Supplemental Materials

Table S1. Yeast strains

Strain	Genotype	Reference
MJY4013	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-ADE2	This study
MJY4014	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TRP1-GALp-TAP-REP1	This study
MJY4024	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TAP-REP1	This study
MJY4023	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TAP-REP1-ADE2	This study
MJY4017	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TRP1-GALp-TAP-REP2	This study
MJY4018	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TAP-REP2	This study
MJY4025	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11* [cir^0] 2µ-TAP-REP2-ADE2	This study
MJY5056	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc8-1::RSC8* [cir^0] 2µ-*CEN*-ADE2	This study
MJY5057	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc8-1* [cir^0] 2µ-*CEN*-ADE2	This study
MJY5058	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc58-1::RSC58* [cir$^+$] 2µ-*CEN*-ADE2	This study
MJY5059	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc58-1* [cir$^+$] 2µ-*CEN*-ADE2	This study
MJY5043	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc8-1::RSC8* [cir^0] 2µ-ADE2	This study

MJY3022	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc8-1* [cir^0] 2μ-ADE2	This study
MJY5044	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc58-1::RSC58* [cir$^+$] 2μ-ADE2	This study
MJY3024	*MAT***a** *his3 ura3 ade2 trp1 leu2 can1 rsc58-1* [cir$^+$] 2μ-ADE2	This study
MJY3162	*MAT***a** *his3 ura3 ade2 trp1 leu2 RSC2-13Myc::kan MX* [cir$^+$]	This study
MJY3219	*MAT***a** *his3 ura3 ade2 trp1 leu2 RSC8-13Myc::HIS3* [cir$^+$]	This study
MJY3221	*MAT***a** *his3 ura3 ade2 trp1 leu2 RSC58-13Myc::HIS3* [cir$^+$]	This study
MJY3220	*MAT***a** *his3 ura3 ade2 trp1 leu2 STH1-13Myc::HIS3* [cir$^+$]	This study
MJY5005	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 KIP1-13MYC::HIS3* [cir$^+$]	Cui et al., 2009
SBY617	*MAT***a** *ade2-1 ura3-1 leu2-3, 112 his3-11 trp1-1 can1-100 bar1⊿ CSE4-MYC12::URA3* [cir$^+$]	Buvelot et al., 2003
MJY5036	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 MCD1-3HA::KanMX RSC2-13Myc::HIS3* [cir$^+$]	This study
MJY5037	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 MCD1-3HA::KanMX RSC8-13Myc::HIS3* [cir$^+$]	This study
MJY5038	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 MCD1-3HA::KanMX RSC58-13Myc::HIS3* [cir$^+$]	This study
MJY5039	*MAT***a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 MCD1-3HA::KanMX STH1-13Myc::HIS3* [cir$^+$]	This study
MJY5040	*MAT* **a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 RSC2-13MYC::KanMX STH1-3HA::HIS3* [cir$^+$]	This study
MJY5041	*MAT* **a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 RSC2-13MYC::KanMX RSC8-3HA::HIS3* [cir$^+$]	This study
MJY5042	*MAT* **a** *ade2-101 ura3-1 leu2-3, 112 trp1 his3-11 RSC2-13MYC::KanMX RSC58-3HA::HIS3* [cir$^+$]	This study
K699	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 GAL psi+* [cir$^+$]	Schwob and Nasmyth, 1993

