Cui et al., http://www.jcb.org/cgi/content/full/jcb.200810130/DC1

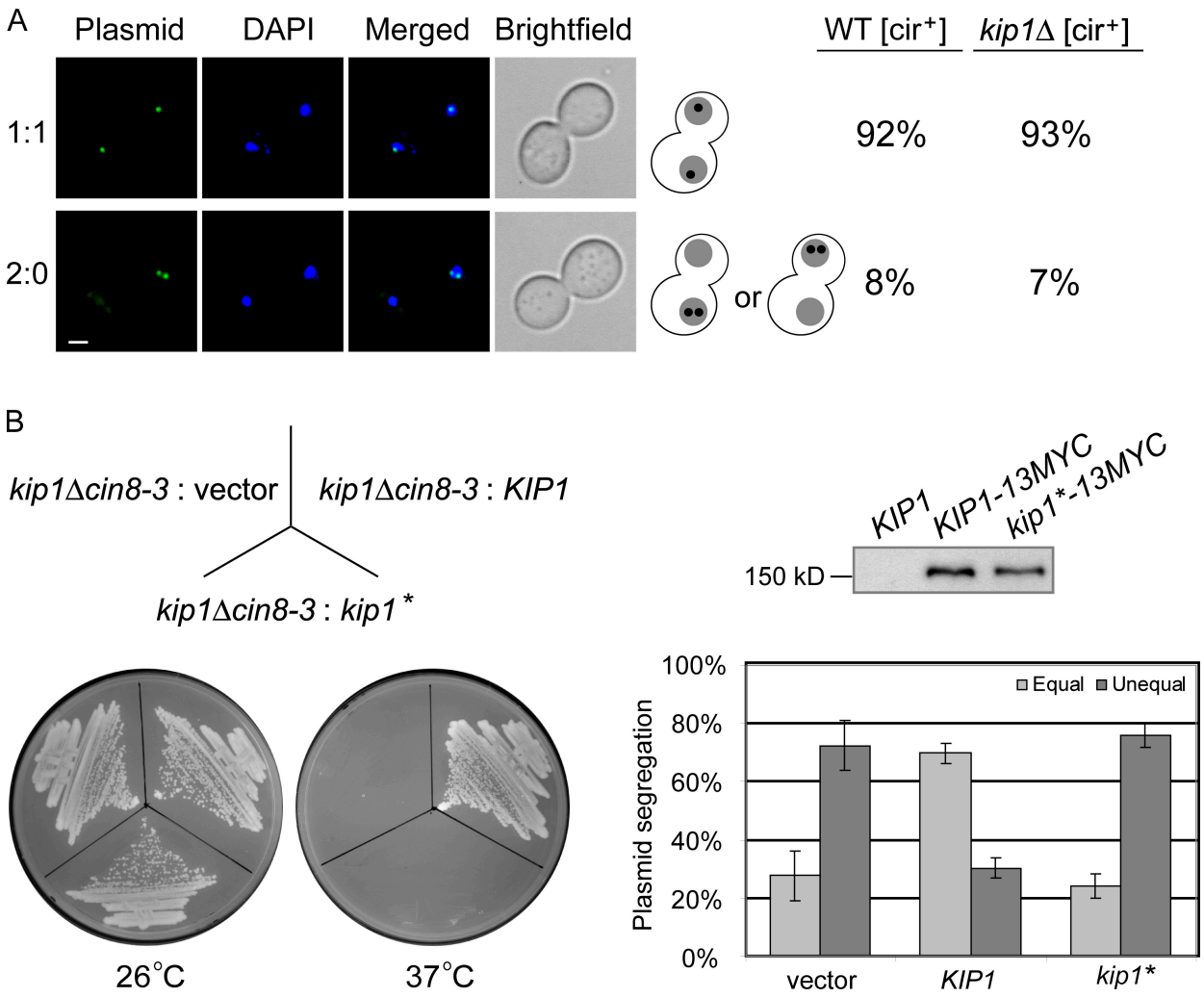
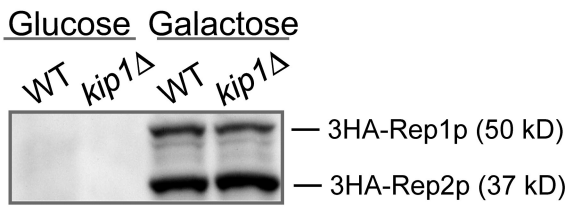


