Seybold et al., http://www.jcb.org/cgi/content/full/jcb.201412050/DC1

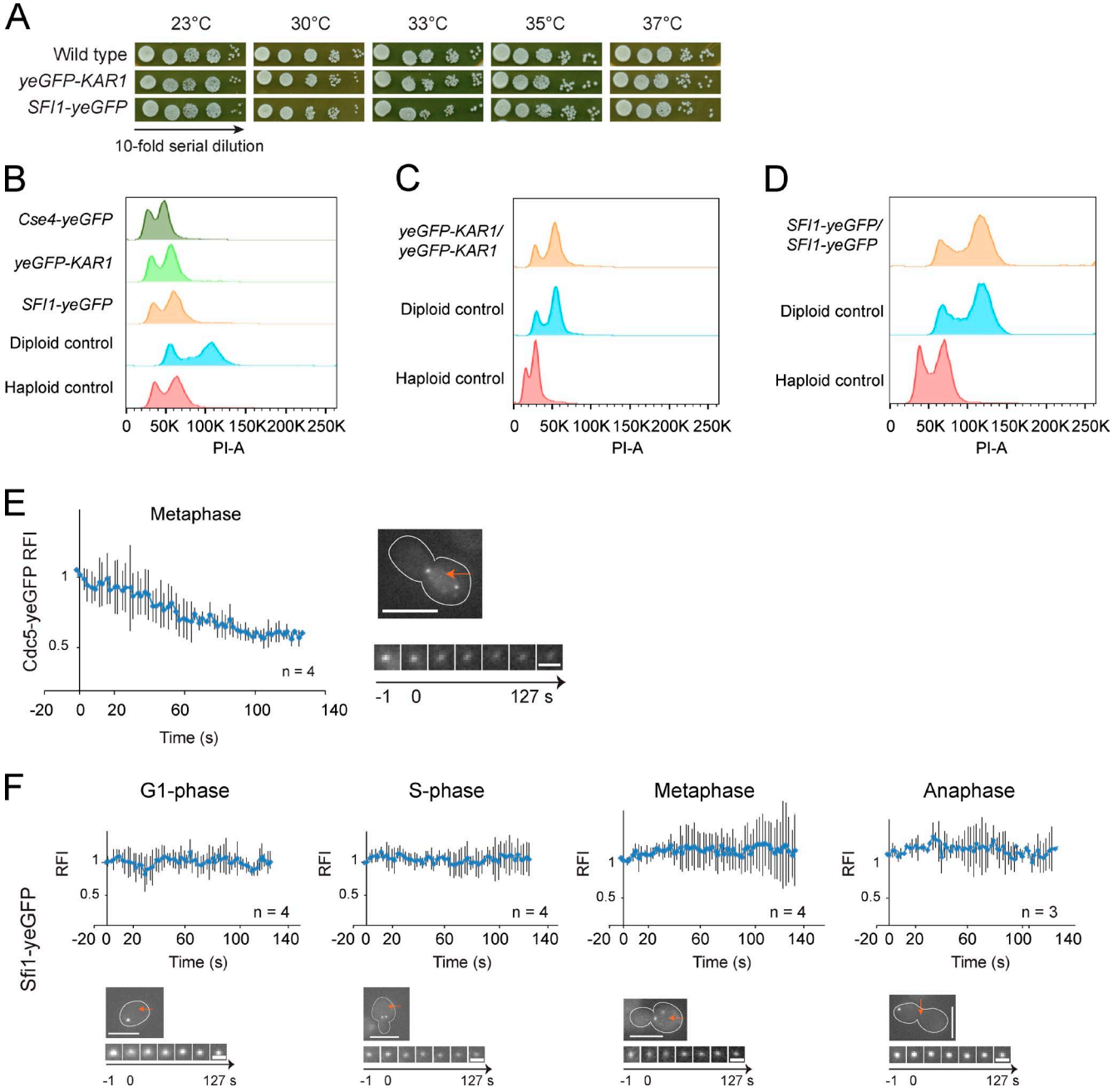

Figure S1. **Coordinated behavior of Kar1 and Sfi1.** (A) Serial dilutions of *yeGFP-KAR1*, *SFI1-yeGFP*, and wild-type cells at different temperatures. (B–D) Flow cytometry analysis of the indicated yeast strains. For each strain, 10,000 cells were counted. (E and F) FLIP of Cdc5-yeGFP (E) or Sfi1-yeGFP (F). The nucleus in E or an area around the SPB in F (indicated by a red arrows) were bleached continuously and the RFI of the yeGFP-tagged protein was measured. Exemplary cells and SPB enlargements at different time points are shown. *n*, number of analyzed SPBs. Error bars indicate SD. Bars: (top panels) 5 µm; (bottom panels) 1 µm.

