Supplemental Materials

Suppl Fig S1 Chin et al

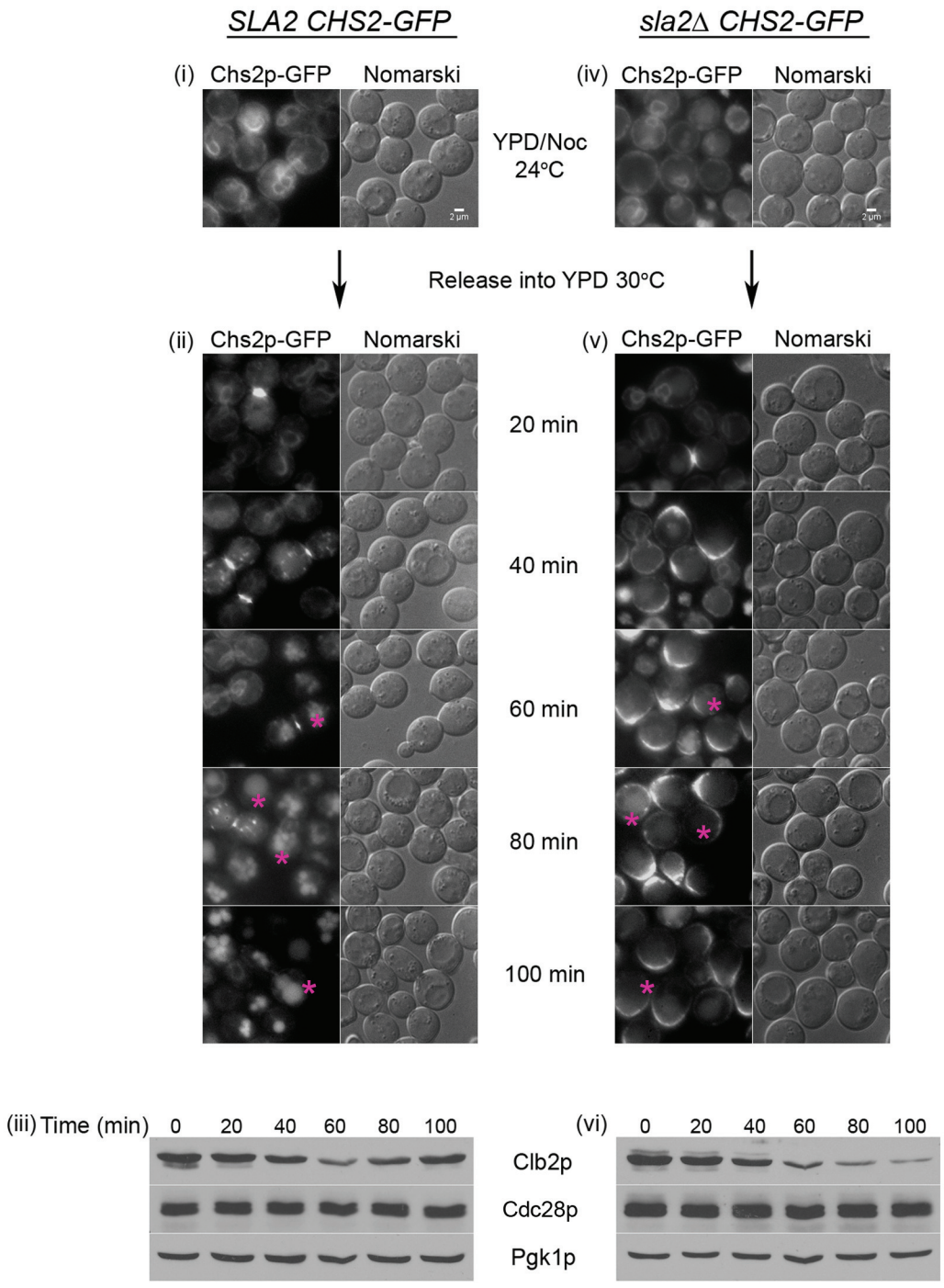

Figure S1. *Chs2p-GFP accumulation at the neck when endocytosis is inhibited by deletion of SLA2.* (i, ii) and (iv, v) *SLA2 CHS2-GFP* and *sla2*Δ *CHS2-GFP* cells were arrested in YPD/Noc at 24°C. After 4.5 hours, the cells were then released from metaphase into YPD pre-warmed at 30°C. Pink asterisk (ii, 60 – 100 min) indicates Chs2p-GFP endocytosed in endosome/vacuoles in *SLA2* cells. Pink asterisk (v, 60 - 100min) indicates absence of Chs2p-GFP endocytosis in the vacuoles in *sla2*Δ cells. Western blot analysis of Clb2, Cdc28 and Pgk1 levels are shown in (iii and vi) to demonstrate equivalent mitotic exit during release from Noc.

