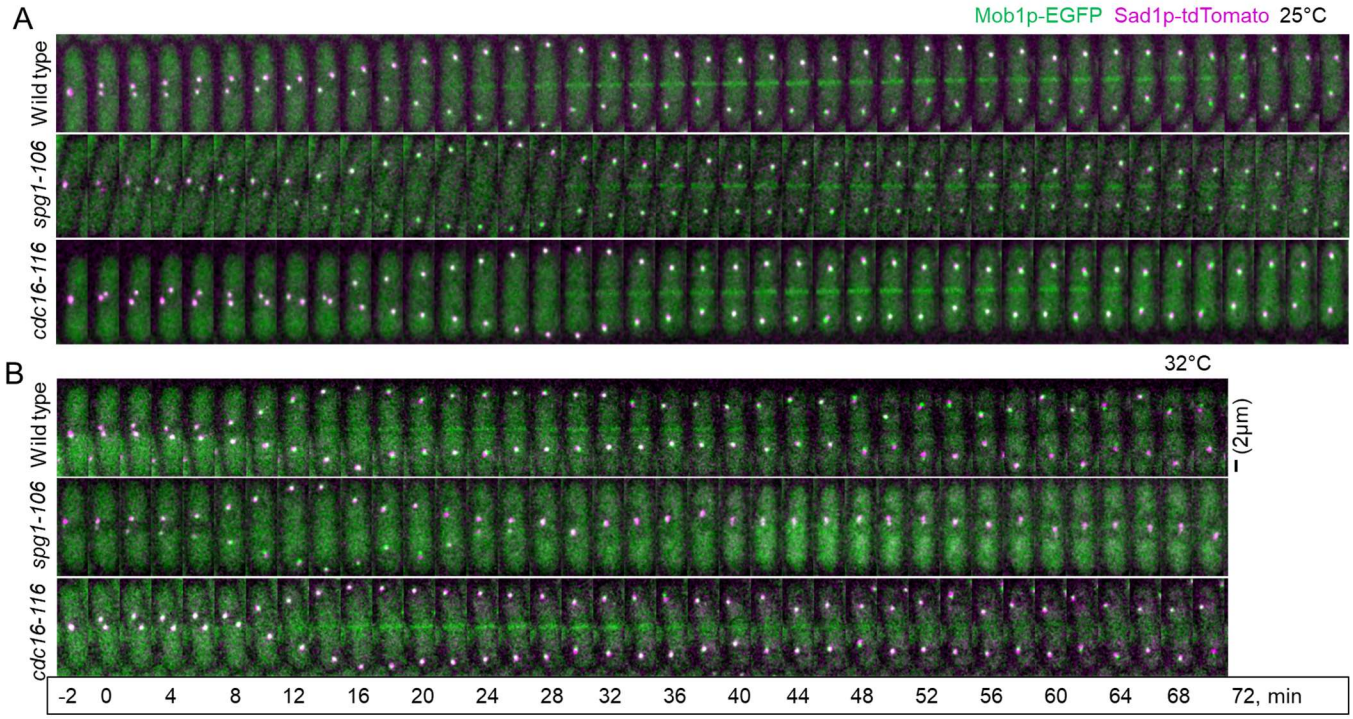
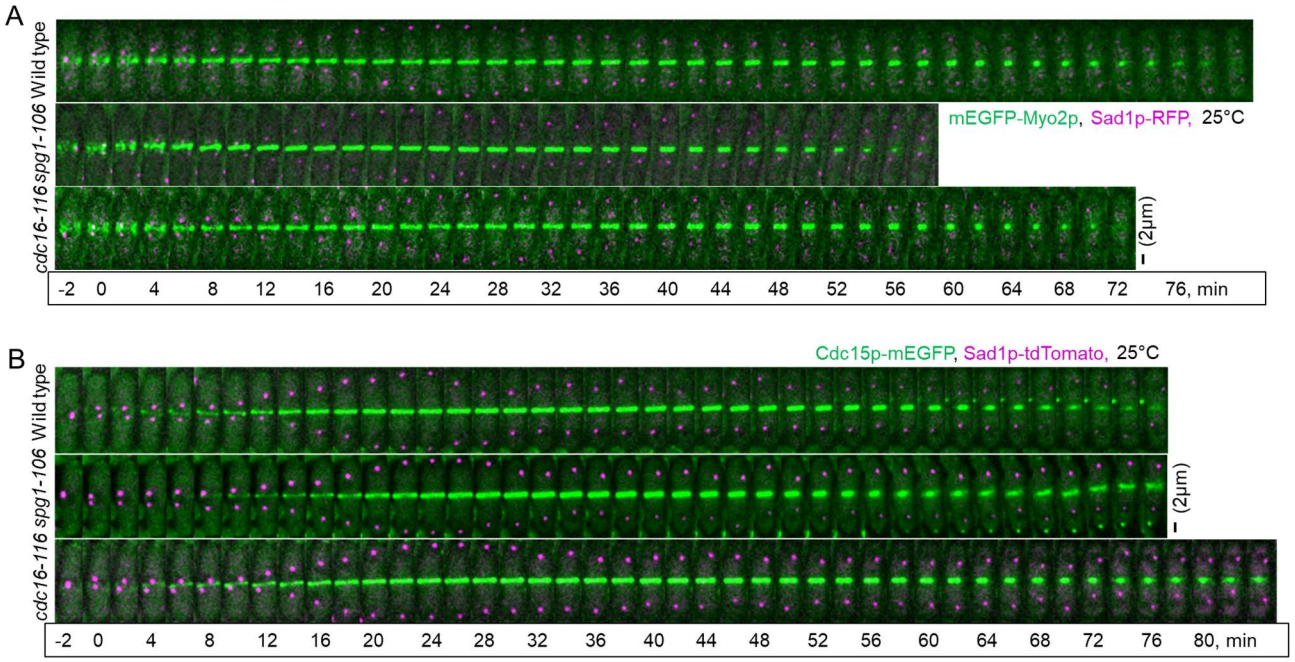
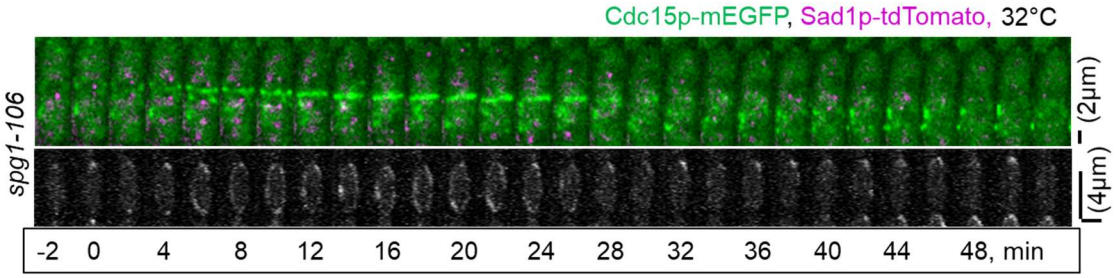


