Supplemental Data

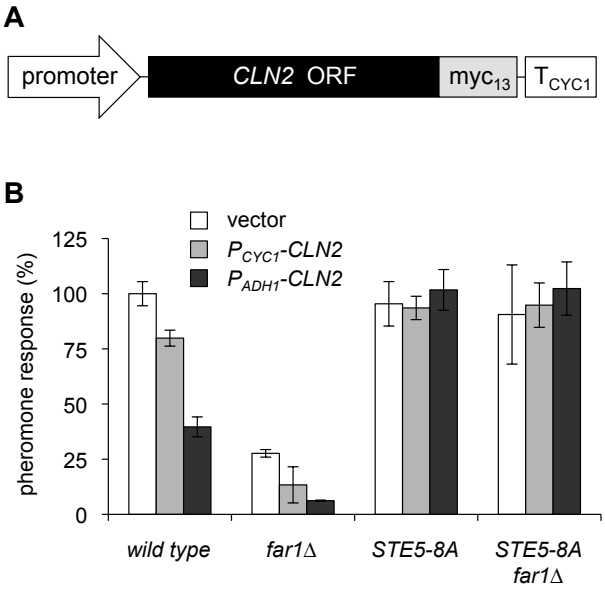

Figure S1. Effects of Cln2 and Far1 on pheromone response (related to Figure 1).

Pheromone signaling is reduced by deleting *FAR1* or expressing *CLN2* from foreign, constitutively-active promoters (P_{CYC1} and P_{ADH1}). Strains with the indicated genotypes (PPY640, PPY892, PPY2075, PPY2076) harbored plasmids expressing *CLN2* from the indicated promoters or a vector control (pPP681, pPP3203, pPP3079). Cells were treated with α factor (5 μM, 2 hr). Pheromone response was assayed using a transcriptional reporter (*FUS1-lacZ*); results (mean ± SD; n = 4) were normalized to the wild-type strain with vector. Note that cells lacking Far1 (*far1*Δ) have reduced signaling, and are sensitive to further reduction by extra *CLN2*. These features are eliminated by the Cdk-resistant *STE5-8A* allele [S1], such that the presence or absence of Far1 (or excess *CLN2*) no longer affects pheromone signaling. Thus, by using *STE5-8A* strains, it is possible to compare the effect of Far1 on Cln2-substrate interactions without complicating secondary effects on pheromone signaling due to the presence or absence of Far1 and/or Cln2.

