Materials and Methods

Strain construction, materials, and Net1 mutagenesis

All strains used are in the W303 background (*can1-100*, *leu2-3*, *his3-11*, *trp1-1*, *ura3-1*, *ade2-1*) except where noted in the strain table (Supplementary Table 1). A strain expressing a stable form of Clb2 lacking both the KEN and destruction boxes ($Clb2C_2DK_{100}$)HA3 was used in over-expression experiments with Clb2 (*1*).

Net1 mutant constructs were created as previously described (*2*). Briefly, a wild type *NET1-myc9* epitope tagged construct was cloned into a modified pRS304 vector containing 300bp upstream of the ATG translation start site using NcoI and EagI. Site-directed mutagenesis of Serine/Threonine to Alanine was carried out using QuikChange Site-Directed mutagenesis kit from Stratagene (La Jolla, CA). The indicated Serine/Threonine were mutated to Alanine in Net1-13m (166,169,212,231,252,259,356,362,384,385,497,611,676), Net1-6m (166,169,212,231,252,259), Net1-3-Cdk (166,212,252), and Net1-6Cdk (62*, 166,212,252,297,304) where * indicates that residue 62 was mutated to ensure complete elimination of all Cdk consensus sites even though it was not determined to be phosphorylated *in vivo*. Mutagenesis was confirmed using restriction digests followed by DNA sequencing. All constructs were targeted by linearization with BstXI to the *trp1* locus in a *NET1*/*net1::his5*$^+$ heterozygous diploid. The strains were sporulated and tetrads were dissected to obtain a haploid isolate of the integrant over *net1::his5*$^+$. Copy number of integrants was estimated by normalizing extract protein from transformed and wild type cells and blotting for Net1 levels. Proper localization of all *net1* phosphosite

mutants was confirmed by indirect immuno-fluorescence against the myc epitope (data not shown).

Production and purification of antibodies made against phosphorylated peptides was performed by Abgent (San Diego, CA). A pair of peptides – phosphorylated or not at the indicated, underlined residue – was synthesized to generate and purify each of the following antibodies against Net1: anti-phosphopeptide A corresponding to aa 159-173 (RSKLNNGSPQSVQPQC); anti-phosphopeptide B corresponding to aa 205-219 (NGSMRVWTPLARQIYC); and anti-phosphopeptide C corresponding to aa 245-259 (PPPTQPQSPPIRISSC). All peptides contained a Cysteine at the C-terminus, and anti-phosphopeptide B was modified by replacing Serine with Tryptophan at the –1 position relative to the underlined Threonine to optimize the phosphoepitope presentation. Antibodies specifically reactive against the phosphopeptides were positively selected on a resin derivatized with the phosphopeptide immunogen and negatively selected by passage through a resin derivatized with the unphosphorylated version of the peptide. Anti-phosphopeptide B (α-PP-B) was used in all experiments described since it generated the strongest signal against Phospho-Net1 (Fig. 2A).

Cell Growth and Synchronization Procedures

Cells were grown in yeast extract-peptone (YP) or in yeast minimal (YM) media containing 2% glucose (YPD,YMD), 2% raffinose (YPR,YMR) or 2% galactose (YPG,YMG) as carbon source. Where appropriate, minimal media were supplemented with leucine, histidine, tryptophan, uracil, and adenine to complement auxotrophies. Synchronization of cells in G1 phase was achieved with α-factor added at 10 μg/ml for

BAR1 cells and 0.1 μg/ml for *bar1*Δ cells for at least 3 hrs at 25°C. Cells were judged to be arrested when greater than 90% of cells displayed the elongated "shmoo" phenotype. Cells were released from α factor by filtration through a 0.2μm filter followed by a wash with 150 ml of YP, then resuspended in the desired volume at a density of 1 $O.D._{600}$/ml. For elutriation, cells were grown overnight in YP containing 2% raffinose and 2% galactose and harvested at log phase. Elutriation was performed as described (*3-5*) for the collection of small, unbudded G1 cells; contamination with budded cells was measured to be no more than 2%. For galactose induction experiments, cells were grown overnight in either YMR or YPR until an $O.D._{600}$ of 1.0 was reached, then induced with 2% galactose followed by time point collection.

Cell Extract Preparation and Western Blotting

Cells were grown to an $O.D._{600}$ of 1.0, and for every time point 2 ml of culture was collected and TCA added to a final concentration of 20%. Cells were collected by centrifugation and washed with 2 ml of Tris-HCl (pH 7.5). SDS loading buffer [70 μl of 100mM Tris-HCl (pH 7.5), 20% glycerol, 4% SDS, 2M Urea, 200mM DTT] was added, tubes were boiled for 3 minutes, and 100μl of acid-washed glass beads (500μm) were added to each tube followed by boiling for an additional 2 minutes. Tubes were vortexed for 45 sec using a Bio 101 multi-bead vortexer at setting 5.5. Tubes were boiled again for 2 minutes and 5μl of sample was fractionated on a 10% SDS-PAGE gel followed by transfer to a nitrocellulose membrane. Western blot analysis was performed with the following primary antibodies at the indicated dilutions: All anti-phospho Net1 antibodies (α-PP-A, α-PP-B, α-PP-C) at 2μg/ml; anti-Clb2 (1:3000), anti-RPA190 (1:5000), anti-

Cdc28 (1:5000), anti-Clb3 (1:2000), anti-myc (9E10) (1:5000), anti-His (1:250), anti-HA (1:5000), and anti-Cdc14 (1:1000).