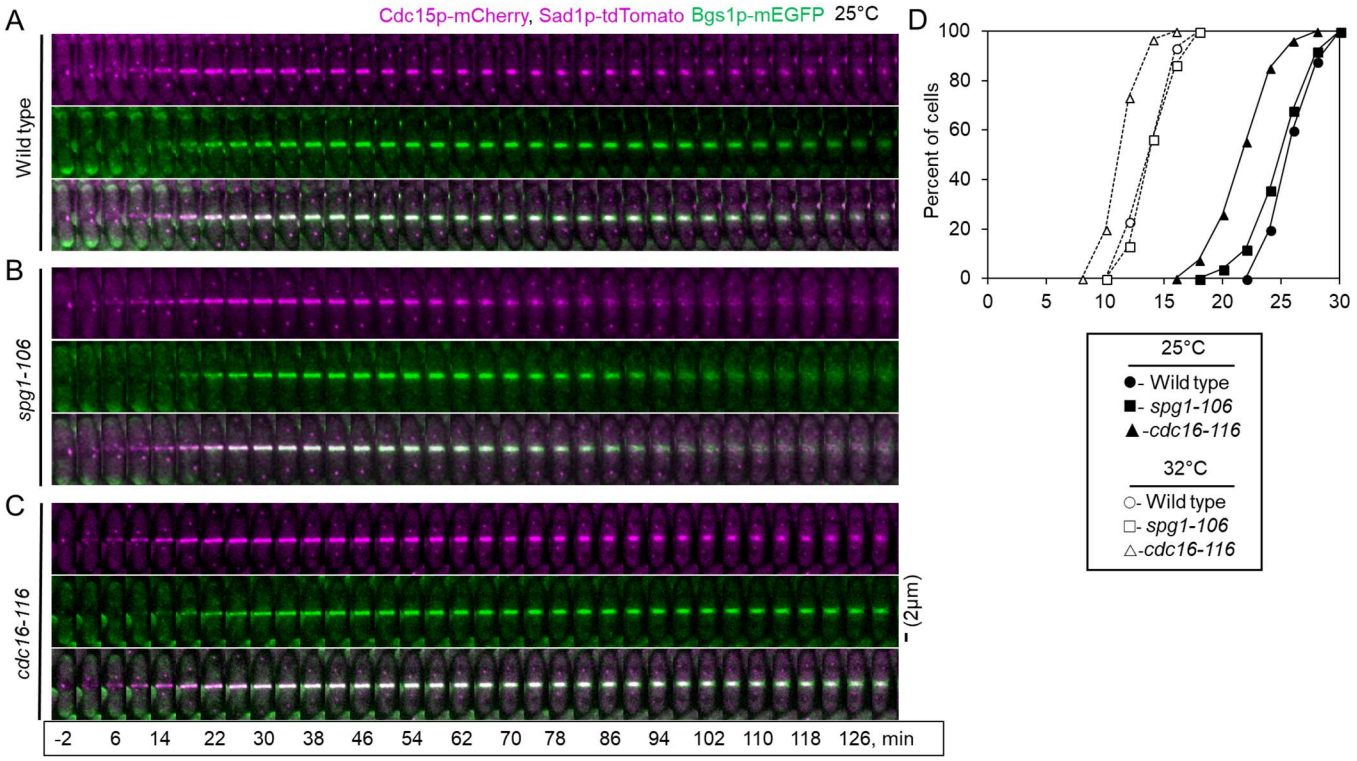
Supplemental Fig S1: Mob1p localization during mitosis in wild-type, *spg1-106* and *cdc16-116* cells expressing (green) Mob1-EGFP and (magenta) Sad1p-tdTomato. (A,B) Time series of maximum intensity projections of fluorescence micrographs at (A) 25°C or (B) after shifting to 32°C for 60 min. (Top panels) wild-type cells, (middle panels) *spg1-106* mutant cells and (lower panels) *cdc16-116* mutant cells. Time 0 min is when the SPBs separated in each cell. Scale bar: 2 µm.