K3415	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 clb5::URA3 clb6::LEU2 GAL psi+* [cir$^+$]	Schwob and Nasmyth, 1993
MJY5045	*MAT***a** *K699 ade2-1 trp-1can1-100 leu2-3, 112 his3-11, 15 ura3 GAL psi+ RSC8-13MYC::HIS3* [cir$^+$]	This study
MJY5046	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 clb5::URA3 clb6::LEU2 GAL psi+ RSC8-13MYC::HIS3* [cir$^+$]	This study
K5601	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 GAL psi+ GPD-TK (thymidine kinase)-URA3 (5 copies) (20.09.92)* [cir$^+$]	Kitamura et al., 2006
MJY5047	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 GAL psi+ GPD-TK (thymidine kinase)-URA3 (5 copies) (20.09.92) ADH-hENT1::LEU2* [cir$^+$]	This study
MJY5048	*MAT***a** *ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 GA L psi+ GPD-TK (thymidine kinase)-URA3 (5 copies) (20.09.92) clb5::TRP1 clb6::HIS3 ADH-hENT1::LEU2* [cir$^+$]	This study
K4675	*MAT***a** *ade2-1 can1-100 leu2-3, 112 his3-11, 15 GAL cdc6::hisG trp1-1 ura3::URA3 GAL-ubiR-CDC6* [cir$^+$]	Piatti et al., 1996
MJY5060	*MAT***a** *ade2-1::pRS402-GFP-LacI, can1-100, (leu2::Gal-RecR::LEU2)X2 his3-11,15::pRS403-RS-ori-STB-LacO-RS::HIS3, trp1-1,ura3-1*	This study
MJY5061	*MAT***a** *ade2-1::pRS402-GFP-LacI, can1-100, (leu2::Gal-RecR::LEU2)X2 his3-11,15::pRS403-RS-ori-STB-LacO-RS::HIS3, trp1-1,ura3-1::pRS406-ADH1-REP1 ADH1-REP2::URA3*	This study
MJY5062	*MAT***a** *ade2-1::pRS402-GFP-LacI, can1-100, (leu2::Gal-RecR::LEU2)X2 his3-11,15::pCM218(pRS403-RS-ori-STB-LacO-RS::HIS3, trp1-1,ura3-1:: pRS406-GAL1-REP1 GAL10-REP2::URA3)*	This study
MJY4122	*MAT***a** *his3 ura3 ade2::GFP-LacI::ADE2 trp1 leu2:: YCp111-LacO::LEU2 can1 rsc8-1* [cir^0]	This study
MJY5051	*MAT***a** *his3 ura3 ade2::GFP-LacI::ADE2 trp1 leu2::YCp111-LacO::LEU2 can1 rsc58-1* [cir$^+$]	This study

The strains constructed by us or obtained from other laboratories for the present studies are listed. The relevant genotypes are indicated. The list is arranged to conform to the sequence in which the results are presented in the main text. Strains MJY4013 to MJY4025 were employed for affinity purification of Rep protein containing complexes and mass spectrometry (Table 1), MJY5056 to MJY3024 for plasmid stability assays (Figure 1), MJY3162 to MJY5042 for short interval chromatin immunoprecipitation (ChIP) assays (Figure 2) and K699 to K4675 for ChIP under replication delayed or blocked conditions (Figures 3 and 4). MY5060 to 5062 were the strains in which the segregation of a reporter plasmid excised during G1 from its integrated state was assayed during the ensuing cell cycle (Figure 5). Strains MJY4122 and MJY5051 were utilized for measurement of fluorescence intensities of plasmid foci in G1 and G2/M cells (Figure S2). The relevant references to a subset of the strains assembled in the Table are listed below.

1. Buvelot, S., Tatsutani, S.Y., Vermaak, D. and Biggins, S. (2003) The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. *J Cell Biol*, **160**, 329-339.

2. Cui, H., Ghosh, S.K. and Jayaram, M. (2009) The selfish yeast plasmid uses the nuclear motor Kip1p but not Cin8p for its localization and equal segregation. *J Cell Biol*, **185**, 251-264.

3. Hajra, S., Ghosh, S.K. and Jayaram, M. (2006) The centromere-specific histone variant Cse4p (CENP-A) is essential for functional chromatin architecture at the

yeast 2-micron circle partitioning locus and promotes equal plasmid segregation. *J Cell Biol*, **174**, 779-790.

4. Kitamura, E., Blow, J.J. and Tanaka, T.U. (2006) Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories. *Cell*, **125**, 1297-1308.

5. Piatti, S., Bohm, T., Cocker, J.H., Diffley, J.F. and Nasmyth, K. (1996) Activation of S-phase-promoting CDKs in late G1 defines a "point of no return" after which Cdc6 synthesis cannot promote DNA replication in yeast. *Genes Dev,* **10**, 1516-1531.

6. Schwob, E. and Nasmyth, K. (1993) CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in Saccharomyces cerevisiae. *Genes Dev*, **7**, 1160-1175.

7. Taneda, T. and Kikuchi, A. (2004) Genetic analysis of RSC58, which encodes a component of a yeast chromatin remodeling complex, and interacts with the transcription factor Swi6. *Molecular genetics and genomics : MGG*, **271**, 479-489.