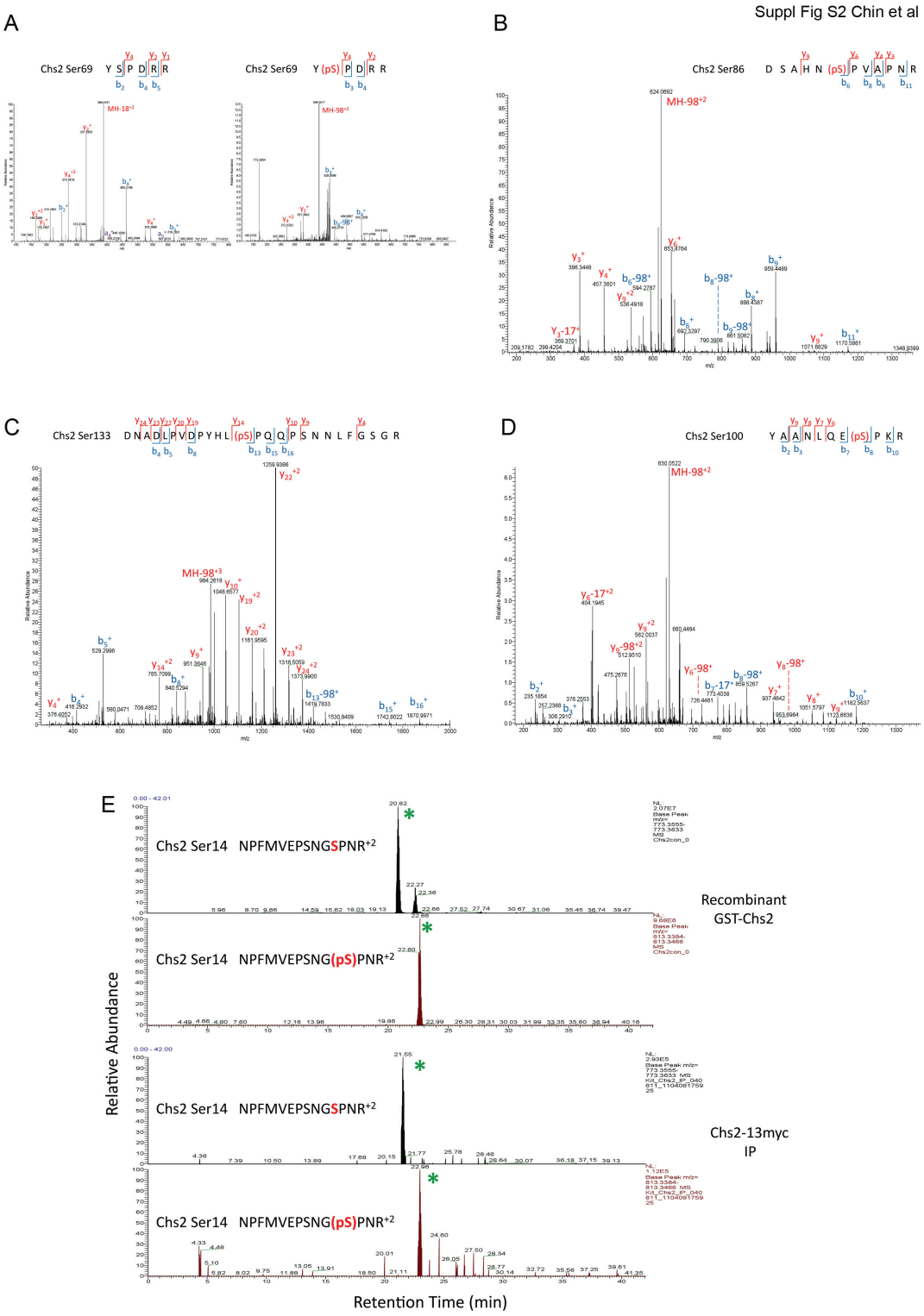

Figure S2. Tandem MS analysis of Chs2-13myc immunoaffinity purified from telophase-arrested *cdc15-2* culture. (A-D) Product ion spectra for the indicated phosphopeptides from Chs2 with the phosphorylated residue in red (all 4 are followed by proline as expected for Cdk1 phosphorylation) and amino acid residue number stated. Only ions from the y and b fragment series are shown to confirm the peptide identity and pinpoint the site of phosphorylation. Neutral loss of phosphate is indicated by "-98" and "MH" refers to the parent ion. Product ion spectra for the unmodified forms of peptides were also acquired but only the spectrum for Chs2pS69 is shown (panel A). (E) Quality product ion spectra were not obtained for the

indicated peptide containing the potential Cdk1 phosphorylation site at position 14 in Chs2. However, significant MS signals were observed for the predicted m/z corresponding to the unmodified and phosphorylated forms of this peptide and are shown here as extracted ion chromatograms (bottom). Since we confirmed this peptide and phosphorylation site in the recombinant protein used for *in vitro* Cdc14 phosphatase assays we compared the XICs and found that the retention times were nearly identical to the unconfirmed signals from the endogenous Chs2-13myc protein. We therefore conclude that this site is also phosphorylated, at an estimated stoichiometry of 30%.