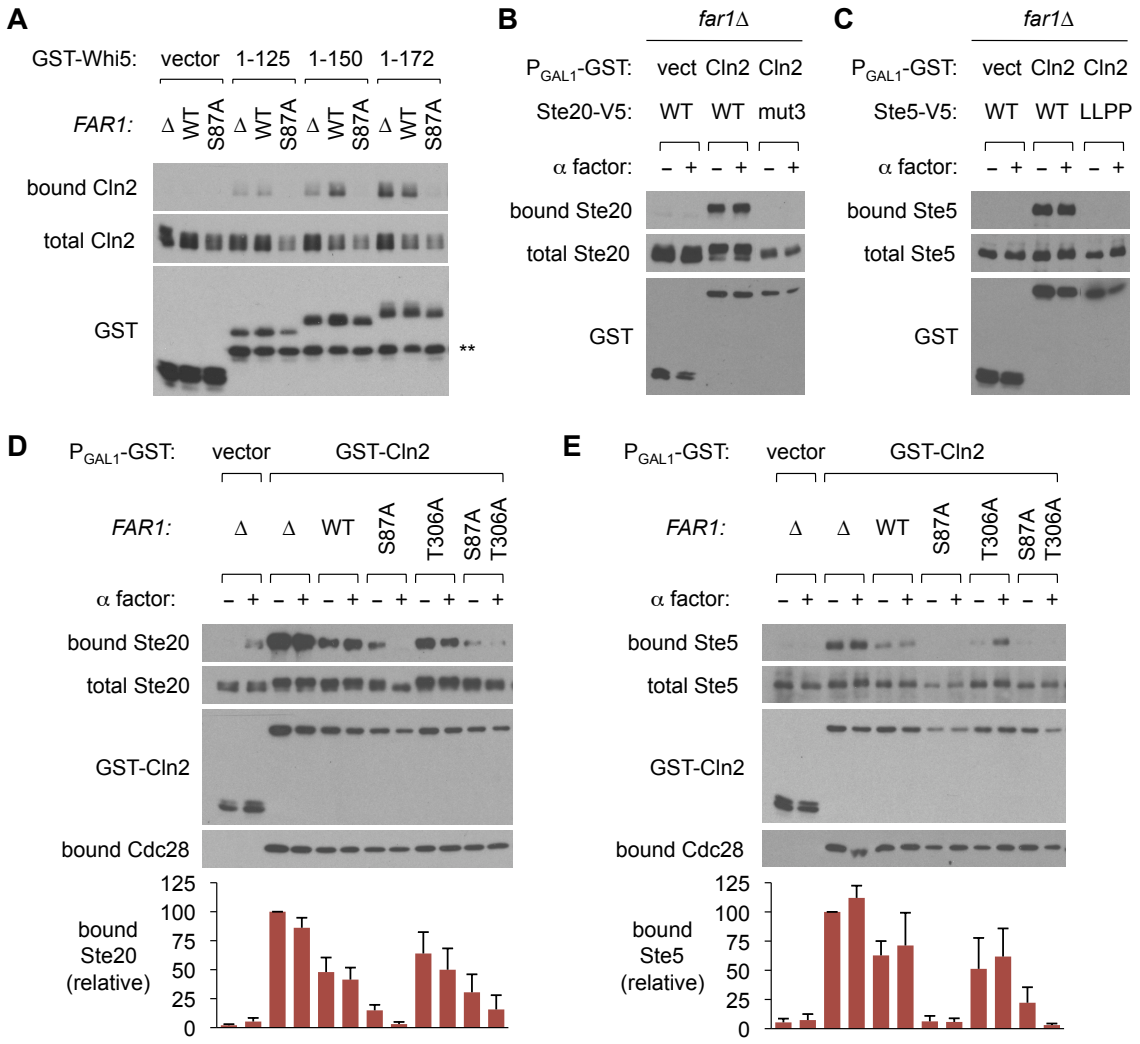

Figure S2. Effects of Far1 on Cln2-substrate binding interactions (related to Figure 2).

(**A**) Far1 inhibits binding of Cln2 to GST-Whi5 fragments. Strains (PPY2322, PPY2326, PPY2329) harbored Cln2-myc (pPP3203) plus GST fusions to Whi5 fragments (pPP4150, pPP4151, pPP4152) or GST alone (pPP2154). Binding was assayed by glutathione-sepharose capture followed by anti-myc and anti-GST blots. Note that Far1-S87A inhibits Cln2 binding to all Whi5 fragments, similar to results using full-length Whi5 (Figure 2E), and with all GST-Whi5 fusions a truncated product is also observed (marked by **). Phosphorylation of Whi5 by Cln2-Cdk depends on docking [S2], and it has at least one docking motif in the 121-150 region [S3], but it could also have additional motifs that confer weak binding to the 1-125 fragment.

(**B, C**) Binding of GST-Cln2 to full-length substrate proteins requires docking sites. Galactose-inducible GST or GST-Cln2 (residues 1-372) was co-expressed in *far1*Δ cells (PPY2327) with V5-tagged forms of full-length substrates (Ste20 or Ste5) expressed from their native promoters. Binding was assayed by glutathione-sepharose capture followed by anti-V5 and anti-GST blots. In each panel, the WT and mutant substrates were analyzed in parallel but were separated by additional lanes in the original gels. Panel B shows binding to full-length Ste20-WT or a docking site mutant (mut3; [S3]). Plasmids were pPP2154, pPP3573, pPP3267, and pPP3368. Panel C shows binding to full-length Ste5-WT or a docking site mutant (LLPP to AAAA; [S3]). Plasmids were pPP2154, pPP3573, pPP3266, and pPP3761.

(**D, E**) Far1 inhibits binding of GST-Cln2 to full-length Ste20 and Ste5. Strains with the indicated *FAR1* genotype (PPY2327, PPY2330, PPY2340, PPY2358, PPY2359) harbored galactose-inducible GST (pPP2154) or GST-Cln2 (pPP3573), plus V5-tagged Ste20 (pPP3267) or Ste5 (pPP3266). Cells were induced with galactose ± pheromone for 90 minutes, and then bound complexes were captured and analyzed by anti-V5, anti-GST, and anti-Cdc28 blots. Graphs show the relative level of substrate binding (mean ± SEM) from three (D) or four (E) experiments. Panel D is identical to Figure 2F and is repeated here for comparison to the other panels.

