Supplementary Materials

Molecular Biology of the Cell

Sherwin *et al*.

SUPPLEMENTAL INFORMATION

Figure S1. Sli15 phosphorylation during cell cycle in WT and *fin1Δ* cells. G_1-arrested WT (781-2-2) and *fin1Δ* (4086-2-3) cells with Sli15-13myc were released into YPD at 30°C. α-factor was added back after 40 min release to block the following cell cycle. Samples were collected every 20 min to prepare protein samples (bottom) and to count budding index (N = 100 cells) (top). Western blotting was performed with anti-myc antibody. Pgk1, loading control.

Figure S2. Abrogating Fin1-PP1 binding restores CPC localization at KTs in phospho-deficient *fin1-5A* cells at metaphase. **(A)** KT localization of Ipl1. *cdc26Δ IPL1-3GFP NUF2-mCherry* cells (4421-3-2) containing either *FIN1* (pMB6) or *fin1-5A-AA* (pMB5) plasmids were grown to log phase in YPD at 25°C then shifted to 36°C for 2 hr to inactivate Cdc26 for metaphase arrest. Both CDK phosphorylation sites and PP1 binding motif are mutated in Fin1-5A-AA. Representative images show localization of Ipl1-GFP and Nuf2-mCherry (outlined in white dotted line). Scale bar, 5 μm. **(B)** Statistical analysis for Ipl1 KT localization. Ipl1 localization was categorized as no KT localization, KT localization, or spindle localization. The results are the average of three experimental repeats (N=3) where 100 cells were counted for each experiment. The bars represent mean values ± SD. *p* values were obtained by using a two-tailed unpaired *t*-test. NS: no statistical significance. **(C)** Statistical analysis for Ipl1 intensity at KTs. The intensity of Ipl1 relative to Nuf2 in 50 cells (100 KTs) was quantified using ImageJ. Statistical significance for relative Ipl1 intensity was analyzed by a two-tailed unpaired *t*-test. NS: no statistical significance.

Figure S3. *sli15-3* mutant cells show CPC dissociation from the KT in metaphase. *IPL1-3GFP NUF2-mCherry* (4516-2-1) and *sli15-3 IPL1-3GFP NUF2-mCherry* (4516-1-1) cells were arrested in G_1 in YPD media at 25°C. Cells were released into the cell cycle at 36°C and samples were collected for imaging. Pictures were taken at 80 min to determine Ipl1 KT localization. Scale bar, 5 μm. Representative images were shown in the top panel, and the cell outlined with dotted line exhibited reduced KT localization of Ipl1. The intensity of Ipl1 relative to Nuf2 in 50 cells (100 KTs) was quantified using ImageJ. Statistical significance for relative Ipl1 intensity was analyzed by a two-tailed unpaired *t*-test, with asterisks indicating **** $p < 0.0001$.

Figure S4. The growth of phospho-deficient *sli15-17A* and *sli15-6A* mutants in combination with *fin1-5A* when syntelic attachment is induced by *CIK1-CC* overexpression. The strains with the indicated genotypes were grown to saturation in synthetic medium then 10-fold serially diluted and spotted onto glucose and galactose plates. The plates were incubated at 30°C for 2 d for imaging**.**

S1 Table. The relevant genotypes of yeast strains used in this study.

S2 Table. The plasmids used in this study.

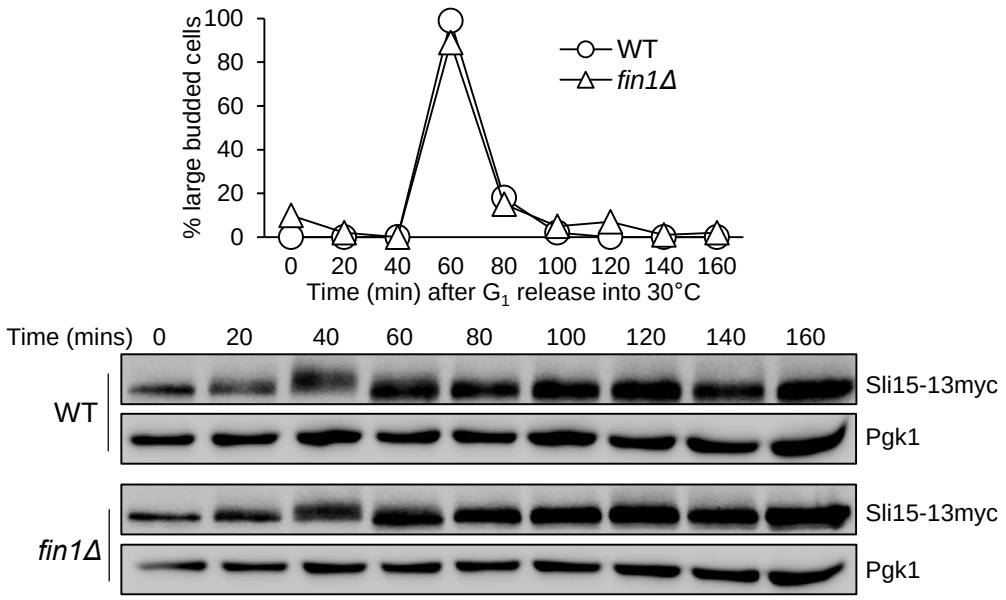
S1 Table. List of relevant genotypes of yeast strains used in this study