Figure S1. **Segregation of a *CEN* plasmid is not significantly affected by *kip1*Δ; a P-loop mutant of Kip1p cannot support *STB*-based plasmid segregation.** (A) The segregation assay for a *CEN*-based reporter plasmid was similar to that described for a multicopy *STB*-based reporter plasmid (see Fig. 1, A and B, and the relevant text). Bar, 2 µm. (B) The P-loop mutant of Kip1p was obtained by alanine substitutions at the conserved GKT triad of the Walker A motif. The wild-type and mutant (indicated by the asterisk) forms of Kip1p were first assayed for their competence in supporting spindle function in a *kip1Δcin8-3* host strain after shifting from the permissive (26°C; left) to the nonpermissive (37°C; right) temperatures. The expression of the cloned *KIP1* or the P-loop mutant, both tagged with the Myc epitope, was confirmed by Western blot analysis using Myc-directed antibodies. As expected, the P-loop mutant could not complement the lack of Cin8p function. The mutant also failed to support the equal segregation of the *STB*-reporter plasmid.

A

Glucose; [cir$^+$] /*CEN* active		
	WT	*kip1*Δ
1:1	91.9%	92.2%
2:0	8.1%	7.8%

C



B

[cir^0] P*GAL*-*3HA-REP1* P*GAL*-*3HA-REP2*				
	Glucose; *CEN* active		Galactose; *CEN* inactive	
	WT	*kip1*Δ	WT	*kip1*Δ
1:1	98.4%	98.0%	66.3%	24.1%
2:0	1.6%	2.0%	33.7%	75.9%

Figure S2. **Lack of *KIP1* function affects segregation of a single-copy reporter plasmid when it is *STB* based and not when it is *CEN* based.** The segregation of a single-copy *CEN-STB* reporter plasmid pSG1was monitored in wild-type and *kip1*Δ strains, either [cir$^+$] or [cir^0]. The protocol for G1 arrest and release into cell cycle was as described under Fig. 1 C. The results for the [cir$^+$] and [cir^0] strains are assembled in A and B, respectively. The plasmid-borne *CEN* was functional during the cell cycle in glucose medium and nonfunctional during that in galactose medium. Galactose also caused the induction of *REP1* and *REP2* in the [cir^0] host strains, as verified by Western blot analysis using antibodies to HA tag present at the N terminus of each protein (C).