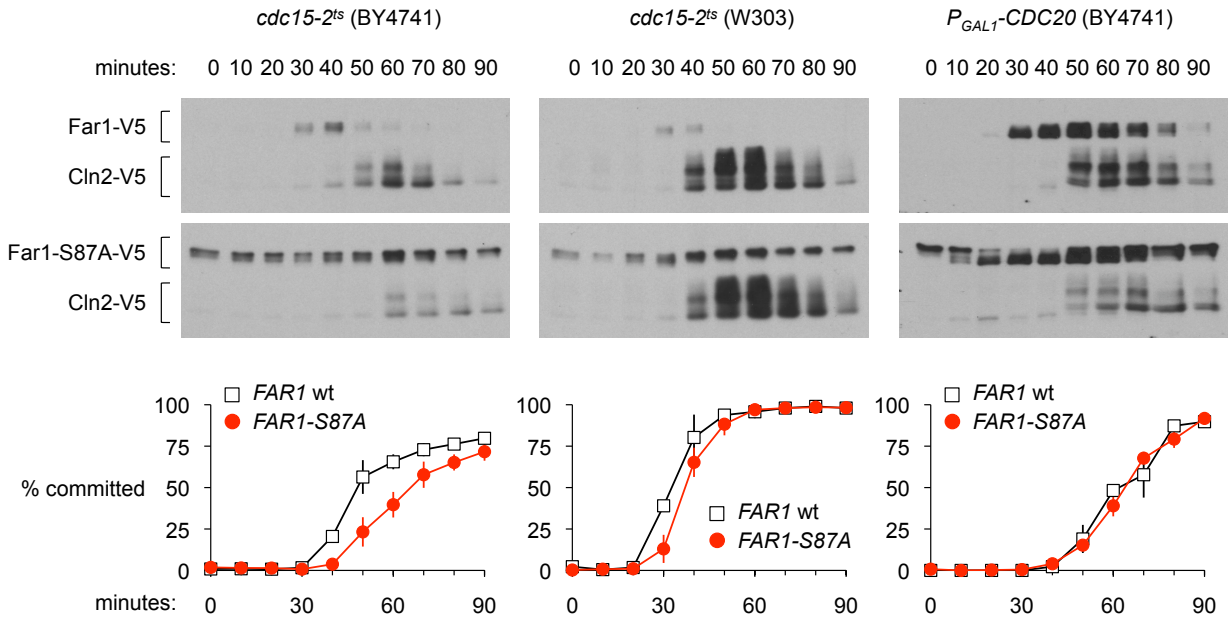

Figure S3. Far1 vs. Cln2 levels in synchronous cultures (related to Figure 3).

Using strains in which Far1 and Cln2 are tagged with the identical 3xV5 tag, cells were synchronized by arrest in mitosis (using either *cdc15-2* or P_{GAL1}-*CDC20*), and then released. At various times after release, aliquots were taken to assess protein levels and then were treated with pheromone to assess whether they could still arrest in G1 or had passed Start (committed). In each column the blots are directly comparable, as the experiments were performed in parallel, the individual gels had the same amount of protein loaded per lane, and the blots were processed in parallel and exposed simultaneously to a single film. For the same reasons, the four *cdc15-2* blots are also directly comparable to each other. Results shown are representative of two or more independent experiments. Graphs show mean ± SEM (n = 4-6; left) or mean ± range (n = 2; middle and right). Note that the degree of delay in commitment caused by the Far1-S87A mutant varied with experimental context; it also varied among independent isolates of BY4741 *cdc15-2* strains, and so we combined results from two *FAR1-wt* strains (PPY2393, PPY2394) and four *FAR1-S87A* strains (PPY2395, PPY2396, PPY2425, PPY2426). The data at left are identical to Figure 3C and are repeated here to facilitate comparison.