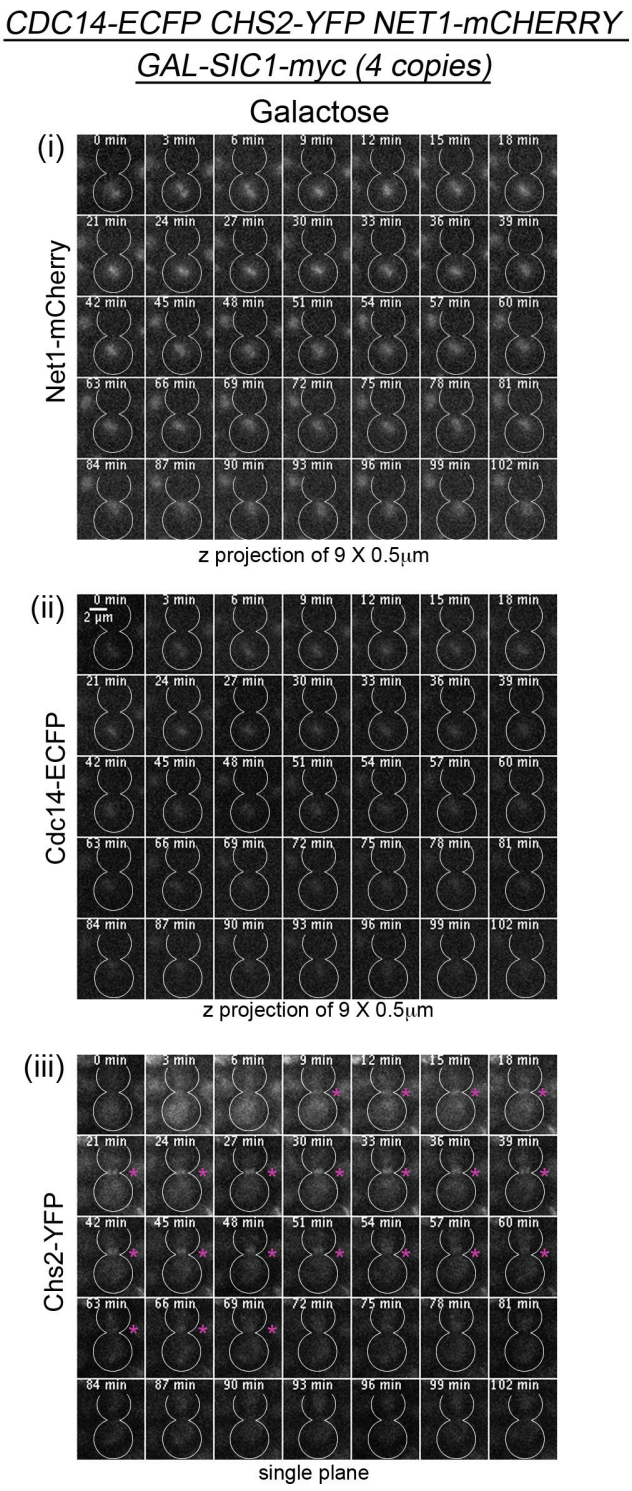

Figure S3. *Premature Cdc14 dispersal and Chs2 ER export from nucleolus at metaphase can be triggered by Sic1 over-expression. CDC14-ECFP CHS2-YFP NET1-mCHERRY GAL-SIC1-myc(4copies)* cells with *SIC1* induction (as described in Figure 4B) showing prolonged but weak Chs2-YFP neck localization (iii, pink asterisks) in the absence of visible Cdc14-ECFP dispersion (ii). Net1-mCherry control for nucleolar position is shown in (i).