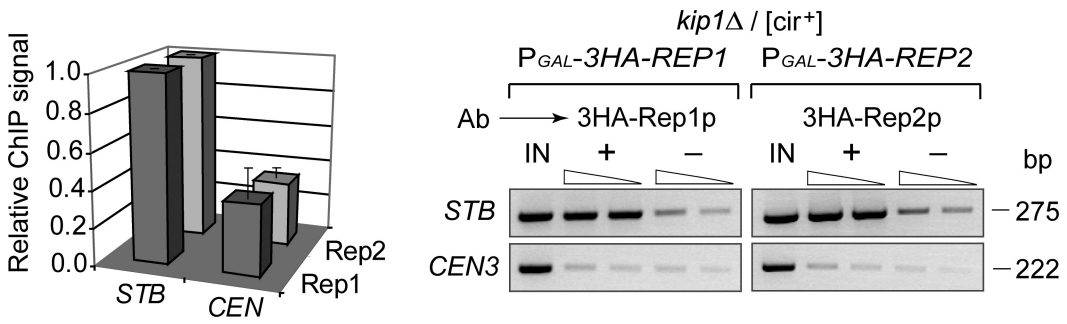


Figure S3. **Association of Rep1p or Rep2p with *STB*, as assayed by chromatin immunoprecipitation (ChIP), is not affected by *kip1*Δ.** The ChIP assays were performed using antibodies to HA-tagged Rep1p or Rep2p expressed by galactose induction in a *kip1*Δ [cir$^+$] host strain. The *CEN3* locus, to which the Rep proteins do not bind, provided the negative control. The data are plotted with ChIP signals at *STB* = 1.

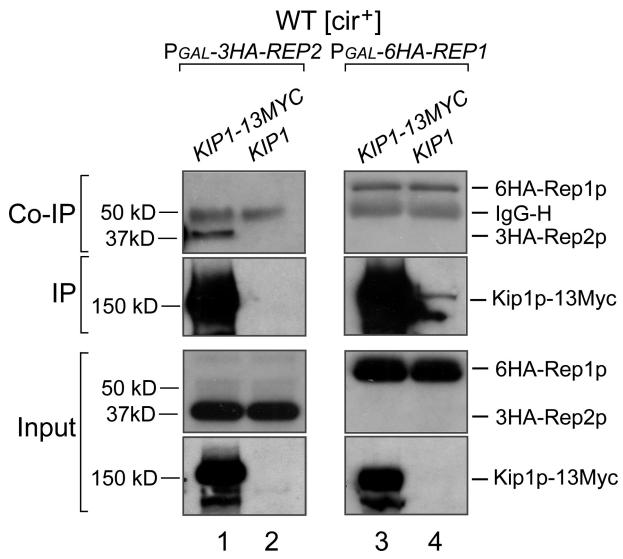


Figure S4. **Immunoprecipitation of Kip1p brings down Rep2p with it.** Immunoprecipitations were carried out in extracts from [cir+] cells expressing Myc-tagged Kip1p constitutively and 6HA-tagged Rep1p or 3HA-tagged Rep2p in a galactose-inducible manner. The three additional HA epitope units in Rep1p helped prevent its comigration with IgG heavy chain (IgG-H) during SDS-PAGE. The presence of Rep1p or Rep2p in the immunoprecipitate obtained with Myc-directed antibodies was probed using HA-directed antibodies. The coimmunoprecipitation of Rep2p, but not Rep1p, with Kip1p is consistent with results from the immunoprecipitation assay carried out in the reverse manner, immunoprecipitating the individual Rep proteins and probing the presence of Kip1p in the immunoprecipitates (see Fig. 5).