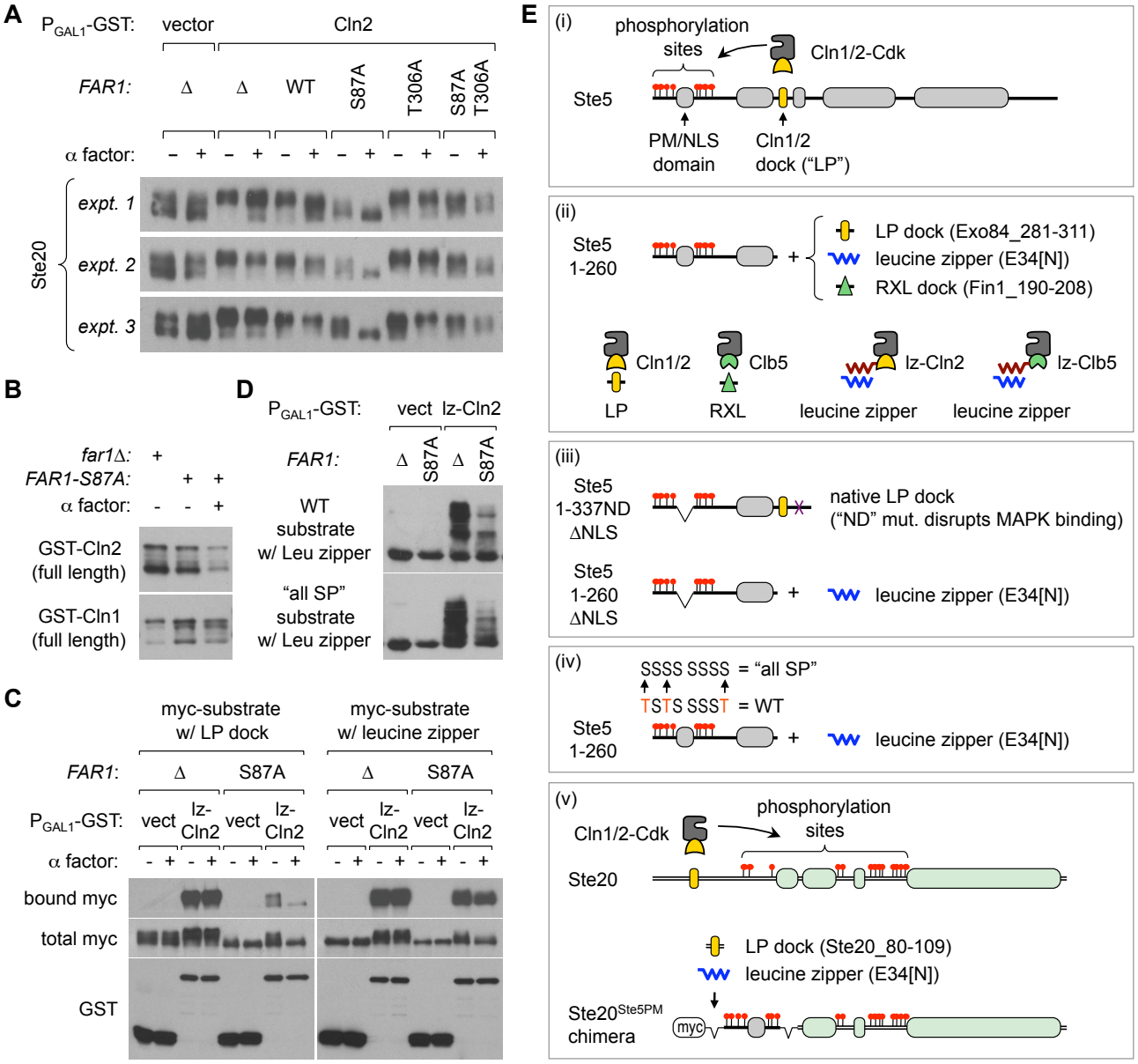

Figure S4. Pheromone/Far1 effects on Cdk phosphorylation in vivo (related to Figure 4).
(**A**) Far1 and pheromone inhibit Ste20 phosphorylation by Cln2-Cdk. Three repetitions of the experiment in Figure 4A are shown to demonstrate reproducibility of the patterns observed.
(**B**) Far1 and pheromone do not affect phosphorylation of Cln1/2 tails. Cultures of *far1*Δ or *FAR1-S87A* strains (PPY2327, PPY2330) harboring P_{GAL1}-*GST-cyclin* plasmids (pPP3572 or pPP3749) were induced with galactose ± α factor. Extracts were analyzed by anti-GST blot. Bands indicate the extent of phosphorylation at Cdk sites in the cyclin C-termini.
(**C**) Far1 and pheromone do not inhibit leucine zipper-mediated binding. Strains (as in B) expressed GST-(lz)-Cln2 or GST alone, plus a myc-tagged Cdk substrate consisting of a Ste20^{Ste5PM} chimera with either an LP-type docking site or a leucine half-zipper (panel Ev). Plasmids: pPP2154, pPP3916; pPP3218, pPP3979. Binding was assayed after induction with galactose ± α factor.
(**D**) The ability of Far1-S87A to partially reduce phosphorylation driven by a leucine zipper was compared for two substrates (see panel Eiv): one with WT phosphorylation sites (5 SP and 3 TP) and one ("all SP") in which the 3 TP sites were converted to SP, to block Cks1 from recognizing phospho-Thr as priming sites [S4, S5]. Strains: PPY2327, PPY2330. Plasmids: pPP2154, pPP3916; pPP3877, pPP4071.
(**E**) Substrates used in Figures 4 and S4. (i) Cdk phosphorylation sites and the "LP"-type Cln1/2 docking site in Ste5 [S3], from which other substrates are derived. (ii) *Top*, substrates used in Figures 4C-D. The native LP docking site in Ste5 is replaced with motifs shown. The recipient fragment, Ste5 1-260, excludes binding and phosphorylation sites for the MAPK Fus3 (in Ste5 267-330) [S6], preventing pheromone-induced phosphorylation from obscuring changes in Cdk-mediated phosphorylation. *Bottom*, cartoons of cyclin docking. (iii) Substrates used in Figure 4F. Deleting the PM/NLS domain [S7] causes the substrate to show only two mobility forms, which simplifies quantification. The top substrate has an "ND" mutation [S6] to prevent MAPK binding. (iv) Substrates used in Figure S4D. In "all SP", the 3 TP (Thr-Pro) sites in the WT Ste5 sequence are changed to SP (Ser-Pro). (v) *Top*, Ste20, which is analyzed in Figures 4A and S4A. *Bottom*, binding partners used in Figure S4C. A Ste20^{Ste5PM} chimera [S3] acted as recipient for an LP dock or a leucine zipper, to compare effects of Far1 on binding by each motif. (These chimeras were used because the phosphorylation substrates analyzed in Figures 4C-D show variable behavior in binding assays.)