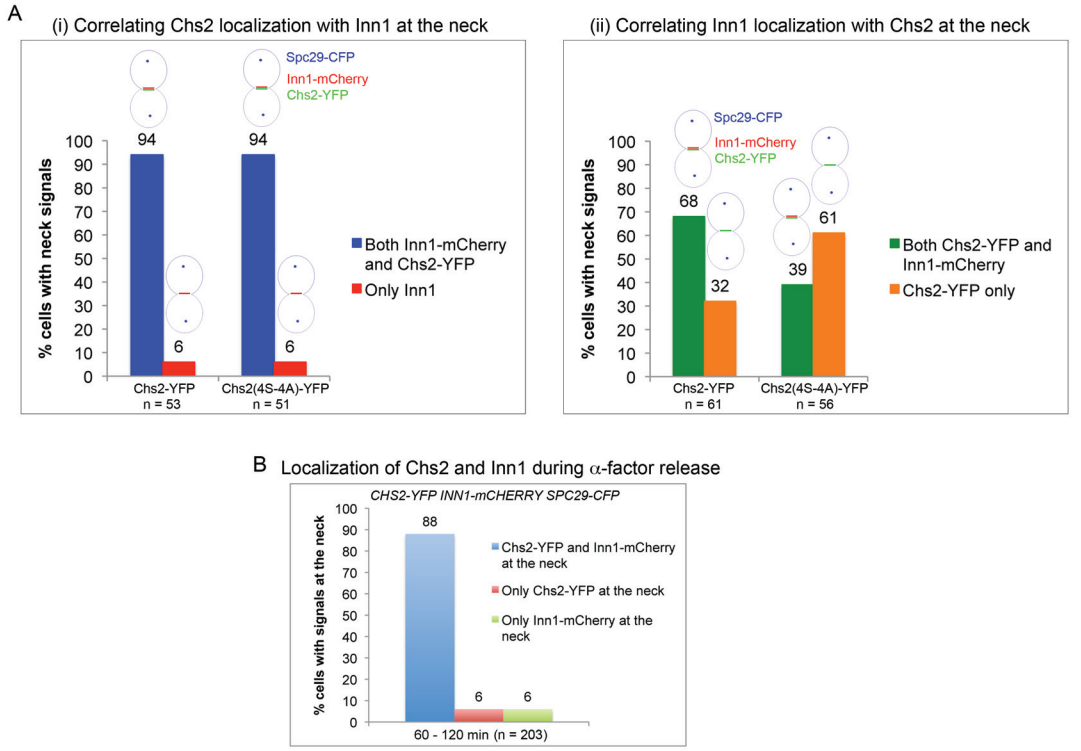

Figure S4 *Dephosphorylation of Chs2p is relevant during unperturbed cell division.* To ascertain if the timely dephosphorylation of Chs2 is important not just in cells released from a metaphase arrest or during a Noc block (eg Fig 4) but also in an unperturbed cell division cycle, we examined *SPC29-ECFP INN1-mCHERRY* cells harbouring *GAL-CHS2-YFP, GAL-CHS2(4S-to-4A)-YFP,* or *GAL-CHS2(4S-to4E)-YFP* in cycling cells for the localization of Chs2-YFP. The *GAL* promoter was used, as the level of endogenous Chs2-YFP is not easily detected in cycling cells given that *CHS2* is transcribed during mitosis (Pammer et al., 1992; Choi et al., 1994; Spellman et al., 1998) and localizes to the neck for a short period of time (Roh et al., 2002; Teh et al., 2009). The separation of the spindle pole bodies (SPBs), marked by an ECFP-fusion of Spc29 (an SPB component) (Elliott et al., 1999), was used as a guide for spindle elongation and progression through mitosis. Inn1-mCherry was used as an indicator of mitotic exit as it localizes to the neck upon mitotic kinase destruction (Meitinger et al., 2010).

To determine the timing of Chs2 neck localization during normal mitosis, we examined large-budded cells for correlations between Inn1-mCherry and Chs2-YFP neck localization based on two questions. First, we asked whether cells that had exited mitosis with Inn1-mCherry at the neck also had Chs2-YFP at the neck. Second, we asked if the reverse correlation would be true, that is, whether cells with Chs2-YFP at the neck also showed Inn1-mCherry neck localization.

Chs2-YFP when induced in the cycling cells was mainly observed to be at the ER (data not shown). When we first looked at cells with Inn1-mCherry neck signals, we found Chs2-YFP localized to the neck in about 94% of the cells (Fig S4Ai). This indicated a tight correlation between Chs2p transport to the neck and mitotic exit in unperturbed cells going through mitosis. When we examined *SPC29-ECFP INN1-mCHERRY GAL-CHS2(4S-to-4A)-YFP* cells with Inn1-mCherry signals at the neck, 94% of the cells also had Chs2(4S-to-4A)-YFP at the neck.

Upon examining Chs2-YFP cells with Chs2-YFP at the neck, about 68% of the cells showed Inn1-mCherry neck signals (Fig S4Aii). The 32% of cells showing