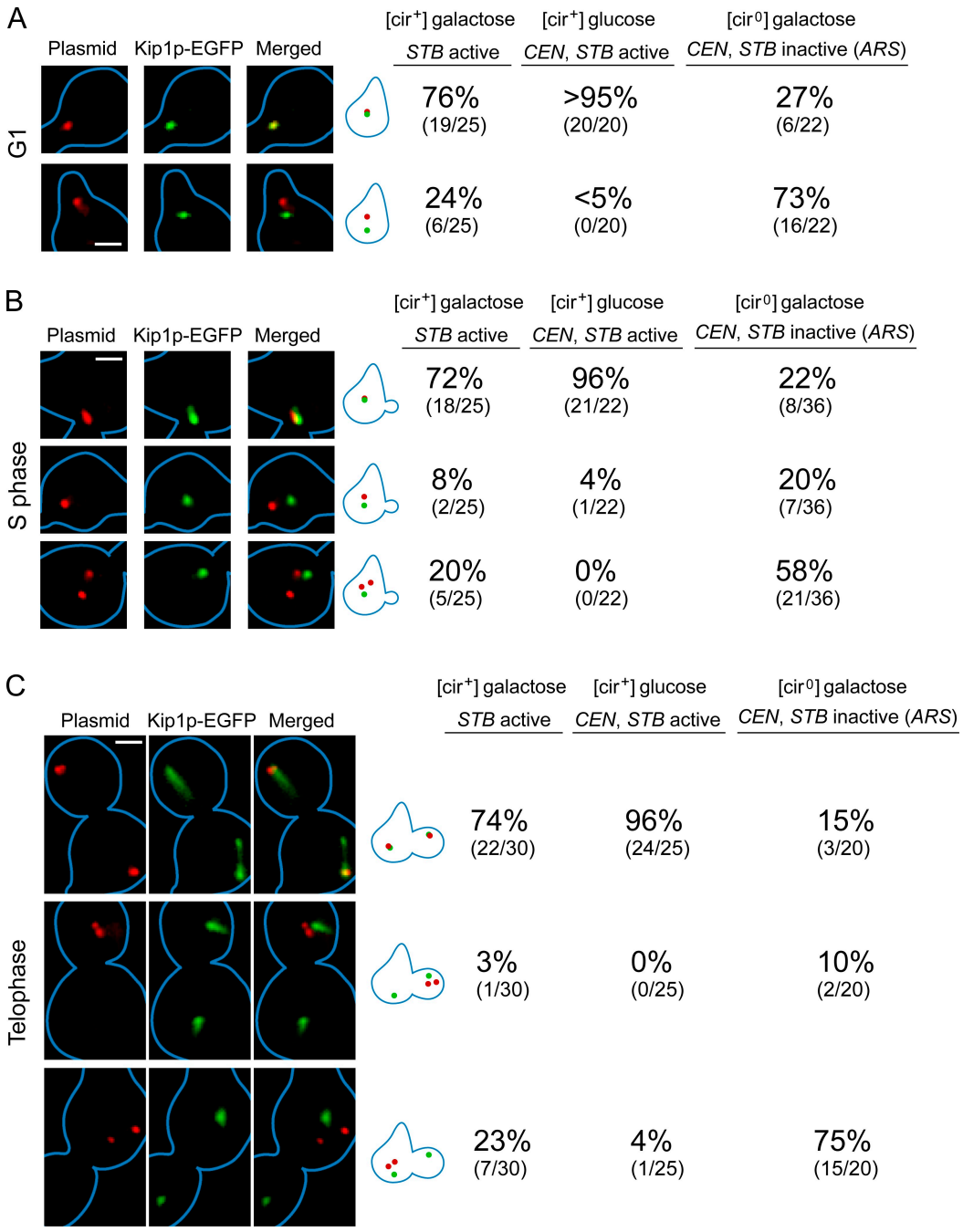


Figure S5. **Localization of a single-copy *CEN-STB* reporter plasmid with respect to Kip1p.** The reporter plasmid was tagged by red fluorescence using $[TetO]_{112}$-RFP-TetR interaction. Kip1p was tagged by green fluorescence by fusing EGFP to its C terminus. The localization of the two entities at distinct stages of the cell cycle was assayed as outlined under Fig. 8 and in the corresponding portions of the text. Assignment of cell cycle stages was according to the characteristic patterns of Kip1p localization described under Fig. 7. The plasmid was made to behave as a *CEN*, *STB*, or *ARS* reporter by appropriately manipulating the carbon source (glucose or galactose) or the $[cir^+]$ or $[cir^0]$ status of the host strain. The data for G1, S, and telophase cells are shown here; those for metaphase and anaphase cells are given in Fig. 8. Each data point was derived from scoring a minimum of 20 cells. Bars, 2 µm. The most significant outcome from these assays was the similarity between the *CEN* and the *STB* reporter plasmids, and their distinction from the *ARS* reporter, in their colocalization with Kip1p at all stages of the cell cycle. There was a high incidence of two plasmid dots for the *ARS* reporter in S phase cells, consistent with a lack of cohesin-mediated pairing of replicated copies of the plasmid. Telophase cells displayed high missegregation frequencies for the *ARS* plasmid. The strong mother bias during missegregation was also evident in these cells.