Strains	Relevant genotypes	Reference
Y300 (WT)	MAT**a** *ura3-1, his3-11, 15 leu2-3,112 trp1-1, ade2-1, can1-100*	Lab stock
781-2-2	MAT**a** *SLI15-13myc-Sphis5*$^{+}$	Lab stock
4175-1-1	MAT**a** *ipl1-321 SLI15-13myc-Sphis5*$^{+}$	This study
4048-1-3	MAT**a** *cdc14-2 SLI15-13myc-Sphis5*$^{+}$	Lab stock
4086-2-3	MAT**a** *SLI15-13myc-Sphis5*$^{+}$ *fin1Δ::KanMX*	This study
4421-3-2	MAT**a** *cdc26::KanMX IPL1-3GFP-Sphis5*$^{+}$ *NUF2-mCherry*	This study
4426-5-3	MAT**α** *cdc26::KanMX sli15-6A-HIS3 IPL1-3GFP-Sphis5*$^{+}$ *NUF2- mCherry*	This study
4437-1-3	MAT**a** *cdc26::KanMX sli15-17A-13myc-KanMX IPL1-3GFP-Sphis5*$^{+}$ *NUF2-mCherry*	This study
3541-2-3	MAT**a** *cdc14-2 IPL1-3GFP-Sphis5*$^{+}$ *TUB1-mCherry-URA3*	This study
4209-1-1	MAT**a** *cdc14-2 ASE1-GFP-HIS TUB1-mCherry-URA3*	This study
4210-1-2	MAT**a** *cdc14-2 SLK19-GFP-Sphis5*$^{+}$ *TUB1-mCherry-URA3*	This study
4370-4-3	MAT**a** *cdc15-2 TUB1-GFP-URA3*	This study
4370-1-2	MAT**a** *cdc15-2 fin1Δ::KanMX TUB1-GFP-URA3*	This study
870-1-2	MAT**a** *cdc15-2 TUB1-GFP-LEU2 bar1-1*	Lab stock
870-2-2	MAT**a** *cdc15-2 slk19::TRP1 TUB1-GFP-LEU2*	Lab stock
4207-1-3	MAT**a** *SLK19-GFP-Sphis5*$^{+}$ *TUB1-mCherry-URA3*	This study
4207-4-1	MAT**a** *fin1::KanMX SLK19-GFP-Sphis5*$^{+}$ *TUB1-mCherry-URA3*	This study
2567-2-1	MAT**a** *ASE1-GFP-HIS TUB1-mCherry-URA3*	Lab stock
4205-5-1	MAT**a** *fin1::KanMX ASE1-GFP-HIS TUB1-mCherry-URA3*	This study
4379-3-3	MAT**a** *promURA3::tetR::GFP-LEU2 CENIV::tetOX448::URA3 SPC110-mCherry::Hygro*	This study
4368-3-1	MAT**a** *sli15-17A-13myc-KanMX promURA3::tetR::GFP-LEU2 CENIV::tetOX448::URA3 SPC110-mCherry::Hygro*	This study
4368-1-1	MAT**a** *sli15-6A-HIS3 promURA3::tetR::GFP-LEU2 CENIV::tetOX448::URA3 SPC110-mCherry::Hygro*	This study
Y3594	MAT**a** *sli15-6A-HIS3*	Uhlmann lab
4053-4-1	MAT**a** *sli15-17A-13myc-KanMX*	Lab stock
4516-2-2	MAT**a** *IPL1-3GFP-Sphis5*$^{+}$ *NUF2-mCherry*	This study
4516-1-1	MAT**a** *IPL1-3GFP-Sphis5*$^{+}$ *NUF2-mCherry sli15-3*	This study
4505-4-3	MAT**a** *mcd1-1 PDS1-18myc-LEU2 sli15-17A-13myc-KanMX*	This study
4517-1-2	MAT**a** *mcd1-1 PDS1-18myc-LEU2 SLI15-13myc-Sphis5*$^{+}$	This study
JBY649	MATa *PDS1-18myc-LEU2*	Lab stock
4595-2-1	MATa *SLI15-13myc-Sphis5*$^{+}$ P_{GAL}*CDC20-TRP1*	This study
4600-2-2	MATa *SLI15-13myc-Sphis5*$^{+}$ P_{GAL}*CDC20-TRP1 ipl1-321*	This study
EGM25	MATa *sli15-6A-13myc-TRP1*	This study