Chs2-YFP at the neck without Inn1-mCherry was likely due to the continued expression of *CHS2-YFP* from the *GAL* promoter such that Chs2-YFP was transported to the neck even after Inn1-mCherry had disappeared from the neck at the end of cytokinesis.

However, compared to Chs2-YFP, when we looked at cells with Chs2(4S-to-4A)-YFP at the neck and asked if Inn1-mCherry would also be present, 61% of the cells showed Inn1-mCherry neck signals (Fig S4Aii), with 39% of the cells showing Chs2(4S-to-4A)-YFP signals at the neck without Inn1-mCherry neck signals. This indicated that the absence of phosphorylation prevents ER retention in mitosis in a normal cell division cycle, resulting in Chs2(4S-to-4A)-YFP neck localization even though cells might not have exited mitosis.

In striking contrast to Chs2-YFP and Chs2(4S-to-4A)-YFP, the phospho-mimetic Chs2p(4S-to-4E)-YFP was observed to be in the ER in the cells at all stages, with very few cells showing neck signals (data not shown). This confirmed our previous finding that the phosphorylated form of Chs2-YFP is retained in the ER and that dephosphorylation is needed for Chs2 to be exported to the neck (Teh et al., 2009).

In an alternative approach to using Gal-induced we also looked at *CHS2-YFP INN1-mCHERRY SPC29-CFP* cells released from α-factor arrest to see if there is a tight association between Chs2-YFP and Inn1-mCherry neck localization during mitotic exit. Such a release experiment from G1, as opposed to a Noc arrest (eg Fig 4B) or Noc release experiment (eg Fig 1B), reflects the normal progression through mitosis and allowed us to examine if Chs2 ER export were regulated by phosphorylation status. We found that in 88% of mitotic cells that both Chs2-YFP and Inn1-mCherry were found together at the neck (Fig S4B). Only in 6% of the cells was Chs2-YFP observed at the neck without Inn1-mCherry signals and vice versa.

Taken as a whole, our data supported the idea that the timely regulation of Chs2p via phosphorylation by Clb2p-Cdc28p is needed for ER retention during an unperturbed mitosis. Furthermore, the dephosphorylation of Chs2 is important for alleviating the restraint on ER export and that this is physiologically relevant during a normal cell division cycle.