Figure S1

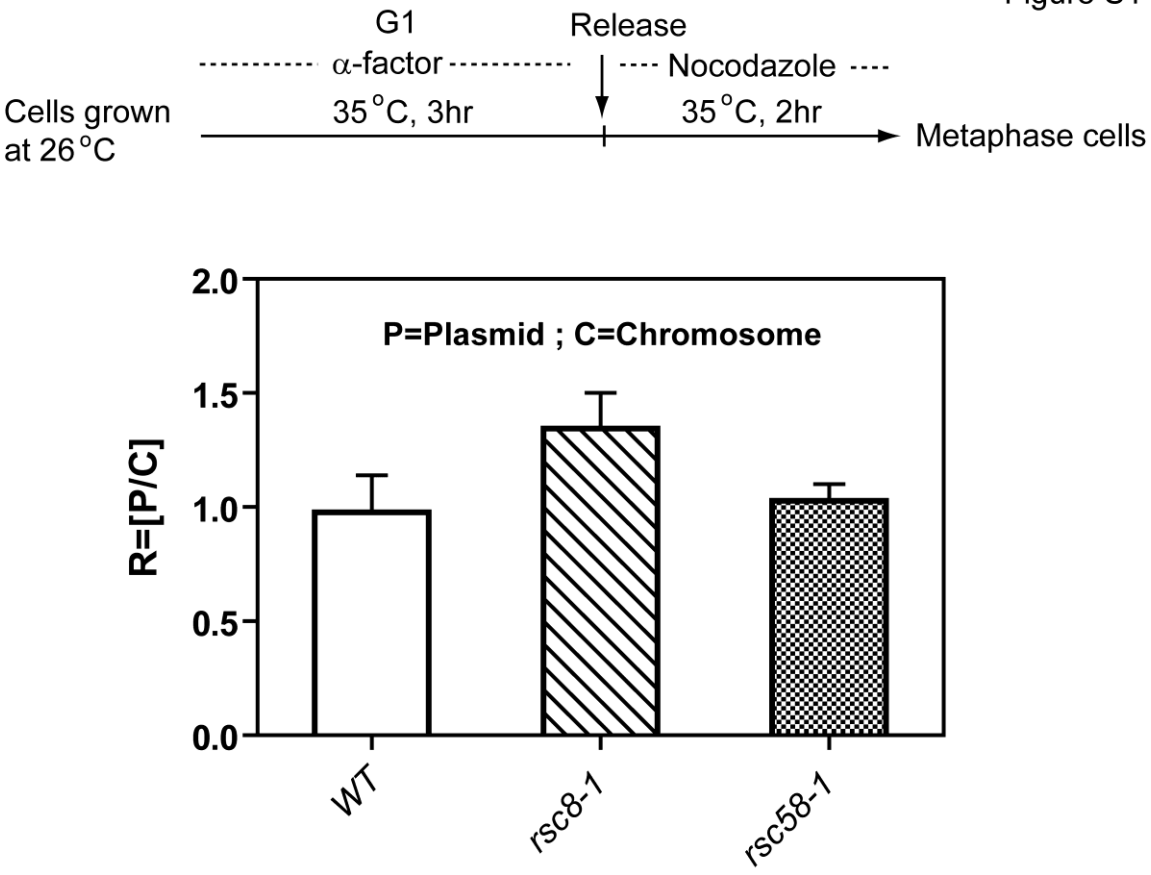

Figure S1. Copy number of the *CEN* reporter plasmid employed in the stability assays summarized in Figure 1. Cells grown to early exponential phase were arrested by α factor in G1, shifted to 35°C for 3 hr, and released from arrest at 35°C (semi-permissive for the mutations) in presence of nocodazole. DNA prepared from G1 cells and metaphase cells harvested at 2 hr following release were employed as templates for PCR. At this time point, ~90 percent of the populations showed large budded cells containing a single nucleus (stained with DAPI) located near the bud neck, the expected metaphase phenotype.

Plasmid copy numbers relative to a single copy chromosomal locus (*MET1*) in the wild type and *rsc* mutant strains were determined by real-time PCR using plasmid-specific and chromosome specific primer pairs. The number of PCR cycles Cp and Cc for the plasmid and the chromosomal locus, respectively, to reach a previously standardized threshold within the exponential region of amplification were determined to estimate ΔC as [Cp-Cc] for G1 and metaphase, respectively. The ΔΔC was derived as the difference between ΔC (metaphase) and ΔC (G1).The relative plasmid copy number R was calculated as $R = 1/(2^{\Delta\Delta C})$. The same value for R between the wild type and a mutant indicates that plasmid replication relative to chromosome replication was unaffected by the mutation. The histograms represent the mean ± SD from assays employing three different template DNA amounts . The strains employed for these assays were MJY5057 (*rsc8-1*), MJY5058 (wild type) and MJY5059 (*rsc58-1*) (Table S1).

