Supporting Information

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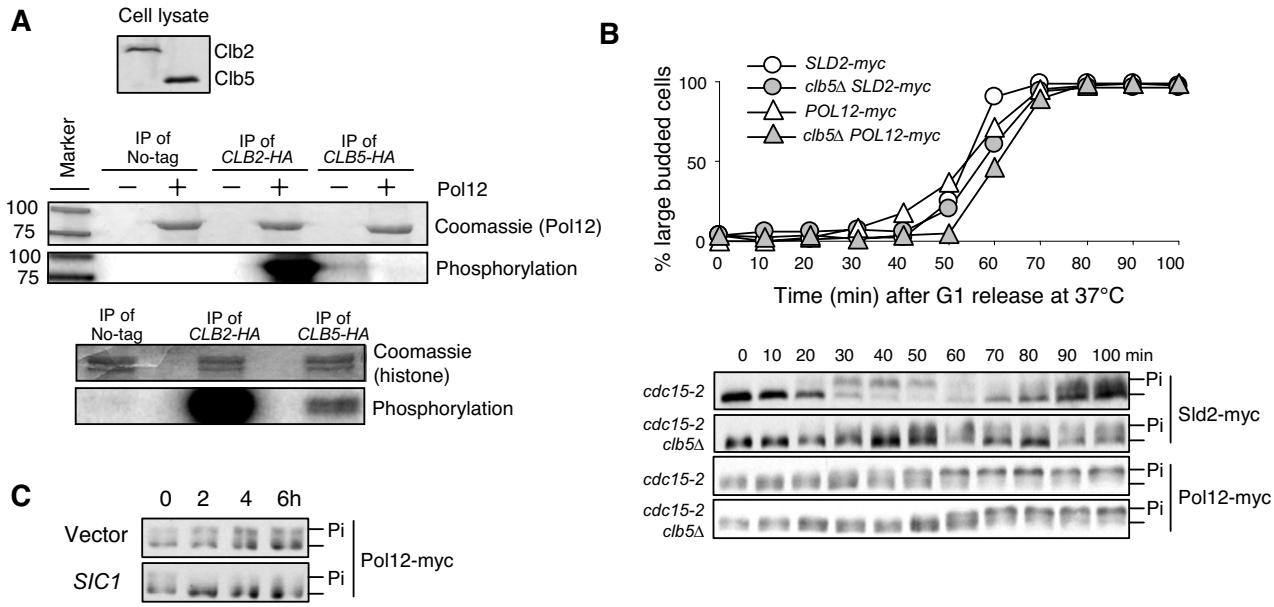

Fig. S1. The substrate specificity of Clb5-Cdk1 and Clb2-Cdk1. (*A*) The phosphorylation of Pol12 and histone by Clb5-Cdk1 and Clb2-Cdk1 *in vitro*. The cell lysates of Y300 (WT), *CLB2-HA,* and *CLB5-HA* strains were immunoprecipitated with anti-HA antibody and protein A beads. The Clb2 and Clb5 protein level before the IP is shown in the top panel. The IP beads, 5 μg of bacteria-expressed Pol12 and 1 μg histone protein were used for the kinase assay as described in *Material and Methods*. After SDS/PAGE, the gels were stained with coomassie blue to determine the loading of Pol12 and histone proteins. Then the gels were dried and exposed to a phosphorimager screen. The protein levels of Pol12 and histone and their phosphorylation are shown in the middle and bottom panel. (*B*) The phosphorylation of Sld2 and Pol12 responds differently to the absence of Clb5. Strains with indicated genotypes were arrested in G_1 phase and then released into cell cycle at 37°C. Cells were collected at the indicated times for protein preparation and the phosphorylation of Sld2 and Pol12 was determined after Western blotting on the basis of the band shift. (*C*) High levels of Cdk inhibitor Sic1 decrease Pol12 phosphorylation. Asynchronous *POL12-myc* cells with vectors or P_{GAL}-*SIC1* plasmids were incubated in raffinose medium to mid-log phase. Galactose was added into the medium to a final concentration of 2% and cells were collected at 0, 2, 4, and 6 h. Pol12 phosphorylation was determined after Western blotting based on the band shift.

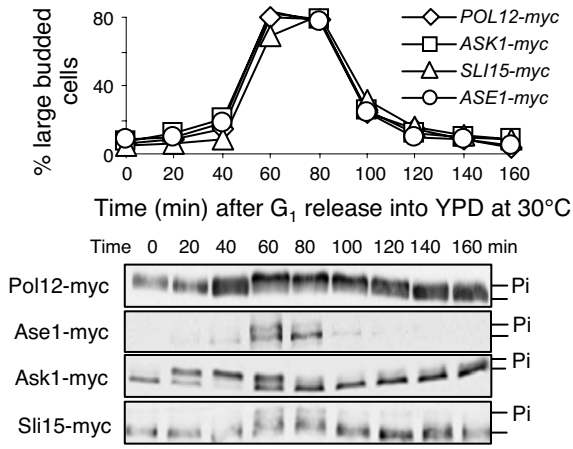

Fig. S2. Cell cycle-regulated phosphorylation and dephosphorylation of Pol12, Ase1, Ask1, and Sli15. G_1-arrested cells were released into cell cycle at 30°C and α factor was added back to block the second cell cycle. Cells were collected every 20 min to prepare protein samples and protein phosphorylation was analyzed after Western blotting.