Supplemental Fig S2: Myo2p and Cdc15p ring assembly at 25°C. (A,B) Time series of maximum intensity projections of fluorescence micrographs of (top panel) wild-type cells, (middle panel) *spg1-106* mutant cells and (lower panel) *cdc16-116* mutant cells. (A) (green) mEGFP-Myo2p and (magenta) Sad1p-RFP. (B) (green) Cdc15p-GFP and (magenta) Sad1p-tdTomato. Time 0 min is when the SPBs separated in each cell. Scale bar: 2 µm.



Supplemental Fig S3: Failure of Cdc15p to form a homogenous contractile ring in a *spg1-106* mutant cell after shifting to 32°C for 60 min. (Upper row) Time series of maximum intensity projections of fluorescence micrographs of a cell expressing (green) Cdc15p-GFP and (magenta) Sad1p-tdTomato. (Lower row) Grey-scale 3D reconstructions of this ring. Time 0 min is when the SPBs separated. Scale bars: 2 μm (upper panel) and 4 μm (lower panel).



Supplemental Fig. S4. Bgs1p-mEGFP localization to the contractile ring at 25°C. (A-C) Time series of maximum intensity projections of fluorescence micrographs of cells expressing (green) Bgs1p-mEGFP and (magenta) Sad1p-tdTomato and Cdc15p-mCherry at 25°C. (A) Wild-type cell. (B) *spg1-106* mutant cell. (C) *cdc16-116* mutant cell. Top panels of A-C (Cdc15p-mCherry and Sad1p-tdTomato), middle panels of A-C (Bgs1p-mEGFP) and lower panels of A-C (Merge). Time 0 min is when the SPBs separated in each cell. Scale bars: 2 µm. (D) Outcome plots of the first appearance of Bgs1p-mEGFP at the division site with time 0 min at SPB separation. Symbols and numbers of cells: (●, n=25, ○, n=30) wild-type cells; (■, n=25, □, n=30) *spg1-106* mutant cells; (▲, n=27, △, n=30) *cdc16-116* mutant cells. Filled symbols and solid lines are at 25°C, and open symbols and dashed lines are observations after 60 min at 32°C.