Supplemental Experimental Procedures

Yeast Strains and Plasmids

Standard procedures were used for growth and genetic manipulation of yeast [S8, S9]. Yeast cultures were grown at 30˚C except as indicated otherwise. Strains and plasmids are listed in Tables below. A two-step (pop-in/pop-out) allele replacement method [S8] was used to introduce the following mutant alleles at their native genomic loci: *STE5-8A*, *FAR1-S87A*, *FAR1-T306A*, *FAR1-S87A T306A*, *cdc15-2*, and *cdc28-as2*. For Cdc28 inhibition we used the Cdc28-as2 [F88A] mutant [S10] because in our strains the more severe mutant Cdc28-as1 [F88G] [S11] caused slow growth and cell shape defects. PCR-mediated gene deletion and tagging used methods described previously [S12]. To add epitope tags at endogenous *FAR1* and *CLN2* loci, a PCR-generated cassette containing the 3xV5 tag and an antibiotic resistance gene (*kanMX6* or *natMX6*) was integrated downstream of the coding sequence. To construct P_{GAL1}-*CDC20* strains, the promoter of the essential cell cycle gene *CDC20* was replaced with a regulated promoter (P_{GAL1}) using a PCR-generated cassette marked with the *K. lactis URA3* gene ($URA3^{KI}$). To promote cyclin interaction with substrates that lack native docking sites, we used a weak leucine zipper (K_d ~ 800 nM) [S13, S14], wherein two hetero-dimerizing sequences called R34 and E34(N) [S14] were fused to the cyclin and substrate, respectively; further details will be described elsewhere (S.B. and P.M.P., in preparation).

GST Co-precipitation Binding Assays

Cultures (25 mL) were grown in synthetic media with 2% raffinose. Expression of GST fusion proteins from P_{GAL1}-*GST* plasmids was induced with 2% galactose, with or without 10 nM α factor, for 1.5 to 3 hours. For experiments using the *cdc28-as2* allele, cultures were also treated with 15 μM 1-NM-PP1. Cells were harvested and stored at -80˚C, then lysed by glass bead beating using a Fast-Prep apparatus (20 sec. at 4 m/s) and a non-ionic detergent buffer described previously [S15]. Aliquots were removed to provide input controls, and then GST fusions and co-bound proteins were collected using glutathione-sepharose beads (GE Healthcare #17-0756-01).

For experiments in which we wished to examine the effects of pheromone signaling on Cln2 binding interactions, one complexity arises from the fact that the mating pathway and Cln1/2-Cdk activity are mutually antagonistic. That is, pheromone-arrested cells do not express the *CLN1/CLN2* genes and hence lack Cln1/Cln2 proteins [S16], whereas Cln1/2-expressing cells inhibit pheromone signaling and degrade Far1 [S17-19]. Therefore, to allow us to vary experimental conditions without affecting protein levels and signaling responses, we circumvented these antagonistic effects as follows: (i) *CLN2* was expressed from a constitutively-active promoter (P_{CYC1} or P_{TEF1}); (ii) the ability of Cln2 to inhibit pheromone signaling (see Figure S1) was prevented by using strains with the *STE5-8A* allele [S1], which encodes a Cdk-resistant form of the pathway scaffold protein, Ste5; and (iii) our initial experiments used strains with the *FAR1-S87A* allele [S20], which is resistant to Cdk-triggered degradation. Subsequent experiments probed the role of Far1 by using strains with *far1*Δ and other *FAR1* alleles.

Synchronous Culture Experiments

For experiments in P_{GAL1}-*CDC20* strains, cells were grown in liquid YPGal medium (containing 2% galactose) and then arrested in M phase by pelleting, resuspending in YPD medium (2% glucose), and incubating for 3 hr. Cultures were released by two rounds of pelleting and washing in YPGal, followed by final resuspension in YPGal. At 10-minute intervals, aliquots were removed to prepare protein samples and test cell cycle commitment. At each time point, 2 mL was pelleted and immediately frozen in liquid nitrogen (and whole cell extracts were prepared later); a separate 3 mL aliquot was treated with α factor (0.2 μM) and incubated at

30˚C, and then at 120 minutes after release all treated aliquots were fixed by adding formaldehyde to 3.7%. Cell cycle commitment was scored as the percentage of cells (n = 200) that failed to arrest in G1 (i.e., as unbudded cells) after the pheromone treatment.

For experiments in *cdc15-2* strains, cultures were grown at 25°C, arrested at 37°C for 3 hr, then released by transfer to 25°C (using shaking water baths). Aliquots were taken at 10-min intervals to prepare protein samples and test cell cycle commitment as described above, except that 10 μM α factor was used for the *BAR1* strains in the W303 background.

