAs obtained from metaphase cells were subjected to topological analysis by electrophoresis in a chloroquine gel (0.3 μg/ml) and Southern hybridization. The ΔLk contributed by the active state of *STB* was derived from the plot of the band intensities shown at the right.

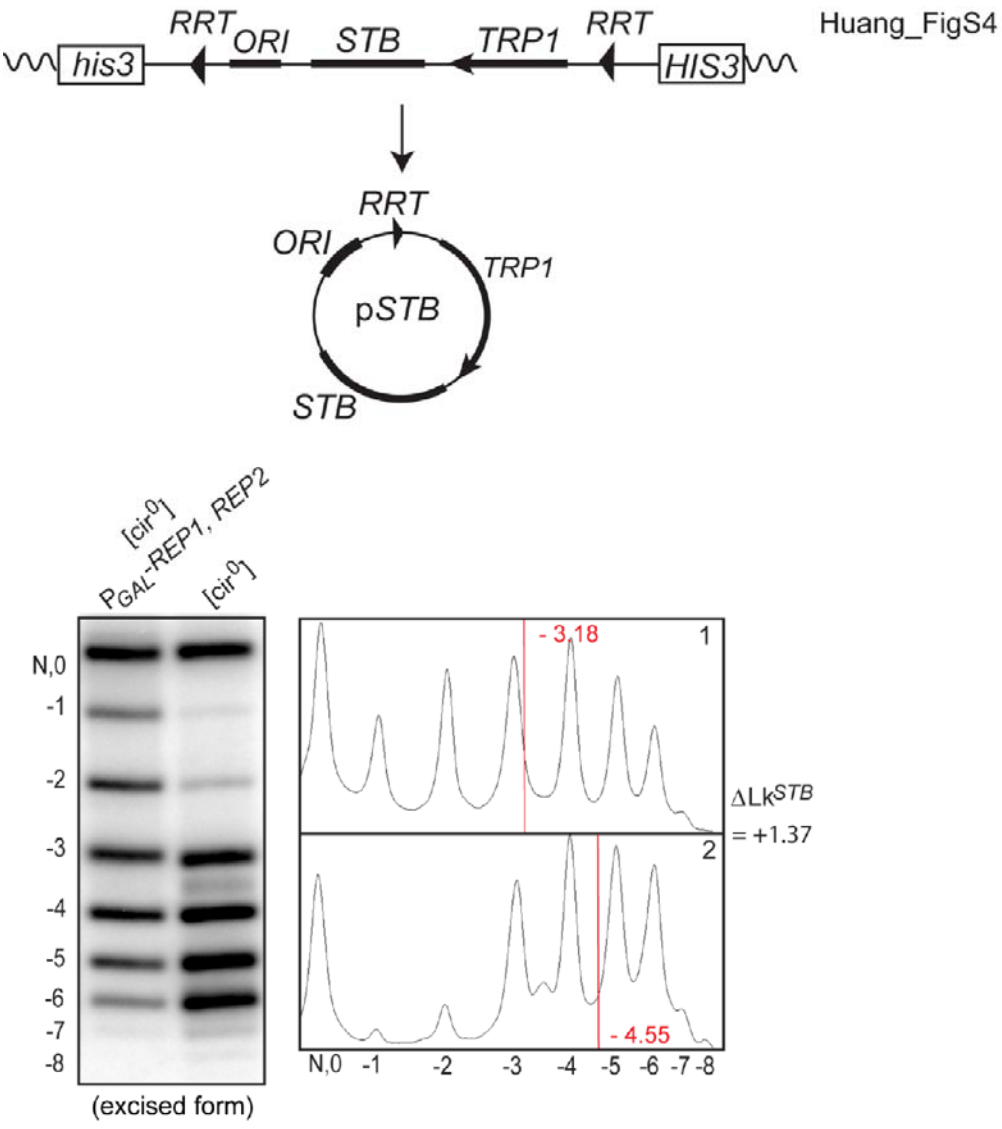

Figure S4. Topology of p*STB* when present at a copy number of precisely one per cell. The single copy circular form of p*STB* was produced in G1 cells from its linear integrated form by the recombination strategy. The topologies of the circle (excised form) in the presence or absence of Rep1 and Rep2 were analyzed at the metaphase stage of released cells. The ΔLk due to the functional *STB* was estimated to be 1.37 from the topoisomer distributions plotted at the right.