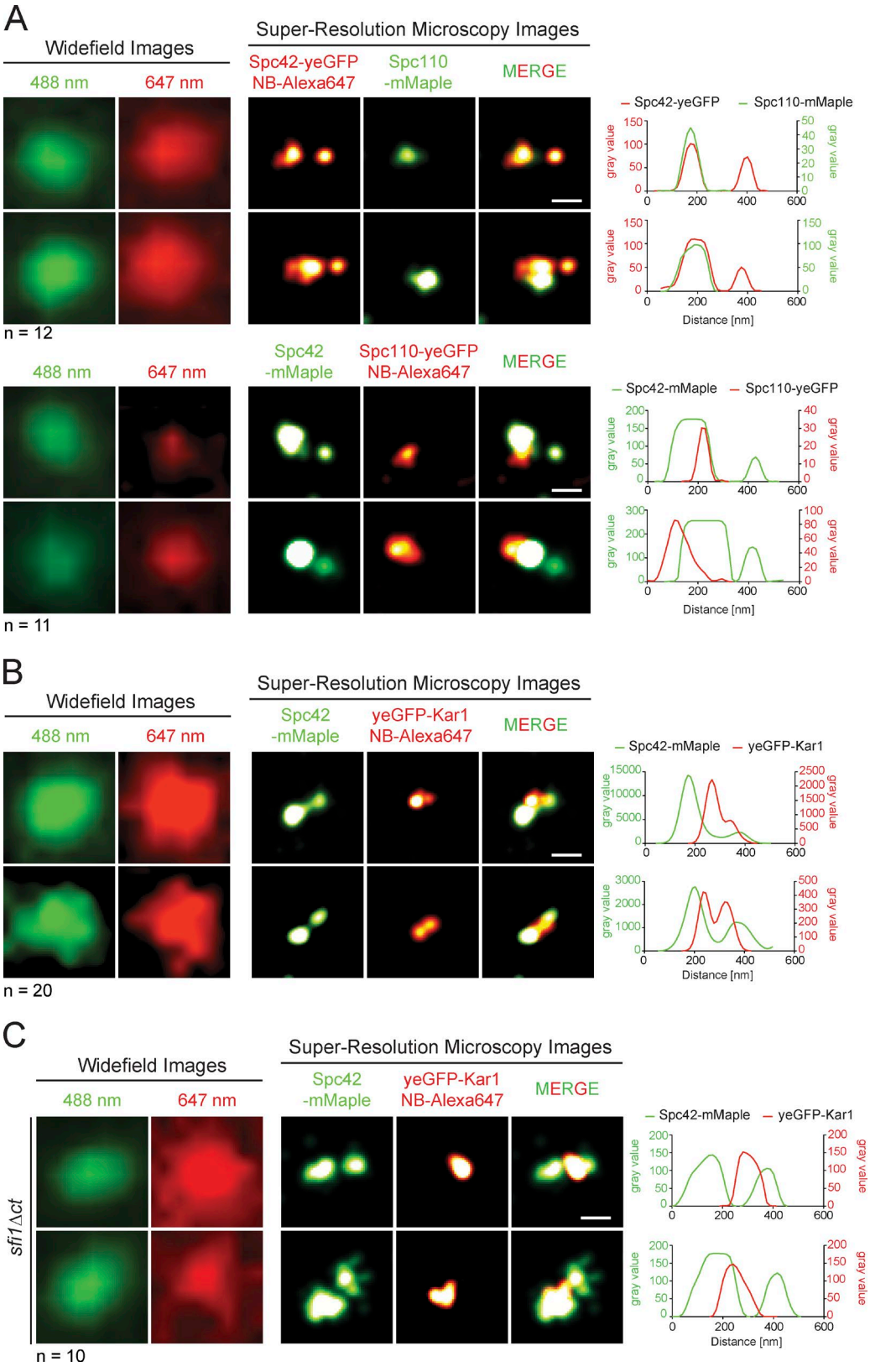

Figure S2. **Kar1 resides in the middle of the bridge in both *SFI1* and *sfi1Δct* cells.** (A) Identification of the mother SPB and its satellite. Combined PALM and dSTORM dual-color images of cells encoding *SPC42-yeGFP* together with *SPC110-mMaple* and vice versa are presented. The corresponding line plot profiles are indicated. (B) The Kar1 signal in the middle of the bridge resolves as two distinct pools. The yeGFP-Kar1 localization was analyzed in *SFI1* cells. Representative pictures of the split Kar1 signal around the bridge center are shown. (C) Kar1 localizes to the bridge center in *sfi1Δct* cells. The yeGFP-Kar1 signal was analyzed relative to the Spc42-mMaple signals and resulted in a single dot, which was centered in the Sfi1 C-terminal overlap zone in all cells investigated. All cells analyzed in A–C were arrested with α-factor before fixation and staining. Data of a single representative experiment out of three repeats are shown. *n*, number of cells analyzed. Bars, 200 nm.