Whole Cell Extracts

Whole cell extracts were prepared by a modified version of a previous protocol [S21]. Here, 300 μL of trichloroacetic acid (TCA) buffer (10 mM Tris-HCl, pH 8.0, 10% TCA, 25 mM ammonium acetate, 1 mM Na_2EDTA) was added directly to frozen cell pellets (usually from 2 mL culture), and incubated on ice for 10 minutes. Samples were pelleted in a microcentrifuge for 10 min at 4˚C. The pellet was resuspended in 75 μL Resuspension Buffer (0.1 M Tris.HCl, pH 11.0, 3% SDS), boiled for 5 min, allowed to cool at room temperature for 5 minutes, then re-centrifuged for 30 sec. The supernatant (60 μL) was transferred to a new tube, 10 μL was reserved to assay protein concentration by a Pierce BCA Protein Assay Kit (#23225), and then 50 μL of 2x SDS Sample Buffer was added to the remainder. Equivalent amounts of total protein (generally 20 μg) were loaded in each lane.

Immunoblotting

Protein samples were analyzed by SDS-PAGE, and transferred to PVDF using a submerged tank device. Myc-tagged proteins were detected with rabbit anti-myc (1:200 Santa Cruz Biotechnologies #sc-789) and HRP-conjugated goat anti-rabbit (1:3000, Jackson ImmunoResearch #111-035-144) antibodies. V5-tagged, HA-tagged, or GST-tagged proteins were detected with mouse anti-V5 (1:5000, Invitrogen #46-0705), anti-HA (1:1000, Covance #MMS101R), or anti-GST (1:1000, Santa Cruz Biotechnologies #sc-138) antibodies, followed by HRP-conjugated goat anti-mouse (1:3000, BioRad #170-6516) antibodies. Cdc28 was detected with goat anti-Cdc28 (1:200 Santa Cruz Biotechnologies #sc-6709) and HRP-conjugated donkey anti-goat (1:3000 Santa Cruz Biotechnologies #sc-2020) antibodies. Enhanced chemiluminescent detection used a Pierce SuperSignal West Pico kit (#34080).

Cyclin-Cdk Phosphorylation Assays

Following methods established in a previous study [S3], cells harboring P_{GAL1}-*GST-cyclin* constructs and HA-tagged Cdk substrates were grown in selective media with 2% raffinose and then induced with 2% galactose (for 40 min. to 2.5 hr) to drive cyclin expression. Where indicated, cultures were pre-treated with pheromone prior to galactose addition. Then, whole cell extracts were prepared, and substrate phosphorylation was assessed by SDS-PAGE and immunoblotting. Cdk substrates are diagrammed in Figure S4E. Note that most substrate fragments contain an NLS, and similar fragments localize to both nucleus and cytoplasm [S1, S7], so in principle they should be accessible to both nuclear and cytoplasmic kinases. Also note that, in order to help express different cyclins at comparable levels, all cyclins in the P_{GAL1}-*GST-cyclin* constructs were truncated to remove destabilizing motifs (see the Plasmids Table for precise boundaries). These truncations remove NLS motifs in some cyclins such as Cln3 [S22] and Clb2 [S23, S24], but the pattern of substrate specificity seen with these truncated cyclins is the same as that seen earlier with full-length cyclins [S3].

Strains and Plasmids Used in Main Figures

Figure 1.
(C) Strain: PPY2296. Plasmids: pPP3203, pPP2163.
(D) Strains: PPY2268, PPY2296. Plasmids: pPP3152, pPP1843, pPP3766, pPP3771.

Figure 2.
(B-F) Strains: PPY2322, PPY2326, PPY2329, PPY2354, PPY2356.
(C-D) Plasmids: pPP2163, pPP3825, pPP3203.
(E) Plasmids: pPP2154, pPP4146, pPP4147, pPP3203.
(F) Plasmids: pPP3573, pPP2154, pPP3267.

Figure 3.
(A) PPY2340, PPY2377, PPY2380, PPY2340 + pPP3266, PPY2340 + pPP3267.
(B) Strains: PPY2377, PPY2380. Plasmids: pPP3267, pPP3267, pPP2154.
(C) Strains: PPY2393, PPY2395.

Figure 4.
(A) Strains: PPY2322, PPY2326, PPY2329, PPY2354, PPY2356. Plasmids: pPP3573, pPP2154, pPP3267.
(B) Strains: PPY2369, PPY2371. Plasmids: pPP3267, pPP3573, pPP2154.
(C) Strains: PPY2327, PPY2340, PPY2330. Plasmids: pPP3916, pPP3807, pPP3877.
(D) Strains: PPY2327, PPY2330. GST-(lz)-cyclin plasmids: pPP2154, pPP3916, pPP3929, pPP3964, pPP3955, pPP3956. HA-tagged substrate plasmids: pPP3807, pPP3877, pPP3571.
(E) Strain: PPY2380. Plasmids: pPP2154, pPP3916, pPP3929, pPP3964, pPP3955, pPP3956.
(F) Strains: PPY2327, PPY2330. Plasmids: pPP3916, pPP3508, pPP3962.