Figure S2

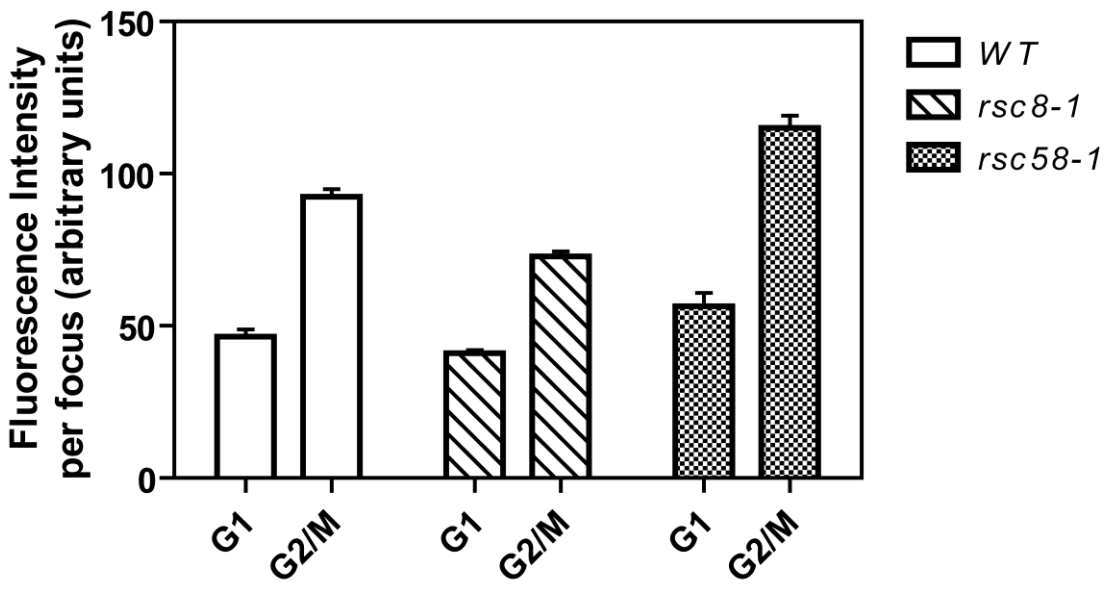

Figure S2. Fluorescence intensities of a *CEN* plasmid harboring *ARS1* From chromosome IV. The scheme for arresting cells in G1, shifting them to 35°C and harvesting metaphase cells after release from arrest were as outlined under Figure S1. Intensities of plasmid foci in G1 and metaphase cells were estimated, and plotted as arbitrary units. The values for each histogram were obtained by scoring 80 each of G1 and metaphase cells. The mean plasmid intensity in metaphase was approximately double that in G1 for the wild type and mutant strains, suggesting that these mutations did not affect the replication potential of *ARS1* present on the plasmid. The analysis was performed in strains MJY4122 (*rsc-8-1*) and MJY5051 *(rsc58-1*) and an isogenic wild type strain.