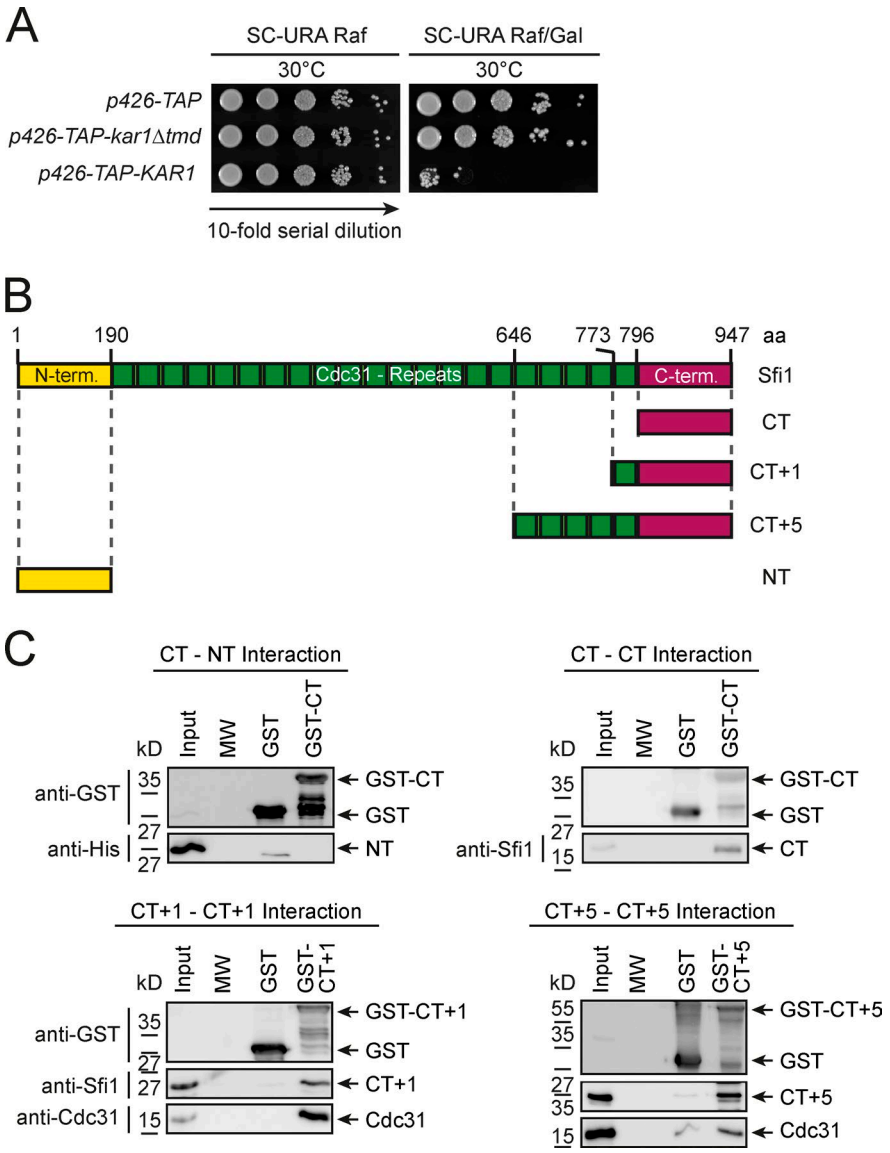

Figure S3. **Sfi1 filaments self-interact via their C termini in vitro.** (A) Overexpression of full-length Kar1 leads to cell inviability. Serial dilutions of cells bearing a galactose-inducible expression plasmid for *TAP-KAR1* or *TAP-kar1Δtmd* were tested for growth on selective media at 30°C. (B) Diagram of Sfrs analyzed in C. (C) Self-interaction ability of Sfrs. The fragments of B were expressed as GST fusions in bacteria, purified, and bound to Glutathione Sepharose. Washed beads were incubated, with *E. coli* lysates expressing the same, but His-tagged, Sfr. All fragments bearing a Cdc31 binding site were coexpressed with Cdc31. Note that this assay does not distinguish between parallel or antiparallel interactions of Sfi1 molecules. Immunoblots with the indicated antibodies are shown. MW, molecular weight marker lane.