Yeast strains used in this study.

Name	Strain Background*	Relevant Genotype
PPY640	(a)	*MAT***a** *FUS1::FUS1-lacZ::LEU2*
PPY892	(a)	*MAT***a** *FUS1::FUS1-lacZ::LEU2 far1::ADE2*
PPY2075	(a)	*MAT***a** *FUS1::FUS1-lacZ::LEU2 STE5-8A*
PPY2076	(a)	*MAT***a** *FUS1::FUS1-lacZ::LEU2 STE5-8A far1::ADE2*
PPY2268	(b)	*MAT***a** *far1Δ::kanMX6*
PPY2296	(b)	*MAT***a** *STE5-8A FAR1-S87A*
PPY2322	(b)	*MAT***a** *bar1Δ::hphMX6*
PPY2326	(b)	*MAT***a** *bar1Δ::hphMX6 far1Δ::kanMX6*
PPY2327	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A far1Δ::kanMX6*
PPY2329	(b)	*MAT***a** *bar1Δ::hphMX6 FAR1-S87A*
PPY2330	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1-S87A*
PPY2340	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A*
PPY2354	(b)	*MAT***a** *bar1Δ::hphMX6 FAR1-T306A*
PPY2356	(b)	*MAT***a** *bar1Δ::hphMX6 FAR1-S87A,T306A*
PPY2358	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1-T306A*
PPY2359	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1-S87A,T306A*
PPY2369	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A far1Δ::kanMX6 cdc28-as2*
PPY2371	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1-S87A cdc28-as2*
PPY2377	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1-3xV5::kanMX6*
PPY2380	(b)	*MAT***a** *bar1Δ::hphMX6 STE5-8A FAR1(S87A)-3xV5::kanMX6*
PPY2384	(a)	*MAT***a** *ade1 CLN2-3xV5::kanR FAR1-3xV5::natMX6 cdc15-2*
PPY2391	(b)	*MAT***a** *bar1Δ::hphMX6 CLN2-3xV5::kanMX6 FAR1-3xV5::natMX6* P_{GAL1}*-CDC20::URA3*^{KI}
PPY2392	(b)	*MAT***a** *bar1Δ::hphMX6 CLN2-3xV5::kanMX6 FAR1(S87A)-3xV5::natMX6* P_{GAL1}*-CDC20::URA3*^{KI}
PPY2393	(b)	*MAT***a** *bar1Δ::hphMX6 CLN2-3xV5::kanMX6 FAR1-3xV5::natMX6 cdc15-2*
PPY2395	(b)	*MAT***a** *bar1Δ::hphMX6 CLN2-3xV5::kanMX6 FAR1(S87A)-3xV5::natMX6 cdc15-2*
PPY2397	(a)	*MAT***a** *ade1 CLN2-3xV5::kanMX6 FAR1(S87A)-3xV5::natMX6 cdc15-2*

* Background: (a) W303 (*ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1*); (b) BY4741 (*his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*).

Plasmids used in this study.