Supplemental Table S1. Ring assembly and constriction times (when rings began to constrict) and Myp2p appearance in minutes after SPB separation measured as the mean times ± SD. In each row the data from experiment-1 is the upper line and the data from experiment-2 is the lower line. Onset of ring constriction defined when ring circumference decrease by 0.6 µm. SDs came from averaging the end points from ~20 cells.

	25°C			32°C		
Marker	Wild type	*spg1-106*	*cdc16-116*	Wild type	*spg1-106*	*cdc16-116*
Times of ring assembly (minutes after SPB separation)						
Mid1p-mEGFP	12.0 ± 1.5 ---	13.1 ± 1.8 # ---	12.7 ± 1.8 # ---	8.3 ± 0.7 # ---	11.4 ± 1.6 *, # (34% failed) ---	5.1 ± 1.3 *, # ---
mEGFP-Myo2p	12.7 ± 1.7 13.4 ± 1.8	16.6 ± 2 * 13.3 ± 1.7	15.5 ± 1.8 * 13.4 ± 1.7	10.3 ± 1.8 10.7 ± 1.7	14.5 ± 2.2 * 13.1 ± 1.8 *	11.3 ± 1.8 9.3 ± 1.6 *
mEGFP-Rng2p	12.6 ± 1.4 11.6 ± 1.5 †	11.7 ± 0.8 # 11.6 ± 1.5 †	13.8 ± 1.4 *, # 11.5 ± 1.4 †	9 ± 1.3 9.6 ± 1.4	14.6 ± 3.3 * (26% failed) 13.5 ± 1.8 * (17% failed)	8.0 ± 1.1 # 9.3 ± 1.4
Cdc12p-3GFP	16.8 ± 3 16 ± 2.5 †	19.7 ± 2.9 *, # 15.3 ± 1.9 †	18.7 ± 2.6 # 15.6 ± 1.8 †	13.5 ± 2.1 # 11.6 ± 1.8	16.3 ± 2.7 *, # (30% failed) 16.2 ± 2.7 †, * (30% failed)	13.2 ± 1.4 # 9.2 ± 1.4 *
Cdc15p-GFP	17.1 ± 2.3 # 15.7 ± 1.5 †	18.0 ± 1.8 # 16.0 ± 1.6 †	17.7 ± 2.1 # 15.7 ± 1.4 †	12.7 ± 1.4 # 11.2 ± 1.6	16.5 ± 2.2 *, # (50% failed) 14.8 ± 2 †, * (20% failed)	12.4 ± 1.5 10.4 ± 1.5 †
Times of Myp2p appearance in fully formed rings (minutes after SPB separation)						
Myp2p-GFP	22.9 ± 1.9 ---	23.7 ± 2.4 ---	25.3 ± 2.4 ---	14.5 ± 1.9 ---	15.8 ± 1.3 ---	15.6 ± 1.7 ---
Times of ring constriction (minutes after SPB separation)						
mEGFP-Myo2p	28.6 ± 3.1 33.5 ± 3	26.5 ± 2.2 * 32.4 ± 3.7	29.6 ± 1.5 31.8 ± 2.8	16.6 ± 2.1 20.9 ± 2.3	18.4 ± 0.8 * (80% failed) 20.4 ± 1.8 (30% failed)	16.2 ± 1.7 19 ± 2.7 *
mEGFP-Rng2p	31.2 ± 3 # 27.9 ± 2.2 †	26.2 ± 2.9 * 27.0 ± 3 †	30.4 ± 2.3 23.5 ± 1.9 †, *	19.8 ± 3.1 # 19.8 ± 3.1	16.6 ± 1.8 *, # (24% failed) 21.2 ± 2 * (45% failed)	21.6 ± 2.4 *, # 16.1 ± 2.1 †, *
Cdc12p-3GFP	32 ± 2.6 # 31.3 ± 1.8 †	30.7 ± 2 # 28.2 ± 2.6 †	33.6 ± 3.2 # 27.8 ± 2.6 †	19.0 ± 2 # 18.7 ± 2 †	21.3 ± 1 *, # (58% failed) 21.3 ± 1 * (57% failed)	17.4± 1.3 * 15.7 ± 1.7 †, *
Cdc15p-GFP	42.3 ± 3.7 # 38.7 ± 4.1 †	43.3 ± 3.3 # 37.0 ± 3.3 †	39.9 ± 2.8 *, # 39.9 ± 2.8 †	26.9 ± 2.8 # 23.4 ± 1.8 †	20.0 *, # (91% failed) 22.0 * (95% failed)	26.6 ± 3 # 24.9 ± 2.7 †
Myp2p-GFP	30.7 ± 2.7 # ---	31.2 ± 3.1 # ---	33.7 ± 3.6 # ---	20.4 ± 2.5 # ---	20.5 ± 3 # (28% failed) ---	21.4 ± 2.1 # ---