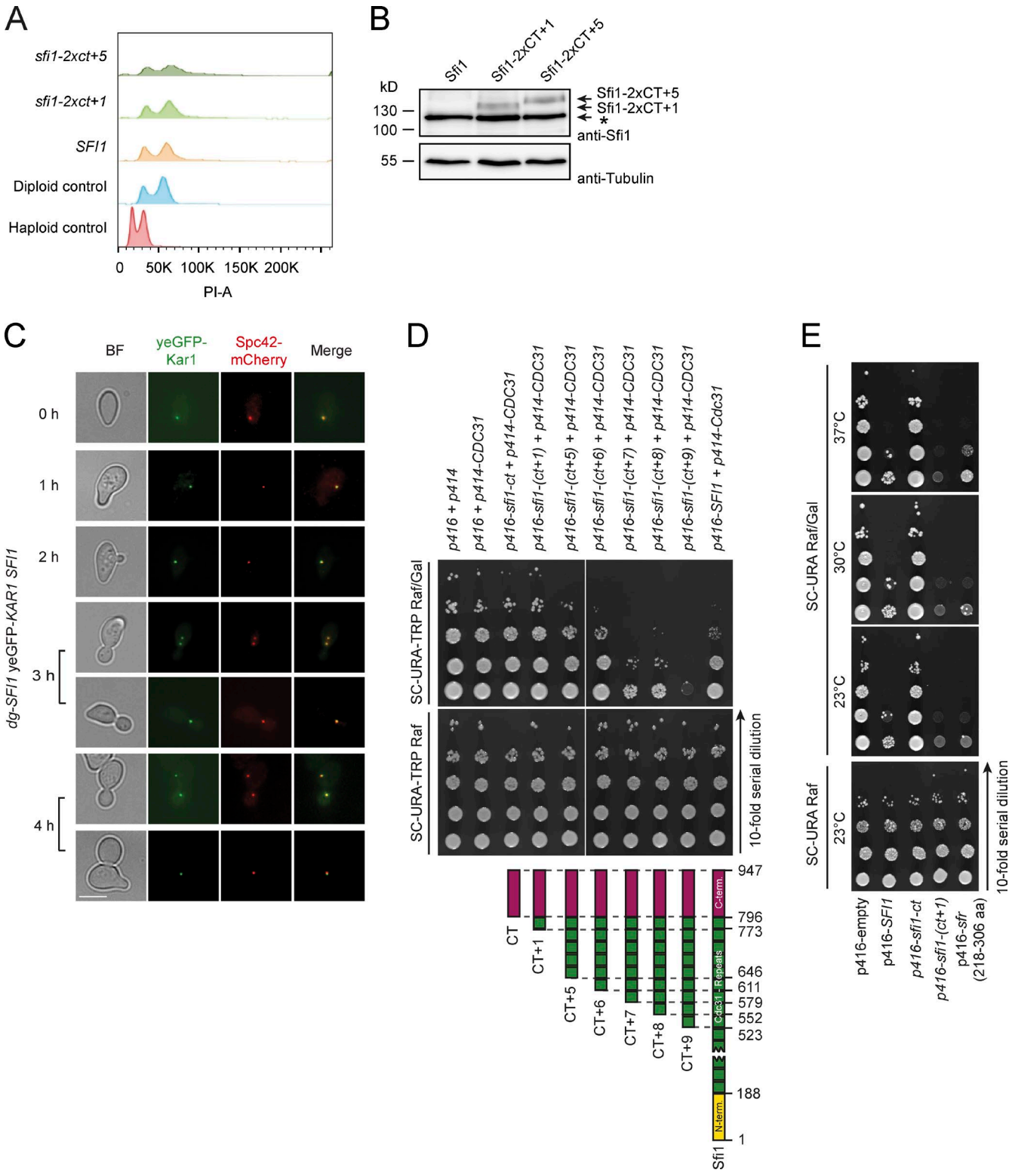

Figure S4. **The C-terminal region of Sfi1 is essential for correct localization of Kar1 to the SPB in vivo.** (A) Flow cytometry analysis of the indicated yeast strains. For each strain, 10,000 cells were counted. (B) Levels of Sfi1-2×CT+1 and Sfi1-2×CT+5. TCA extracts of *SFI1* wild type, *sfi1-2xct+1*, and *sfi1-2xct+5* cells were blotted and detected with the anti-Sfi1 antibody. Tubulin served as a loading control. A cross-reacting band (asterisk) runs with the same size as Sfi1. (C) Localization of yeGFP-Kar1 in *dg-SFI1* cells, expressing a wild-type copy of *SFI1*. yeGFP-Kar1 localization was analyzed upon degradation of *dg-SFI1* at 37°C in cells bearing a wild-type copy of *SFI1*. All cells analyzed (n = 44, per time point) showed no mislocalization of Kar1 from the SPB. Bar, 5 µm. (D) Overexpression of C-terminal *SFI1* fragments in yeast. The indicated fragments were overexpressed in the presence of galactose. Cell growth of serial dilutions was monitored at 23°C on the indicated media plates. All fragments were co-overexpressed with *CDC31*. (E) Overexpression of some *SFI1* constructs without *CDC31* co-overexpression is toxic. Indicated constructs were expressed as in F but without *CDC31* co-overexpression.