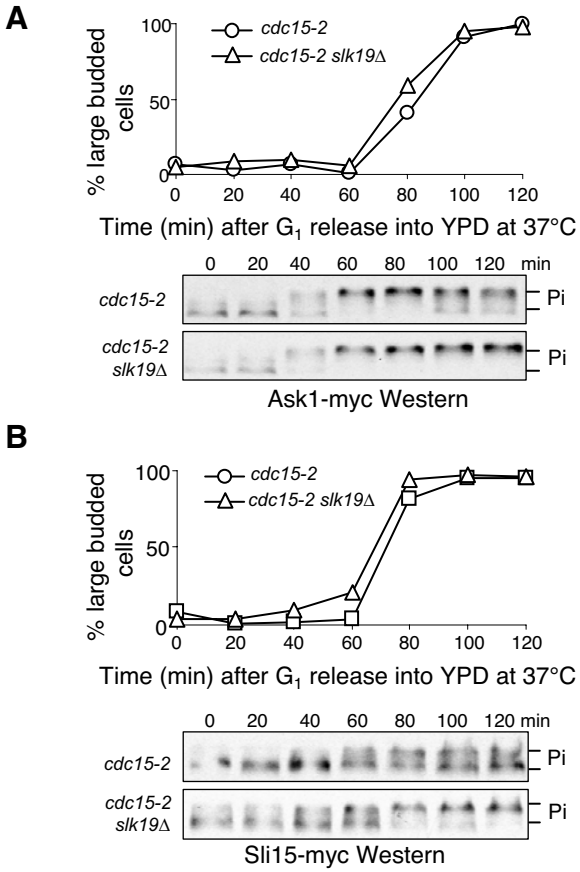

Fig. S3. The dephosphorylation of Ask1 and Sli15 in *cdc15–2* mutants depends on FEAR. G_1-synchronized *cdc15–2* and *cdc15–2 slk19*Δ mutants with myc-tagged Ask1 or Sli15 were released into YPD medium at 37°C. Cells were harvested every 20 min for the preparation of protein samples. The phosphorylation of Ask1 and Sli15 was examined after Western blotting and their phosphorylation is shown in (*A* and *B*), respectively.

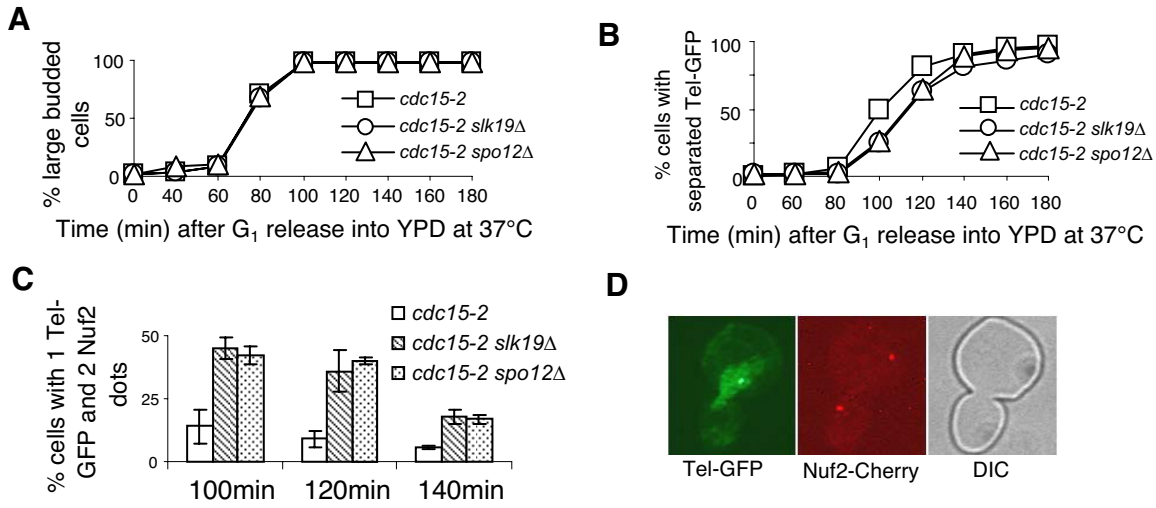

Fig. S4. FEAR mutant cells show telomere separation defects. G_1-arrested *cdc15–2*, *slk19Δ cdc15–2,* and *spo12Δ cdc15–2* cells with Cherry-tagged Nuf2 and GFP-marked telomere of chromosome V were released into YPD medium at 37°C. (*A*) shows the budding index. The telomere separation kinetics is shown in (*B*). The percentage of cells with asynchronous separation of Tel-GFP and Nuf2-Cherry is shown in *C*. *D* shows the representative cells with asynchronous telomere and centromere separation. The experiments are repeated for three times.