Table S1. **Yeast strains and plasmids used in this study**

Strain or plasmid	Genotype or salient features	Source/reference
MJY124	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11* [cir$^+$]	Mehta et al., 2002
MJY125	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11* [cir^0]	Mehta et al., 2002
MJY3016	*MAT***a** *ade2::GFP-LacI::ADE2 his3-11 leu2-3, 112 trp1 ura3-1* [cir$^+$]	Ghosh et al., 2007
MJY3017	*MAT***a** *ade2::GFP-LacI::ADE2 his3-11 leu2-3, 112 trp1 ura3-1* [cir^0]	Ghosh et al., 2007
MJY3020	*MAT***a** *his3-11 leu2::GFP-LacI::LEU2 trp1 ura3-1 RFPTetR* [cir$^+$]	Ghosh et al., 2007
MJY3021	*MAT***a** *his3-11 leu2::GFP-LacI::LEU2 trp1 ura3-1 RFPTetR* [cir^0]	Ghosh et al., 2007
JT107	*MAT***a** *ade2-101 ura3-1 his3-11 leu2-3,112 trp1 KIP1-EGFP::URA3 SPC42-CFP::TRP1* [cir$^+$]	Tytell and Sorger, 2006
SBY617	*MAT***a** *ade2-1 ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 bar1Δ CSE4-MYC12::URA3* [cir$^+$]	Buvelot et al., 2003
MJY5020	*MAT***a** *ade2-1 ura3-1 leu2-3,112 his3-11 trp1-1 can1-100 bar1Δ CSE4-MYC12::URA3 kip1::His3MX* [cir$^+$]	Jayaram laboratory collection
MJY311	*MAT***a** *ura3-52 his3-200 ade2-101 lys2-801 leu2-3,112 trp1-901 tyr1-501 gal4-Δ512 gal80-Δ538 ade5::hisG::STB$_{UAS}$-HIS3::HIS3* [cir$^+$]	Yang et al., 2004
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1* [cir$^+$]	Stratagene
MJY5001	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 kip1::His3MX* [cir$^+$]	This study
MJY5002	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 cin8::His3MX* [cir$^+$]	This study
MJY5003	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 kar3::His3MX* [cir$^+$]	This study
MJY5004	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 kip3::TRP* [cir$^+$]	This study
MJY5005	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11 KIP1-13MYC::His3MX* [cir$^+$]	This study
MJY5006	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11 KIP1-13MYC::His3MX* [cir^0]	This study
MJY5007	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11 CIN8-3HA::TRP1* [cir$^+$]	Jayaram laboratory collection
MJY5008	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KAR3-13MYC::His3MX* [cir$^+$]	This study
MJY5009	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KIP3-13MYC::His3MX* [cir$^+$]	This study
MJY5010	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 MCD1-3HA::TRP1* [cir$^+$]	This study
MJY5011	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 MCD1-3HA::TRP1 kip1::His3MX* [cir$^+$]	This study
MJY5012	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 MCD1-3HA::TRP1 cin8::His3MX* [cir$^+$]	This study
MJY5013	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 MCD1-3HA::TRP1 kar3::His3MX* [cir$^+$]	This study
MJY5021	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 MCD1-3HA::KanMX kip3::TRP1* [cir$^+$]	This study