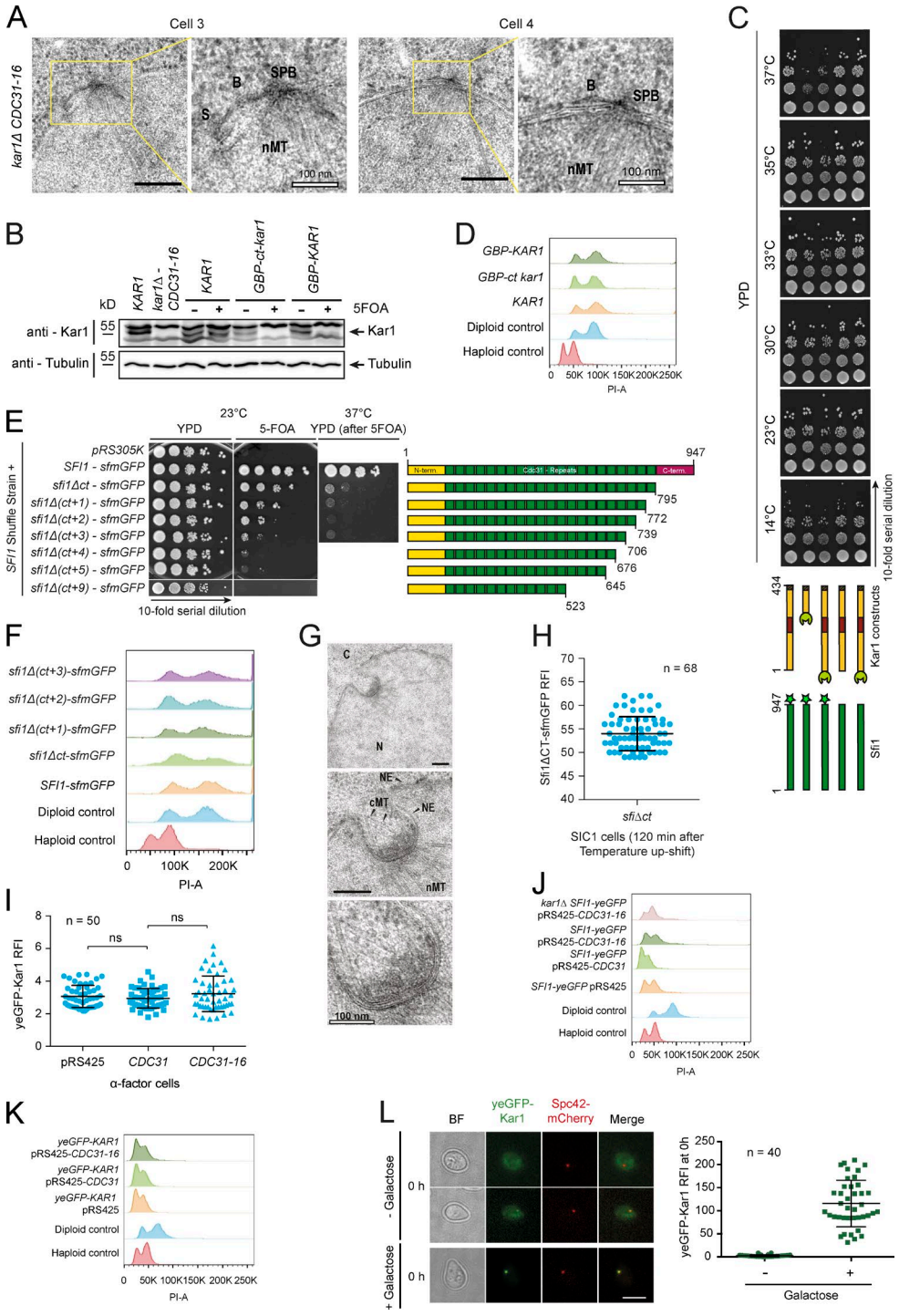

Figure S5. **Bypassing the essential role of *KAR1*.** (A) Additional electron micrographs of *kar1Δ CDC31-16* cells. Bars, 200 nm unless otherwise indicated. See Fig. 5 A for further information. nMT, nuclear microtubules; B, bridge; S, satellite. Boxed regions are enlarged on the right. (B) The presence of *KAR1* in *GBP-kar1* truncation cells. TCA extracts of indicated yeast strains before (–) and after (+) selection on 5-FOA were analyzed by immunoblotting with anti-Kar1 and anti-Tubulin antibodies. (C) Temperature profile of selected *GBP-kar1* strains after 5-FOA selection. See Fig. 6 A for further description. (D) Flow cytometry analysis of the indicated yeast strains. (E) The importance of the C-terminal region of Sfi1 for cell survival. Serial dilutions of cells genomically bearing the indicated *SFI1* truncations were tested for growth on selective media. Survivors on 5-FOA were tested for temperature sensitivity. (F) FACS analysis of strains after 5-FOA from E. (G) Further serial sections of cell 2 shown in Fig. 7 G. The invagination occurring at the duplicated SPBs is indicated. Bars, 200 nm, unless otherwise indicated. (H) Distribution of Sfi1 molecules in *SIC1* arrested *sfi1Δct* cells after 120 min at 37°C. The RFI of *sfi1Δct-sfmGFP* at split SPBs was determined to check the distribution of Sfi1 after SPB splitting. For details see Fig. 7, H and I. (I) Influence of *CDC31* or *CDC31-16* expression on yeGFP-Kar1 abundance at the SPB. The RFI of yeGFP-Kar1 was measured at 23°C in indicated α-factor–arrested cells as described in Fig. 8 A. (J and K) FACS of the indicated yeast strains. (L) Galactose-induced overexpression of Kar1. The SPB-located yeGFP-Kar1 signal intensity was determined 45 min after the addition (+) or no addition (–) of galactose. Representative pictures as well as the respective quantification of the RFI are shown. Kar1 signal intensity at the SPBs increased ~44-fold. ns, $P > 0.05$. Error bars indicate SD. *n*, number of analyzed SPBs. For each strain analyzed by FACS, 10,000 cells were counted. Bar, 5 µm.

Table S1. **Computed binding free energies of the Sfi1–Cdc31 complex for *CDC31* and its mutants**

Force field	Structure	Mutation	ΔG_{ele}^{desolv}	E_{ele}^{P1-P2}	$\Delta G_{apolar}^{desolv}$	ΔG_{ele}^{bind}	ΔG^{bind}
Amber99	wt	–	+8.9	−14.2	−4.3	−5.4	−9.6
	Cdc31-12	E148A	+6.8	−13.8	−4.1	−7.0	−11.1
	Cdc31-14	D107Y	+9.0	−16.4	−4.6	−7.4	−12.0
	Cdc31-16	D131N	+8.3	−16.0	−4.4	−7.7	−12.1
	Cdc31-17	E148Q	+8.0	−13.5	−4.3	−5.6	−9.9
Charmm22	wt	–	+8.3	−15.0	−4.3	−6.8	−11.1
	Cdc31-12	E148A	+7.7	−14.7	−4.1	−6.9	−11.1
	Cdc31-14	D107Y	+9.7	−17.2	−4.7	−7.5	−12.2
	Cdc31-16	D131N	+8.8	−16.9	−4.4	−8.1	−12.4
	Cdc31-17	E148Q	+8.6	−13.8	−4.3	−5.2	−9.5
Parse	wt	–	+4.1	−7.9	–	−3.8	–
	Cdc31-12	E148A	+4.1	−7.9	–	−3.8	–
	Cdc31-14	D107Y	+5.1	−9.5	–	−4.4	–
	Cdc31-16	D131N	+4.6	−8.8	–	−4.2	–
	Cdc31-17	E148Q	+4.8	−7.2	–	−2.4	–

Values computed for the different components of the binding free energy are given in kcal/mol for computations with three different force fields: Amber99, CHARMM22, and PARSE. ΔG_{ele}^{desolv}, electrostatic desolvation energy; E_{ele}^{P1-P2}, interaction energy between binding partners P1 and P2; $\Delta G_{apolar}^{desolv}$, nonpolar desolvation energy; ΔG_{ele}^{bind}, electrostatic binding free energy; ΔG^{bind}, total binding free energy; wt, wild type.