Table S1. Yeast strains used in this study

Strain	Relevant genotypes	Source
Y300	*MATa ura3–1 his3–11, 15 leu2–3,112 trp1–1 ade2–1 can1–100*	Lab stock
133–1–3	*MATa cdc15–2*	Lab stock
702–5–3	*MATa SLD2–9myc::LEU2*	Lab stock
730–4–4	*MATa cdc15–2 SLD2–9myc::LEU2*	This study
730–8–2	*MATa cdc15–2 slk19Δ::TRP1 SLD2–9myc::LEU2*	This study
784–16–2	*MATa cdc15–2 spo12Δ::KanMX SLD2–9myc::LEU2*	This study
689–4–2	*MATa cdc55Δ::Sphis5 + SLD2–9myc::LEU2*	This study
764–15–2	*MATa bfa1Δ::Sphis5 + SLD2–9myc::LEU2*	This study
769–3–2	*MATa cdc14–1 SLD2–9myc::LEU2*	This study
YYW28	*MATa cdc55Δ::Sphis5*$^{+}$	Lab stock
YFH240	*MATa bfa1Δ::Sphis5*$^{+}$	Lab stock
395–6–3	*MATa POL12–13myc::KanMX*	This study
745–1–2	*MATa cdc15–2 POL12–13myc::KanMX*	This study
745–3–1	*MATa cdc15–2 slk19Δ::TRP1 POL12–13myc::KanMX*	This study
768–6–4	*MATa cdc14–1 POL12–13myc::KanMX*	This study
446–3–3	*MATa ade2 can1 his3 trp1 ura3 cdc55Δ::Sphis5 + slk19Δ::TRP1*	This study
733–4–3	*MATa ade2 can1 his3 trp1 ura3 slk19Δ::TRP1*	This study
FLY63	*MATa mob1–77 ura3–52 his-D200 leu2–3,112 trp1–1*	Luca Lab
906–6–2	*MATa ade2 can1 his3 trp1 ura3 bfa1Δ::Sphis5 + slk19Δ::TRP1*	This study
785–2–4	*MATa spo12Δ::KanMX*	This study
798–1–1	*MATa cdc55Δ::Sphis5 + spo12Δ::KanMX*	This study
907–3–4	*MATa bfa1Δ::Sphis5 + spo12Δ::KanMX*	This study
768–6–4	*MATa cdc14–1 POL12–13myc::KanMX*	This study
728–1–1	*MATa cdc55Δ::Sphis5 + POL12–13myc::KanMX*	This study
YYW136–1	*MATa ura3–1 his3–11 15 leu2–3 112 trp1–1 ade2–1 can1–100 ASE1–13myc::Sphis5+*	This study
Y1113	*MATa ask1Δ::LEU2 ASK1–9myc-HIS3*	Elledge lab
746–4–1	*MATa cdc15–2 ask1Δ::LEU2 ASK1–9myc-HIS3*	This study
YYW109	*MATa ura3–1 his3–11 15 leu2–3, 112 trp1–1 ade2–1 can1–100 SLI15–13myc::Sphis5+*	This study
782–2–2	*MATa cdc15–2* SLI15–13myc::Sphis5+	This study
348–1–2	*MATa CLB2-HA*	Lab stock
911–12–3	*MATa spo12Δ::KanMX* CLB2-HA	This study
229–3–2	*MATa CLB5-HA*	Lab stock
990–5–4	*MATa spo12Δ::KanMX* CLB5-HA	This study
870–1–2	*MATa cdc15–2 TUB1-GFP::LEU2*	This study
870–2–2	*MATa cdc15–2 slk19Δ::TRP1 TUB1-GFP::LEU2*	This study
900–7–1	*MATa cdc15–2 spo12Δ::KanMX TUB1-GFP::LEU2*	This study
899–4–3	*MATa cdc15–2 promURA::tetR::GFP::LEU2 TELV::tetOX448::URA3 NUF2-mCherry*	This study
899–28–3	*MATa cdc15–2 slk19Δ::TRP1 promURA::tetR::GFP::LEU2 TELV::tetOX448::URA3 NUF2-mCherry*	This study
898–33–1	*MATa cdc15–2 spo12Δ::KanMX promURA::tetR::GFP::LEU2 TELV::tetOX448::URA3 NUF2-mCherry*	This study
683–15–3	*MATa MTW1–3GFP::HIS3*	This study
733–2–1	*MATa spo12Δ:: KanMX MTW1–3GFP::HIS3*	This study
920–24–1	*MATa slk19Δ::TRP1 MTW1–3GFP::HIS3*	This study
1055–8–3	*MATa cdc15–2 clb5Δ::URA3 SLD2–9myc::LEU2*	This study
1059–5–1	*MATa cdc15–2 clb5Δ::URA3 POL12–13myc::KanMX*	This study
1062–9–4	*MATa cdc15–2 clb2Δ::LEU2 POL12–13myc::KanMX*	This study
1004–29–1	*MATa CLB5Δdb SLD2–9myc::LEU2*	This study
1004–35–3	*MATa cdc15–2 CLB5Δdb SLD2–9myc::LEU2*	This study