Surpporting Information

Supplemental Table 1. Yeast strains used in this study

Strain	Genotype	Reference
W303	*MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL*	(11)
A1467	*MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc34-2*	(11)
A1469	*MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc53-1*	(11)
A2594	*MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc28-4, MET-*	(11)
A17188	*MATα,ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc34-2, cdc28-as1*	(11)
GY3706	*MATa,, his3-11, leu2-3,112, trp1-1, ura3-52, ade2-1, can1-100, GAL+, bar1, EXO84-12MYC::LEU2*	This study
DMY349	*MATa, his3-11, leu2-3,112, trp1-1, ura3-52, ade2-1, can1-100, GAL+, bar1*	(54)
GY3701	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, EXO84, LEU2)*	This study
GY3702	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, exo84-5A, LEU2)*	This study
GY3703	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, exo84-5E, LEU2)*	This study
GY3774	*MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc34-2, exo84Δ::HIS3 (CEN, EXO84, LEU2)*	This study
GY3775	*MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc34-2, exo84Δ::HIS3 (CEN, EXO84-5E, LEU2)*	This study
GY3776	*MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, cdc34-2, exo84Δ::HIS3 (CEN, EXO84-5E, LEU2)*	This study
GY3846	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, EXO84, LEU2), EXO70-GFP::URA3*	This study
GY3847	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, exo84-5A, LEU2), EXO70-GFP::URA3*	This study
GY3848	*Mata, ura3-52, leu2-3,112, his3Δ200, trp1, LA-, exo84Δ::HIS3 (CEN, exo84-5E, LEU2), EXO70-GFP::URA3*	This study
GY3791	*MATa sec3-2 ura3-52 leu2-3,112, sec3-2, exo84Δ:: KanMX6, (CEN, EXO84, LEU2)*	This study
GY3792	*MATa sec3-2 ura3-52 leu2-3,112, sec3-2,, exo84Δ:: KanMX6, (CEN, exo84-5A, LEU2)*	This study
GY3793	*MATa sec3-2 ura3-52 leu2-3,112, sec3-2, exo84Δ:: KanMX6, (CEN, exo84-5E, LEU2)*	This study

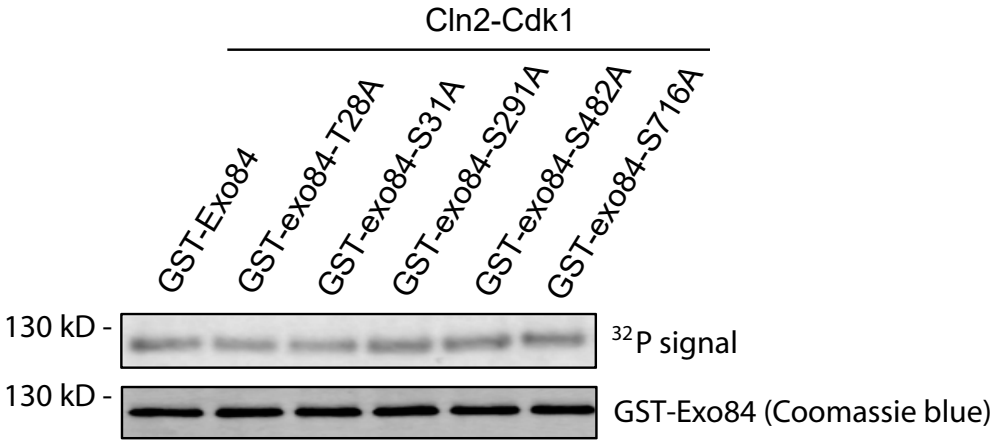
Supplemental Table 2. Plasmids used in this study

Plasmid	Description	Reference
pG338	*pRS315-EXO84, LEU2, CEN*	(68)
pG1621	*pRS315-EXO84-5A (T28A, S31A, S291A, S482A, S716A), LEU2, CEN*	This study
pG1623	*pRS315-EXO84-5E (T28E, S31E, S291E, S482E, S716E), LEU2, CEN*	This study
pG778	*pRS305-EXO84-12MYC, LEU2*, integration into *EXO84* by cutting with BglII	This study
pG465	*pGeX2T–EXO84*	This study
pG1849	*pGeX2T–exo84-5A (T28A, S31A, S291A, S482A, S716A)*	This study
pNB880	*pRS306-EXO70-GFP*, *URA3,* integration into *EXO70* gene by cutting with BstEII	Peter Novick lab

FIGURE LEGENDS

Supplemental Figure S1. Mutation of single Cdk1 consensus site did not affect Exo84 phosphorylation by Cln2-Cdk1. *In vitro* Cln2-Cdk1 kinase assays were performed with wild type Exo84 and e*xo84-A* mutants, which lack one of the five Cdk1 phosphorylation sites (T^{28}, S^{31}, S^{291}, S^{482}, S^{716}). The phosphorylation level of *Exo84-A* mutants by Cln2–Cdk1 is similar to that of wild type Exo84. GST-Exo84 and GST-*exo84-A* mutants were purified from *E. coli* and incubated with Cln2–Cdk1in the presence of γ-[^{32}P]ATP. Exo84 phosphorylation was detected by autoradiography (top). The Corresponding Coomassie blue–stained gel is shown on the bottom.

Fig. S1


Cln2-Cdk1
GST-Exo84
GST-exo84-T28A
GST-exo84-S31A
GST-exo84-S291A
GST-exo84-S482A
GST-exo84-S716A
130 kD -
32P signal
130 kD -
GST-Exo84 (Coomassie blue)