Table S2. **Yeast strains used in this study**

Strain	Genotype	Source/reference	Figure
AS001-8	MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 CSE4-yeGFP-klTRP1	This study	1
CS017-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 sfi1Δ::His3MX6/ sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*SFI1-sfmGFP/leu2Δ1*::pRS305K-*SFI1-sfmGFP SPC42-mCherry-hghNT1/SPC42-mCherry-hghNT1*	This study	7
CS018-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 sfi1Δ::His3MX6/ sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*sfi1Δct-sfmGFP/leu2Δ1*::pRS305K-*sfi1Δct-sfmGFP SPC42-mCherry-hghNT1/SPC42-mCherry-hghNT1*	This study	7
CS038	*MATa/MATa trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 sfi1Δ::His3MX6/sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*sfi1Δct-sfmGFP/leu2Δ1*::pRS305K-*sfi1Δct-sfmGFP SPC42-mCherry-hghNT1/ SPC42-mCherry-hghNT1 ura3-52::pGal1-sic1T5V T33V S76A-HA/ura3-52*	This study	7
CS040	*MATa/MATa trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 sfi1Δ::His3MX6/sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*SFI1-sfmGFP/leu2Δ1*::pRS305K-*SFI1-sfmGFP SPC42-mCherry-hghNT1/ SPC42-mCherry-hghNT1 ura3-52::pGal1-sic1T5V T33V S76A-HA/ura3-52*	This study	7
CS045-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 sfi1Δ::His3MX6 SPC42-mCherry-hghNT1* pRS316-*SFI1 cin8Δ::NatNT2*	This study	7
CS046-2	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 sfi1Δ::His3MX6 SPC42-mCherry-hghNT1* pRS316-*SFI1 kip1Δ::NatNT2*	This study	7
CS047-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 sfi1Δ::His3MX6 SPC42-mCherry-hghNT1* pRS316-*SFI1 dyn1Δ::NatNT2*	This study	7
CS068	*MATa/MATα ura3-52/ura3-52 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 trp1Δ63/trp1Δ63 SFI1-yeGFP-hphMX4/SFI1-yeGFP-KanMX4*	This study	1
CS092	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 kar1Δ::His3MX6 SFI1-yeGFP-KanMX4* pRS316-*KAR1*	This study	
CS104-1	*MATa ura3-52 trp1Δ63 his3Δ200 kar1Δ::His3MX6 SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*GBP-kar1(Δ2-276aa)* pRS316-*KAR1*	This study	6, S5
CS130-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 kar1Δ::His3MX6/ kar1Δ::His3MX6 SFI1-yeGFP-KanMX4/SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*GBP-kar1(Δ2-276aa)/ leu2Δ1*::pRS305-*GBP-kar1(Δ2-276aa)*	This study	6, S5
CS134-1	*MATa ura3-52 his3Δ200 kar1Δ::His3MX6 SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*GBP-kar1(Δ2-276aa) trp1Δ63*::pRS304-*SPC42-mCherry-hphMX4* pRS316-*KAR1*	This study	6, S5
CS142-1	*MATa ura3-52 trp1Δ63 his3Δ200 kar1Δ::His3MX6 SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*KAR1* pRS316-*KAR1*	This study	6, S5
CS143-1	*MATa ura3-52 trp1Δ63 his3Δ200 kar1Δ::His3MX6 SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*GBP-KAR1* pRS316-*KAR1*	This study	6, S5
CS148-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 kar1Δ::His3MX6/ kar1Δ::His3MX6 SFI1-yeGFP-KanMX4/SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*KAR1/ leu2Δ1*::pRS305-*KAR1*	This study	6, S5
CS149-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 kar1Δ::His3MX6/ kar1Δ::His3MX6 SFI1-yeGFP-KanMX4/SFI1-yeGFP-KanMX4 leu2Δ1*::pRS305-*GBP-KAR1/ leu2Δ1*::pRS305-*GBP-KAR1*	This study	6, S5
CS150-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 kar1Δ::His3MX6 leu2Δ1*::pRS305-*KAR1*	This study	6, S5
CS151-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 kar1Δ::His3MX6 leu2Δ1*::pRS305-*GBP-KAR1*	This study	6, S5
CS166	*MATa/MATa ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/trp1Δ63 yeGFP-KAR1/yeGFP-KAR1 sfi1Δ::His3MX6/sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*SFI1/ leu2Δ1*::pRS305K-*SFI1*	This study	4
CS167	*MATa/MATa ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/trp1Δ63 yeGFP-KAR1/yeGFP-KAR1 sfi1Δ::His3MX6/sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*sfi1-2xct+1/leu2Δ1*::pRS305K-*sfi1-2xct+1*	This study	4
CS168	*MATa/MATa ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/trp1Δ63 yeGFP-KAR1/yeGFP-KAR1 sfi1Δ::His3MX6/sfi1Δ::His3MX6 leu2Δ1*::pRS305K-*sfi1-2xct+5/leu2Δ1*::pRS305K-*sfi1-2xct+5*	This study	4
CS219-1	*MATa/MATa ura3-52/ura3-52 trp1Δ63/trp1Δ63 sfi1Δ::His3MX6/sfi1Δ::His3MX6 yeGFP-KAR1/ yeGFP-KAR1 SPC42-mMaple-klTRP1/SPC42-mMaple-klTRP1 leu2Δ1*::pRS305K-*sfi1Δct-3HA/ leu2Δ1*::pRS305K-*sfi1Δct-3HA*	This study	S2
DR017	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP-KanMX4 SPC42-mMaple-klTRP1*	This study	2
DR019	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-KAR1 SPC42-mMaple-klTRP1*	This study	2
DR023	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC42-mMaple- klTRP1*	This study	2
DR108-1	*MATa trp1Δ63 his3Δ200 leu2Δ1 SPC42-yeGFP-hghNT1 ura3-52*::pRS306-*mMaple-KAR1*	This study	2
DR109-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC110-yeGFP-KanMX4 SPC42-mMaple-TRP1*	This study	S2
DR110-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC42-yeGFP-hghNT1 SPC110-mMaple-TRP1*	This study	S2
DR119-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-SFI1 SPC42-mMaple-TRP1*	This study	2
ESM2540-9	*MATa/MATα ura3-52/ura3-52 leu2Δ1/leu2Δ1 his3Δ200/ his3Δ200 trp1Δ63/trp1Δ63*	E. Schiebel	FACS figures
ESM356-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1*	Pereira et al., 2001	All
FY1679	*MATa/MATα ura3-52/ura3-52 trp1Δ63/TRP1 leu2Δ1/LEU2 his3Δ200/HIS3 GAL2+/GAL2+*	EUROSCARF	FACS figures
GPY107-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 CDC5-GFP-KanMX6*	G. Pereira (German Cancer Research Center DKFZ-ZMBH Alliance, Heidelberg, Germany)	S1