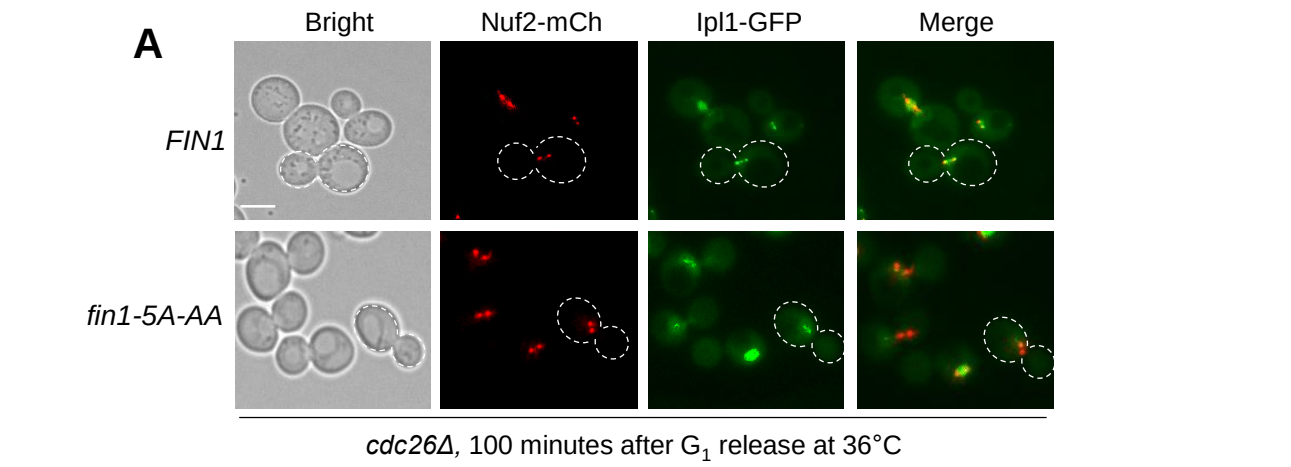
S2 Table. List of plasmids used in this study

Name	Relevant genes	Reference
p1218	*CEN-TRP1* vector	Lab stock
pHL002	P_{GAL}-*myc-CIK1-CC-TRP1*	Lab stock
pRS416	*CEN-URA3* vector	Phil Hieter
pYW200	P_{GAL}-*myc-CIK1-CC-URA3*	Lab stock
pMB6	P_{FIN1}-*FIN1-TRP1*	Lab stock
pMB7	P_{FIN1}-*fin1-5A-TRP1*	Lab stock
pMB5	P_{FIN1}-*fin1-5A-AA-TRP1*	Lab stock
pSB1252	P_{FIN1}-*FIN1-GFP-LEU2*	Biggins lab
pSB1359	P_{FIN1}-*fin1-5A-GFP-LEU2*	Biggins lab
pSB1361	P_{FIN1}-*fin1-5A-AA-GFP-LEU2*	Biggins lab

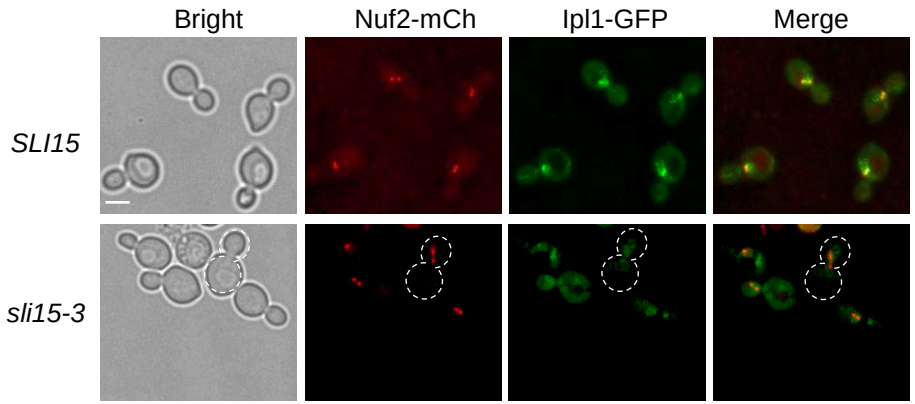
Supplemental Fig. S1



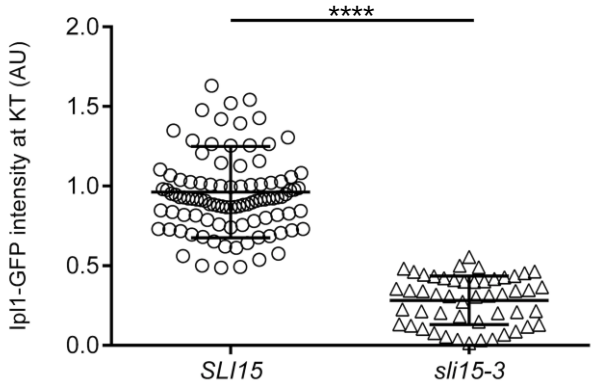
Supplemental Fig. S2



Supplemental Fig. S3



80 minutes after G_1 release at 36°C



**** $p < 0.0001$, N = 100 KTs

Supplemental Fig. S4