Table S1. Values for the linkage change between the functional and non-functional states of *STB* [$\Delta Lk^{STB} = Lk_f - Lk_{nf}$] estimated from the topological assays performed in this study (Figures 2-4) are summarized. The cumulative results signifying $\Delta Lk = +(1.35 \pm 0.23)$, Mean ± SD, would be consistent with the loss of one unit of positive writhe when a Cse4 containing nucleosome is removed from the plasmid-borne *STB* to be replaced in a significant subset of the molecules with a histone H3 containing nucleosome (that induces a unit of negative writhe). The DNA twist is assumed to be maintained constant between the two types of nucleosomes.

Conditions for establishing Cse4-free *STB*	**ΔLk^{STB} (Lk_f - Lk_{nf})**	**Figures displaying relevant assays**
Absence of Rep1, Rep2	+ 1.75	Figure 2
G1 arrested cells	+ 1.20	Figure 3
Nocodazole treated cells	+ 1.25	Figure 3
Absence of Rep1, Rep2	+ 1.23 + 1.32	Figure 4
	+ (1.35 ± 0.23)	

Table S2. Yeast strains employed for this study. The genotypes of the experimental strains used in the present study are listed. The absence of endogenous 2 micron circles in a strain is denoted by the [cir^0] designation. Reporter plasmids harbored by the strains are indicated. These plasmids are schematically drawn in Figure 1 of the manuscript, and their constructions are described above. The strain MJY9001 contained a copy of the p*STB* plasmid integrated at the *HIS3* locus. The circular form of p*STB* could be generated by the R recombinase mediated deletion reaction between a pair of target DNA sites in head-to-tail orientation.

Strains	Genotype
MJY5778	*MATa ade2-1 can1-100 his3-11,15 trp1-1 ura3-1 leu2::(P_{GAL}-RecR::LEU2)X2* [cir^0, p*STB-CEN4*]
MJY5808	*MATa ade2-1 can1-100 trp1-1 ura3-1 leu2::(P_{GAL}-RecR::LEU2)X2 his3-11,15::P_{GAL}-REP1,REP2::HIS3* [cir^0, *pSTB-CEN4*]
MJY5751	*MATa ade2-1 leu2-3,112 his3-11,15 trp1-1 can1-100 ura3*-1::CSE4-*MYC12::URA3 ndc10-1* [cir^0, p*STB-CEN4'*]
MJY5761	*MATa ade2-1 leu2-3,112 trp1-1 can1-100 ura3-1::CSE4-MYC12::URA3 his3-11,15::P_{GAL}-REP1,REP2::HIS3 ndc10-1* [cir^0, p*STB-CEN4'*]
MJY5839	*MATa ade2-1 can1-100 his3-11 trp1-1 ura3-1 leu2::(P_{GAL}-RECR::LEU2)X2 his3-11,15::P_{GAL}-REP1,REP2::HIS3 ndc10-1* [cir^0, p*STB-CEN4*]
MJY5840	*MATa ade2-1 can1-100 his3-11,15 trp1-1 ura3-1 leu2::(P_{GAL}-RECR::LEU2)X2 ndc10-1* [cir^0, p*STB-CEN4*]
MJY9001	*MATa ade2-1 can1-100 leu2::(P_{GAL}-RecR::LEU2)X2 his3-11,15::pSTB::HIS3 ura3-1::P_{GAL}-REP1,REP2::URA3* [cir^0]

Reference

1. Ghosh SK, Huang CC, Hajra S, Jayaram M (2010) Yeast cohesin complex embraces 2 micron plasmid sisters in a tri-linked catenane complex. *Nucleic Acids Res* 38: 570-584.