Table S2. **Yeast strains used in this study** *(Continued)*

Strain	Genotype	Source/reference	Figure
MEN001	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 kar1Δ::His3MX6* pRS316-*KAR1*	This study	
MEN002	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-KAR1*	This study	1, S1
MEN010	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 Δkar1:His3MX6 SFI1-yeGFP-hphMX4* pRS425-*CDC31-16*	This study	5, 8, S5
MEN022	*MATa ura3-52 trp1Δ63 leu2Δ1 SPC42-mCherry-NatNT2 pCup1-td-SFI1-klTRP1 his3Δ200::pGal1-3HA-UBR1-His3MX6 yeGFP-KAR1*	This study	4
MEN025	*MATa ura3-52 trp1Δ63 SPC42-mCherry-NatNT2 pCup1-td-SFI1-klTRP1 his3Δ200::pGal1-3HA-UBR1-HIS3 yeGFP-KAR1 leu2Δ1::*pRS305K-*SFI1*	This study	S4
MEN046	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP* pRS425	This study	5,8, S5
MEN047	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP* pRS425-*CDC31-16*	This study	5,8, S5
MEN057	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP* pRS425-*CDC31*	This study	5,8, S5
MEN058	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-Kar1* pRS425	This study	S4
MEN059	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-Kar1* pRS425-*CDC31*	This study	S4
MEN060	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 yeGFP-Kar1* pRS425-*CDC31-16*	This study	S4
MEN111	*MATa ura3-52 trp1Δ63 leu2Δ1 SPC42-mCherry-NatNT2 pCup1-td-SFI1-klTRP1 his3Δ200::pGal1-3HA-UBR1-His3MX6 yeGFP-KAR1 NIC96-mCherry-hphMX4*	This study	4
MEN128	*MATa/MATα ura3-52/ura3-52 leu2Δ1/leu2Δ1 his3Δ200/his3Δ200 trp1Δ63/ trp1Δ63 yeGFP-KAR1/ yeGFP-KAR1*	This study	1, S1
MEN132	*MATa lys2-801 cdc31-1 trp1Δ63 his3Δ200 leu2Δ1 ura3-52::*pRS306-*yeGFP-SFI1 SPC42-eqFP-KanMX4 ade2-101::ADE2*	This study	8
MEN133	*MATa lys2-801 trp1Δ63 his3Δ200 leu2Δ1 ura3-52::*pRS306-*yeGFP-SFI1 SPC42-eqFP-KanMX4 ade2-101::ADE2*	This study	8
MEN145.1	*MATa/MATa trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 ura3-52::*pRS306-*CDC31-mCherry/ ura3-52::*pRS306-*CDC31-mCherry kar1Δ::His3MX6/kar1Δ::His3MX6 SFI1-yeGFP-KanMX4/ SFI1-yeGFP-KanMX4 leu2Δ1::*pRS305-*GBP-kar1(Δ2-276aa)/leu2Δ1::*pRS305-*GBP-kar1(Δ2-276aa)*	This study	6
MEN146.1	*MATa/MATa trp1Δ63/trp1Δ63 his3Δ200/his3Δ200 ura3-52::*pRS306-*CDC31-mCherry/ ura3-52::*pRS306-*CDC31-mCherry kar1Δ::His3MX6/kar1Δ::His3MX6 SFI1-yeGFP-KanMX4/ SFI1-yeGFP-KanMX4 leu2Δ1::*pRS305-*GBP-KAR1/leu2Δ1::*pRS305-*GBP-KAR1*	This study	6
MEN159.1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP-KanMX4 SPC42-mCherry-NatNT2* p413-*pGAL-KAR1*	This study	9
MEN166.1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SPC42-mCherry-NatNT2* p413-*pGAL-yeGFP-KAR1*	This study	S5
MIS008	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 sfi1Δ::His3MX6 SPC42-mCherry-hghNT1* pRS316-*SFI1*	Elserafy et al., 2014	
MIS059	*MATa ura3-52 trp1Δ63 leu2Δ1 SPC42-mCherry-NatNT2 pCup1-td-SFI1-klTRP1 his3Δ200::pGal1-3HA-UBR1-His3MX6*	Elserafy et al., 2014	
MIS088	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 SFI1-yeGFP-KanMX4 SPC42-mCherry-NatNT2*	Elserafy et al., 2014	1
YR019-1	*MATa ura3-52 trp1Δ63 his3Δ200 leu2Δ1 CSE4-yeGFP-klTRP1 SPC42-eqFP-hphMX4*	This study	1