MJY5014	*MAT***a** *ura3 leu2 ndc10-2 KIP1-13MYC::His3MX* [cir$^+$]	This study
MJY5017	*MAT***a** *his3-11 leu2::GFP-LacI::LEU2 trp1 ura3-1 RFPTetR KIP1-EGFP::His3MX* [cir$^+$]	This study
MJY5018	*MAT***a** *his3-11 leu2::GFP-LacI::LEU2 trp1 ura3-1 RFPTetR KIP1-EGFP::His3MX* [cir^0]	This study
MJY5020	*MAT***a** *Lys2-801 trp1-1 ura3-52 leu2-3,112 his3-Δ200 kip1::HIS3 cin8-3*	Clarence Chan, UT Austin
MJY5022	*MAT***a** *ade2-101 ura3-1 leu2-3,112 trp1 his3-11 kip1::His3MX* [cir^0]	This study
MJY5023	*MAT***a** *his3 leu2-3,112 ura3 trp1-1 can1-100 SPC110-RFP::KanMX* [cir$^+$]	Clarence Chan, UT Austin
MJY5024	*MAT***a** *his3 leu2-3,112 ura3 trp1-1 can1-100 SPC110-RFP::KanMX kip1::His3MX* [cir$^+$]	This study
MJY5025	*MAT***a** *his3 leu2-3,112 ura3 trp1-1 can1-100 SPC110-RFP::KanMX* [cir^0]	This study
MJY5026	*MAT***a** *his3 leu2-3,112 ura3 trp1-1 can1-100 SPC110-RFP::KanMX kip1::His3MX* [cir^0]	This study
pSV1	256 copies of Lac operator sequence cloned in YEpLac181 (*LEU2*)	Mehta et al., 2002
pSV4	GFP-Lac repressor cloned in YCpLac33 (*URA3*)	Mehta et al., 2002
pSV5	256 copies of Lac operator sequence cloned in YEpLac112 (*TRP1*)	Mehta et al., 2002
pSG1	P_{GAL1}-*CEN3-STB-ORI* cloned in pSV5	Ghosh et al., 2007
pSG2	P_{GAL1} -*CEN3-STB-ORI* cloned in pRS306XTetO$_{112}$ (*URA3*)	Ghosh et al., 2007
pSV30	pRS402-GFPLacI (*ADE2*)	Hajra et al., 2006
pSV31	pRS406-GFPLacI (*URA3*)	Ghosh et al., 2007
pSV32	pRS405-GFPLacI (*LEU2*)	Ghosh et al., 2007
pSV2	256 copies of Lac operator sequence cloned in YCpLac111 (*LEU2*)	Velmurugan et al., 2000
pSTB	2 micron circle-derived plasmid harboring *ADE2*	Velmurugan et al., 1998
pCM26	P_{GAL1}-*3HA-REP1* in pRS406 (*URA3*)	Jayaram laboratory collection
pCM2	P_{GAL1}-*3HA-REP2* in pRS405 (*LEU2*)	Jayaram laboratory collection
pXY93	*CEN* plasmid expressing class I Rep1p mutant Rep1p (Y317I) by galactose induction	Jayaram laboratory collection
pXY76	*CEN* plasmid expressing class II Rep1p mutant Rep1p (A50D) by galactose induction	Jayaram laboratory collection
pSTB2	*REP2* (under native promoter) cloned into pYES2 vector (*ADE2* and *LEU2*)	Velmurugan et al., 1998
pKT128	EGFP tagging vector (*SpHIS5*)	EUROSCARF
pGAD424	Vector for generating activation domain fusion protein	Clontech Laboratories, Inc.

pHC1	*KIP1-13MYC* (under native promoter) cloned into the *CEN* plasmid pRS414 (*TRP*1)	This study
pHC2	*AD-KIP1* (under *ADH1* promoter) cloned into pGAD424	This study
2μ-ADE2	2 micron plasmid containing *ADE2* insertion	Tsalik and Gartenberg, 1998
pHC3	P-loop mutant of Kip1p-13Myc (under native promoter) in pRS414 (*TRP1*)	This study
pHC4	P_{GAL1}-*6HA-REP1* in pRS406 (*URA3*)	This study

The tabulated yeast strains and plasmids pertain to experiments in the manuscript and Supplemental material.

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