* Significant differences ($p < 0.05$) by log-rank tests from wild-type cells at the same temperature. # Significant difference ($p < 0.05$) by Student's T-test from Myo2p marker in the same column from experiment-1. † Significant difference ($p < 0.05$) by Student's T-test from Myo2p marker in the same column from experiment-2.

Supplemental Table S2: Maturation times (interval between completion of ring assembly and ring constriction) in minutes after SPB separation measured as the mean times ± SD. SDs came from averaging the end points from ~20 cells. In each row the data from experiment-1 is the upper line and the data from experiment-2 is the lower line. The data for Mid1p-mEGFP is from experiment-1.

	25°C			32°C		
Marker	Wild type	*spg1-106*	*cdc16-116*	Wild type	*spg1-106*	*cdc16-116*
Ring maturation times (between assembly and constriction)						
mEGFP-Myo2p	15.8 ± 3.9 20.1 ± 2.8	9.9 ± 2.6 * 19 ± 3.7	14.2 ± 1.8 18.4 ± 2.8	6.3 ±2.5 10.2 ± 2.3	4.2 ± 1.9 * (80% failed) 8 ± 2 (30% failed)	4.9 ± 2.2 9.7 ± 2.5
mEGFP-Rng2p	18.5 ± 4 # 16.3 ± 2.3 †	14.5 ± 3.3 *, # 15.3 ± 3 †	16.5 ± 2.5 *, # 16.3 ± 2.3 †	7.5 ± 1.8 10.2 ±2.3	7.5 ± 2.4 # (24% failed) 8.3 ± 2.1 (45% failed)	7.5 ± 1.8 # 6.8 ± 2.4 †, *
Cdc12-3GFP	15.2 ± 2.7 15.3 ± 2 †	11 ± 3.1 * 12.9 ± 1.4 †, *	14.9 ± 4.2 12 ± 2.7 †, *	5.5 ± 2.1 7.1 ± 1.6 †	5.3 ± 2 (58% failed) 6 ± 1.7 (57% failed)	4.1 ± 0.5 6.5 ± 1.3 †
Cdc15-GFP	25.4 ± 4.4 # 22.4 ± 3.9 †	26 ± 3.8 # 21 ± 3.1	21.2 ± 2.6 *, # 24.2 ± 3.3 †	14.1 ± 2.8 # 12.2 ± 2.5 †	6 *, # (91% failed) 10 * (95% failed)	14.6 ± 2.5 # 14.5 ± 2.5 †
Mid1p disappearance from rings						
Mid1p-mEGFP	23.1 ± 1.9 ---	22.1 ± 2.3 ---	23.1 ± 1.7 ---	12.4 ± 1.4 ---	13 ± 1.1 ---	9.2 ± 1.8 * ---

*Significant differences ($p < 0.05$) by log-rank tests from wild-type cells at the same temperature.
Significant difference ($p < 0.05$) by Student's T-test from Myo2p marker in the same column from experiment-1. † Significant difference ($p < 0.05$) by Student's T-test from Myo2p marker in the same column from experiment-2.

Supplemental Table S3. Ring constriction rates in µm/min ± SD, measured from the linear part of the slops in the graphs. SDs came from averaging the constriction rates from ~15 cells. The data for Myp2p-GFP come from experiment-1.