Table S3. **Plasmids used in this study**

Name	Description	Source/reference
p414-GAL1	*pGAL1* inducible plasmid	Mumberg et al., 1994
p416-GAL1	*pGAL1* inducible plasmid	Mumberg et al., 1994
pCS017-8	pETDuet-1 where His6 tag was replaced by GST tag	This study
pCS050-4	p414-*pGal1-CDC31*	This study
pCS081	pRS305K-*SFI1-sfmGFP*	This study
pCS089	pRS305K-*sfi1Δct-sfmGFP*	This study
pCS116-1	pRS305K-*SFI1*	This study
pCS118	pRS305K-*SFI1-3HA*	This study
pCS119	pRS305K-*sfi1Δct-3HA*	This study
pCS168-1	pRS305K-*sfi1-2xct+1*	This study
pCS169-1	pRS305K-*sfi1-2xct+5*	This study
pCS177-1	p423-*pGal1-sfi-ct+5*	This study
pCS178-1	p426-*pGal1-TAP*	This study
pCS180-1	p426-*pGal1-TAP-kar1Δtm*	This study
pCS234-1	pRS305K-*sfi1Δ(ct+9)-sfmGFP*	This study
pCS263	pRS315-*CDC31-12 (E148A)*	This study
pCS264	pRS315-*CDC31-14* (D107Y)	This study
pCS266-1	pRS315-*CDC31-17* (E148Q)	This study
pCS275	p413-*pGal1-KAR1*	This study
pD361	Yiplac211-*pGal1-sic1T5V T33V S76A-HA*	E. Schwob (Institut of Molecular Genetics, CNRS UMR5535, and University of Montpellier, Montpellier, France)
pDR018	pYM23-*mMaple-klTRP1*	This study
pETDuet-1	Dicistronic bacterial expression vector	EMD Millipore
pFA6a-His3MX6	For gene disruption using *His3MX6* cassette	Addgene
pFA6a-natNT2	For gene disruption using *NatNT2* cassette	Addgene
pGEX-5X-1	GST expression vector	GE Healthcare
pGex-6p-2rbs	Dicistronic bacterial expression vector	A. Musacchio (Institut de Génétique Moléculaire de Montpellier, CNRS-Université Montpellier 1 et 2, Montpellier, France)
pKL187	*KanMX6-pCup1-Ubi-R-dhfr(ts)-Myc* cassette	K. Labib (College of Life Sciences, University of Dundee, Dundee)
pKS133-6	*NatMX4* disruption cassette	M. Knop (Center for Molecular Biology of the University of Heidelberg, DKFZ-ZMBH Alliance, Heidelberg, Germany)
pKS144-1	yeGFP-hphMX4	M. Knop
pLG162	pRS304-*SPC42-mCherry-hphMX4*	This study
pMaM56	pFA6a-*mCherry-NatNT2*	M. Knop
pMEN29.1	p413-*pGal1-yeGFP-KAR1*	This study
pMK295-1	pRS315-*CDC31-16 (D131N)*	M. Knop
pMK296-2	pRS425-*CDC31-16 (D131N)*	M. Knop
pRS204	*ADE2*-based integration vector	Brachmann et al., 1998
pRS305	*LEU2*-based integration vector	Sikorski and Hieter, 1989
pRS305K	Single integration vector into *LEU2* locus with *KanMX4* selection	Taxis and Knop, 2006
pRS425	*LEU2*-based 2 µm yeast/*E. coli* shuttle vector	Christianson et al., 1992
pSM825-1	For *yeGFP-klTRP1* cassette amplification	Janke et al., 2004
pUF10	pRS315-*CDC31*	This study
pYA20-1	pRS306-*yeGFP-KAR1*	This study
pYA483-1	pRS425-*CDC31*	This study

References

Brachmann, C.B., A. Davies, G.J. Cost, E. Caputo, J. Li, P. Hieter, and J.D. Boeke. 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast.* 14:115–132. http://dx.doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2

Christianson, T.W., R.S. Sikorski, M. Dante, J.H. Shero, and P. Hieter. 1992. Multifunctional yeast high-copy-number shuttle vectors. *Gene.* 110:119–122. http://dx.doi.org/10.1016/0378-1119(92)90454-W

Elserafy, M., M. Šarić, A. Neuner, T.C. Lin, W. Zhang, C. Seybold, L. Sivashanmugam, and E. Schiebel. 2014. Molecular mechanisms that restrict yeast centrosome duplication to one event per cell cycle. *Curr. Biol.* 24:1456–1466. http://dx.doi.org/10.1016/j.cub.2014.05.032

Janke, C., M.M. Magiera, N. Rathfelder, C. Taxis, S. Reber, H. Maekawa, A. Moreno-Borchart, G. Doenges, E. Schwob, E. Schiebel, and M. Knop. 2004. A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast.* 21:947–962. http://dx.doi.org/10.1002/yea.1142

Mumberg, D., R. Müller, and M. Funk. 1994. Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res.* 22:5767–5768.

Pereira, G., T.U. Tanaka, K. Nasmyth, and E. Schiebel. 2001. Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. *EMBO J.* 20:6359–6370. http://dx.doi.org/10.1093/emboj/20.22.6359

Sikorski, R.S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae. Genetics.* 122:19–27.

Taxis, C., and M. Knop. 2006. System of centromeric, episomal, and integrative vectors based on drug resistance markers for *Saccharomyces cerevisiae. Biotechniques.* 40:73–78. http://dx.doi.org/10.2144/000112040