Name	Alias	Description	Source
pPP681	pRS316	CEN URA3 vector	[S25]
pPP1843	pUG-GST-GFP	2μm URA3 P_{GAL1}-GST-GFP vector	[S7]
pPP2154	pHG-GST	CEN HIS3 P_{GAL1}-GST vector	[S3]
pPP2155	pHG-GST-GFP	CEN HIS3 P_{GAL1}-GST-GFP vector	this study
pPP2163	pHGT-S20A	CEN HIS3 P_{GAL1}-GST-ste20(1-333)	[S26]
pPP2330	p306-S5-8A	integrating URA3 STE5-8A	[S1]
pPP3025	pFA6a-KlacURA3-PGAL1	PCR template for $URA3^{KI}$-P_{GAL1} promoter insertion	this study
pPP3079	pADH1-CLN2-myc	CEN URA3 P_{ADH1}-CLN2-myc13 T_{CYC1}	this study
pPP3152	pH-TEFpr-CLN2-myc	CEN HIS3 P_{TEF1}-CLN2-myc13 T_{CYC1}	[S3]
pPP3203	pCYC1-CLN2-myc	CEN URA3 P_{CYC1}-CLN2-myc13 T_{CYC1}	[S3]
pPP3218	pM20-BXA5N-U	CEN URA3 myc13-[(Ste20 80-109)-(Ste5 1-85)-(Ste20 312-939)]	[S3]
pPP3266	pS5kV5	CEN URA3 STE5-3xV5 T_{CYC1}	this study
pPP3267	pRL116V5	CEN URA3 3xV5-STE20	[S3]
pPP3368	pRL116V5-Ala5	CEN URA3 3xV5-STE20(mut3 = docking site mutant)	[S3]
pPP3369	pRL116V5-Ala13	CEN URA3 3xV5-STE20(Ala13 = Cdk site mutant)	[S3]
pPP3508	pH5n-1-337ΔNLS-ND	CEN URA3 P_{STE5}-(Ste5 1-337[ΔNLS, ND])-3xHA T_{CYC1}	this study
pPP3571	pH5n-1-260-fRXL	CEN URA3 P_{STE5}-(Ste5 1-260)-(Fin1 190-208)-3xHA T_{CYC1}	[S3]
pPP3572	pt-HGT-CLN2-F	CEN HIS3 P_{GAL1}-GST-CLN2(full length) T_{CYC1}	[S3]
pPP3573	pt-HGT-CLN2-t	CEN HIS3 P_{GAL1}-GST-CLN2(1-372) T_{CYC1}	this study
pPP3630	pACL437	integrating URA3 cdc28-as2	[S10]
pPP3640	pJS21	integrating URA3 FAR1-S87A	[S27]
pPP3749	pt-HGT-CLN1	CEN HIS3 P_{GAL1}-GST-CLN1(full length) T_{CYC1}	[S3]
pPP3766	pUG-GST-F20L-wt	2μm URA3 P_{GAL1}-GST-GFP-[ste20(72-118) + ste20(120-333)]	this study
pPP3761	pS5kV5-Nhe-P4	CEN URA3 ste5(LLPP-AAAA)-3xV5 T_{CYC1}	[S3]
pPP3771	pUG-GST-F20M-5CSM-wt	2μm URA3 P_{GAL1}-GST-GFP-[ste5(263-335) + ste20(120-333)]	[S3]
pPP3807	pH5n-1-260-dExo84	CEN URA3 P_{STE5}-(Ste5 1-260)-(Exo84 281-311)-3xHA T_{CYC1}	[S3]
pPP3825	pHG-GST-S20M-5CSM-WT	CEN HIS3 P_{GAL1}-GST-[ste5(263-335) + ste20(120-333)]	this study
pPP3877	pS5kHA-1-260-E34(N)	CEN URA3 P_{STE5}-(Ste5 1-260)-(E34[N] zipper)-3xHA T_{CYC1}	this study
pPP3916	pt-HGT-CLN2-t-R34up	CEN HIS3 P_{GAL1}-GST-(R34 zipper)-CLN2(1-372) T_{CYC1}	this study
pPP3929	pt-HGT-CLN1-t-R34up	CEN HIS3 P_{GAL1}-GST-(R34 zipper)-CLN1(1-388) T_{CYC1}	this study
pPP3955	pt-HGT-Rup-CLB5-t	CEN HIS3 P_{GAL1}-GST-(R34 zipper)-CLB5(133-435) T_{CYC1}	this study
pPP3956	pt-HGT-Rup-CLB2-t	CEN HIS3 P_{GAL1}-GST-(R34 zipper)-CLB2(198-491) T_{CYC1}	this study
pPP3962	pS5kHA-1-260ΔNLS-E34N	CEN URA3 P_{STE5}-(Ste5 1-260[ΔNLS])-(E34[N] zipper)-3xHA T_{CYC1}	this study
pPP3964	pt-HGT-CLN3-t-R34up	CEN HIS3 P_{GAL1}-GST-(R34 zipper)-CLN3(1-395) T_{CYC1}	this study
pPP3979	pM20-BXA5N-E34N	CEN URA3 myc13-[(E34[N] zipper)-(Ste5 1-85)-(Ste20 312-939)]	this study
pPP4032	pIU-FAR1-T306A	integrating URA3 FAR1-T306A	this study
pPP4033	pIU-FAR1-S87A,T306A	integrating URA3 FAR1-S87A,T306A	this study
pPP3265	pFA6a-3xV5-kanMX6	3xV5::kanMX6 C-terminal tagging cassette	[S28]
pPP4011	p2275	integrating URA3 cdc15-2 (= G206D)	†
pPP4034	pFA6a-3xV5-natMX6	3xV5::natMX6 C-terminal tagging cassette	this study
pPP4071	pS5kHA-1-260-E34N-3sp	pPP3877 w/ Ste5 T4S,T29S,T102S	this study
pPP4146	pHGT-SIC1-1-214	CEN HIS3 P_{GAL1}-GST-Sic1 (1-214)	this study
pPP4147	pHGT-WHI5-FL	CEN HIS3 P_{GAL1}-GST-Whi5 (full length)	this study
pPP4150	pHGT-WHI5 1-125	CEN HIS3 P_{GAL1}-GST-Whi5 (1-125)	this study
pPP4151	pHGT-WHI5 1-150	CEN HIS3 P_{GAL1}-GST-Whi5 (1-150)	this study
pPP4152	pHGT-WHI5 1-172	CEN HIS3 P_{GAL1}-GST-Whi5 (1-172)	this study

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