Supplemental Table 1. Strains list

Name	Genotype	Source
JKY5	*MAT α his3-d leu2-d mef15-d lys2-d ura3-d cdc14-Δ::CDC14:LEU2*	(Bembenek et al., 2005)
JKY8	*MAT α his3-d leu2-d mef15-d lys2-d ura3-d cdc14-Δ::cdc14_L359A, I360A, L362A::LEU2*	(Bembenek et al., 2005)
HCY115	*Mat a LEU2::GAL-HA-CDC14::LEU2*	(Hall et al., 2008)
HCY116	*Mat a LEU2::GAL-HA-CDC14(C283S)::LEU2*	(Hall et al., 2008)
YL1051	*cdc14-degron GAL-sic1NTΔ::URA*	(Zhai et al., 2010)
FM1997	*MAT a GAL-sic1NTΔ::URA CHS2-GFP::KAN*	This study
FM1999	*MAT a cdc14-3 GAL-sic1NTΔ::URA CHS2-GFP::KAN*	This study
FM1603	*MAT α his3-d leu2-d mef15-d lys2-d ura3-d cdc14-Δ::CDC14:LEU2 CHS2-YFP::HIS*	This study
FM1605	*MAT α his3-d leu2-d mef15-d lys2-d ura3-d cdc14-NES::LEU2 CHS2-YFP::HIS*	This study
FM1779	*MAT a cdc14-NES::LEU2 CHS2-YFP::HIS GAL-CDC14-9MYC::URA*	This study
FM1709	*MAT a cdc14-NES::LEU2 GAL-CHS2-YFP::TRP SEC63-CFP::SpHIS*	This study
FM1710	*MAT a cdc14-NES::LEU2 GAL-CHS2(4S-to-4A)-YFP::TRP SEC63-CFP::SpHIS*	This study
FM1850	*MAT a GAL-CHS2-13MYC::TRP*	This study
FM1893	*MAT a GAL-CHS2(4S-to-4A)-13MYC::TRP*	This study
FM1866	*Mat a LEU2::GAL-HA-CDC14::LEU2 CHS2-3MYC::TRP*	This study
FM1867	*Mat a LEU2::GAL-HA-CDC14(C283S)::LEU2 CHS2-3MYC::TRP*	This study
FM1906	*Mat a LEU2::GAL-HA-CDC14::LEU2 pep4Δ::HYGRO*	This study
FM1909	*Mat a LEU2::GAL-HA-CDC14(C283S)::LEU2 pep4Δ::HYGRO*	This study
FM1846	*MAT a CHS2-3MYC::TRP*	This study
FM2017	*MAT a CHS2-GFP::KAN CDC14-mCHERRY::HYGRO cdc55Δ::NAT2*	This study
FM2003	*MAT a CHS2-GFP::KAN CDC14-mCHERRY::HYGRO bub2Δ::NAT*	This study
FM1880	*MAT a cdc15-2 CHS2-3MYC::TRP*	This study
FM2120	*Mat a leu2 his3 trp1 ura3 bar- CHS2-YFP(Citrine)::SpHIS5 CDC14-ECFP::KAN*	This study
FM2133	*Mat a CHS2-YFP::SpHIS5 GAL-SIC1(myc)::URA (4COPIES) NET1-mCHERRY::HYGRO CDC14-ECFP::KAN ADE bar-*	This study
FM2138	*Mat a CHS2-GFP::KAN sla2Δ::HYGRO*	This study

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Name	Genotype	Source
FM2199	*Mat a INN1-mCHERRY::HYGRO SPC29-ECFP::KAN GAL-CHS2-YFP::URA*	This study
FM2201	*Mat a INN1-mCHERRY::HYGRO SPC29-ECFP::KAN GAL-CHS2(StoA)-YFP::URA*	This study
FM2203	*Mat a INN1-mCHERRY::HYGRO SPC29-ECFP::KAN GAL-CHS2(StoE)-YFP::URA*	This study
FM2207	*Mat a INN1-mCHERRY::HYGRO SPC29-ECFP::KAN Endogenous promoter-CHS2-YFP::TRP*	This study