Figure S3

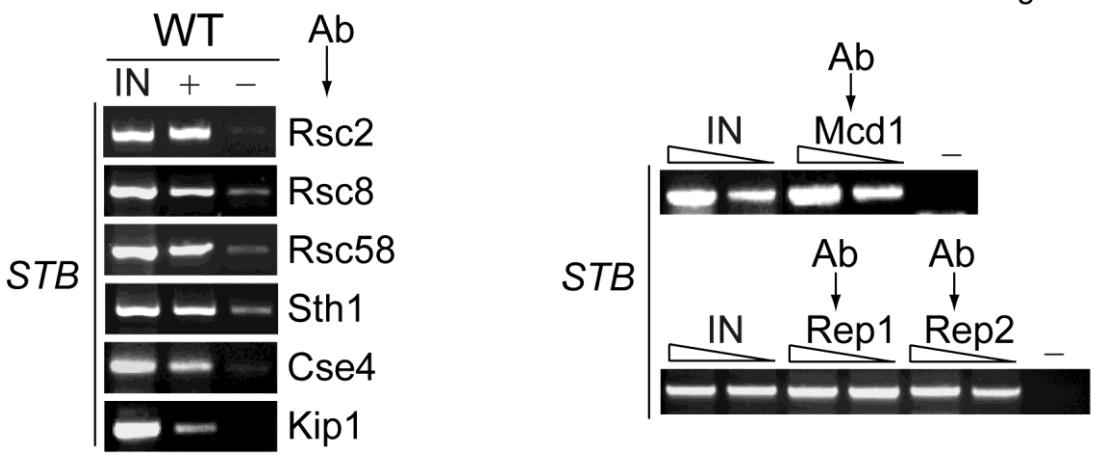

Figure S3. The associations of Rep1, Rep2 and host proteins with the *STB* locus detected by chromatin immunoprecipitation (ChIP). The ChIP assays were carried out in cell populations grown to mid-log phase. Rep1 and Rep2 were immunoprecipitated with antibodies to the native protein. All the other proteins were epitope tagged by the Myc epitope, and were immunoprecipitated with antibodies directed to Myc. The input DNA (IN) was isolated from one-fiftieth the number of cells used for ChIP (+) or for mock immunoprecipitation (-). The DNA preparations were dissolved in the same final volume. In the panel to the left, the signals in each lane of a row were obtained using equal aliquots of DNA as template in the PCR reactions. In the panel to the right, the template DNA amount used for PCR in the left lanes of the input and immunoprecipitated samples were twice that in the right lanes. The negative controls utilized the higher of the two aliquots.

Figure S4

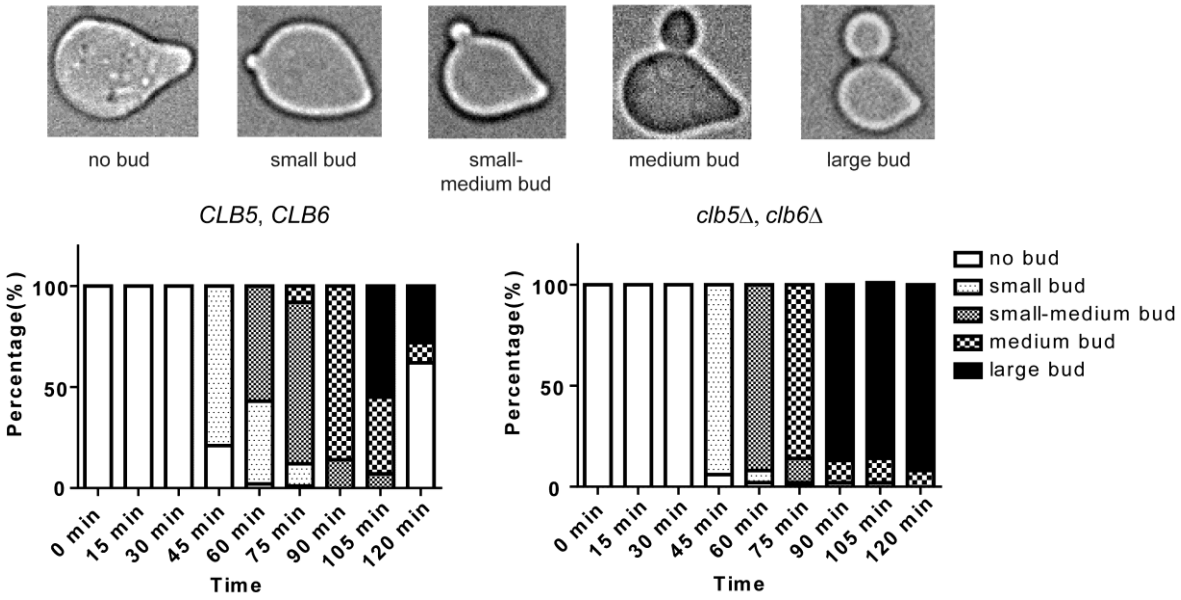

Figure S4. Budding patterns of wild type and *clb5*Δ, *clb6*Δ strains after release from G1 arrest. Cells were scored for their phenotypes at time zero (just prior to release) and at various times after release from G1. The patterns categorized as 'no bud' to 'large bud' are depicted in the top row. The percentages were derived from at least 100 cells analyzed at each time point.