	25°C			32°C		
Marker	Wild type	*spg1-106*	*cdc16-116*	Wild type	*spg1-106*	*cdc16-116*
mEGFP-Myo2p	0.25 ± 0.06	0.25 ± 0.03	0.26 ± 0.04	0.48 ± 0.06	0.29 ± 0.06 * (partial constriction)	0.43 ± 0.04 *
mEGFP-Rng2p	0.28 ± 0.02	0.31 ± 0.03 †, *	0.31 ± 0.04 †, *	0.44 ± 0.06	0.31 ± 0.10 * (partial constriction)	0.48 ± 0.05 †
Cdc12p-3GFP	0.27 ± 0.03	0.28 ± 0.06	0.30 ± 0.03 †, *	0.51 ± 0.07	0.31 ± 0.09 * (partial constriction)	0.50 ± 0.1 †
Cdc15p-GFP	0.30 ± 0.04 †	0.30 ± 0.03 †	0.26 ± 0.02 *	0.37 ± 0.09 †	0.30 * (partial constriction)	0.45 ± 0.07 *
Myp2p-GFP	0.29 ± 0.03	0.29 ± 0.02	0.28 ± 0.03	0.51 ± 0.06	0.29 ± 0.13 * (partial constriction)	0.48 ± 0.05

*Significant differences ($p < 0.05$) by Student's T-test from wild-type cells at the same temperature. †Significant difference ($p < 0.05$) by Student's T-test from Myo2p marker in the same column from experiment-2.

Supplemental Table S4: Strain list

Strain	Genotype	Source
CL181	h+ KanMX6-Pmyo2p-mEGFP-Myo2p Sad1p-RFP-KanMX6 ade6-M216 his3-D1 leu1-32 ura4-D18	Lab stock
IRT69	h- KanMX6-Prng2p-mEGFP-Rng2p Sad1p-RFP-KanMX6 ade6-M21X leu1-32 ura4-D18	Lab stock
SD8	h+ KanMX6-Pmyo2p-mEGFP-Myo2p Sad1p-RFP-kanMX6 *cdc16-116* ade6-M216 leu1-32 ura4-D18	This study
SD21	h+ KanMX6-Pmyo2p-mEGFP-Myo2p Sad1p-RFP-KanMX6 *spg1-106* ade6-M210 his3-D1 leu1-32 ura4-D18	This study
SD23	h- Cdc7p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD24	h+ Mob1p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD25	Cdc7p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This study
SD27	Cdc7p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This study
SD31	h+ Myp2p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD34	Myp2p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This study
SD35	Myp2p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This study
SD37	KanMX6-Prng2p-mEGFP-Rng2p Sad1p-RFP-KanMX6 *cdc16-116* ade6-M21X leu1-32 ura4-D18	This study
SD38	KanMX6-Prng2p-mEGFP-Rng2p Sad1-RFP-KanMX6 *spg1-106* ade6-M21X leu1-32 ura4-D18	This study
SD40	h- Cdc12p-3GFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD41	h- Cdc15p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD42	h- Cdc12p-3GFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This study
SD43	h+ Cdc12p-3GFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This study
SD44	Cdc15p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This study
SD45	Cdc15p-GFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This study
SD46	h- Mid1p-mEGFP-KanMX6 Sad1p-tdTomato-NatMX6 ade6-M210 leu1-32 ura4-D18	This study
SD47	Mid1p-mEGFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This study
SD48	Mid1p-mEGFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This study
SD82	Cdc15p-GFP-KanMX6 Rlc1p-tdTomato-NatMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This Study
SD83	h- KanMX6:Pbgs1-mEGFP-Bgs1p Cdc15p-mCherry-KanMx6 Sad1p-tdTomato-NatMX6 ade6-M21x leu1-32 ura4-Δ18	This Study
SD85	KanMX6:Pbgs1-mEGFP-Bgs1p Cdc15p-mCherry-KanMx6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M21x leu1-32 ura4-Δ18	This Study
SD86	KanMX6:Pbgs1-mEGFP-Bgs1p Cdc15p-mCherry-KanMx6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M21x leu1-32 ura4-Δ18	This Study
SD91	Mob1p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 *spg1-106* ade6-M210 leu1-32 ura4-D18	This Study
SD92	Mob1p-EGFP-KanMX6 Sad1p-tdTomato-NatMX6 *cdc16-116* ade6-M210 leu1-32 ura4-D18	This Study