Immunoprecipitation and Clb2–Cdk release/kinase assay.

To prepare extracts for immunoprecipitation, 10 $O.D._{600}$ units of a log phase cell culture was harvested and washed with 2 ml of Tris-HCl (pH 7.5). Cells were re-suspended in 500µl lysis buffer [25mM HEPES/KOH (pH7.5), 150mM NaCl, 1mM DTT, 0.2% Triton, 1mM EDTA, 1mM PMSF, 1mM Benzamidine, 1x Protease Inhibitor Cocktail (Aprotinin, Chymostatin, Leupeptin, and PepstatinA all at 5µg/ml in 90% DMSO)], transferred to a flat-bottom 2 ml tube and supplemented with 100µl of acid-washed glass beads (500µm). Samples were vortexed using a Bio 101 multi-beads vortexer at setting 5.5 (speed) and 45sec (time). Tubes were then centrifuged for 5 minutes at 14,000 rpm and the supernatant was collected. Clarified extract (400µl) was incubated with 60µl of 9E10-coupled protein A beads for 1 hour on a rotator at 4°C. Beads were collected and washed ten times in wash buffer [25mM HEPES/KOH (pH7.5), 150mM NaCl, 1mM DTT, 0.2% Triton], and divided to approximately 15µl beads per reaction condition. For protein kinase assays, 3µl of either Clb2–Cdk or Clb5–Cdk was used with either 1µg of myc9-Net1 (purified from insect cells infected with a recombinant baculovirus) (*6*), or 5µg of Histone H1. For assays that monitored release of Cdc14 from bead-bound Net1-myc9, varying concentrations of *in vitro* assembled Clb2–Cdk in 30µl kinase buffer [25mM Tris-HCl (pH 7.5), 10mM $MgCl_2$, 1mM ATP, 1mM DTT, 0.1mg/ml BSA, 50mM NaCl] were mixed with 15µl 9E10 beads coated with RENT complex. Reactions were allowed to proceed for 30 minutes on a rotator at 25°C.

Supernatant and beads were processed for Western blot analysis as previously described (*2*). For *in vitro* assays with bacterially expressed constructs, approximately 116ng of Net1 per 20µl of Ni^{+2}-NTA beads, 10ng Cdc14, and 5µl of roughly 30ng/µl stock of Clb2–Cdk was used per reaction.

Immuno-fluorescence and Cdc14 release quantification

Immuno-fluorescence was performed as previously described (*2, 7*). The analysis of Cdc14 localization for (fig. S1) was performed in haploid cells carrying the transposon-mutagenized *net1* allele. Rabbit anti-Cdc14 (1/3000) and rat anti-tubulin monoclonal antibody YL1/34 (1/1000) were used at the indicated dilutions. Images of synchronized cells at 70-110 minutes following release from α factor were collected on a Zeiss Axioskop or Axiovert 200M microscope using a Hamamatsu CCD digital camera. Spindle length measurements were performed using Zeiss Axiovision software.

Supplementary fig. 1. Cell cycle-regulated binding site for Cdc14 resides in the N-terminal half of Net1

The numbers adjacent to each construct indicate where a transposon insertion occurred in the *NET1* locus (*8, 9*) with the exception of the aa 1-621 HA-fragment of Net1 in which endogenous *NET1* was replaced by the truncated allele (RJD1783). The localization of Cdc14 and the length of microtubule spindles in asynchronous cell populations were determined by indirect immuno-fluorescence using anti-Cdc14 and anti-tubulin antibodies, respectively. Cell cycle position was estimated from the length of the microtubule spindle. The hatched bars indicate fragments of Net1 with proper nucleolar localization as reported in the TRIPLES database (*9*). 'N+C' refers to both nuclear and cytoplasmic staining.

Supplementary fig. 2. Net1 phosphosite mutants are defective in Cdc14 release in early anaphase.

(A) Mutant *cdc15-2* cells carrying either *net1-13m* (RJD2611) or *net1-6m* (RJD2612) alleles were synchronized with／ factor at 25°C and released in Yeast-Peptone 2% glucose at 37°C. Cells were collected for analysis by indirect immuno-fluorescence at 10 to 15 min time intervals. Staining was performed with DAPI, anti-Cdc14, and anti-tubulin antibodies for determination of nuclear position, Cdc14 localization, and spindle length, respectively. Cell outlines are indicated.

(B) Mutant *cdc15-2* cells carrying the *net1-6m* allele were synchronized with／ factor at 25°C and released in Yeast-Peptone 2% glucose at 37°C. Cells collected at 70 to 110 min after／ factor release were double-labeled with anti-Cdc14 and anti-tubulin antibodies. Release of Cdc14 from the nucleolus was determined to be either complete (black boxes) or partial (white

boxes; see legend to Fig. 1A) and was plotted against spindle length. Over 350 cells were counted for each panel. The wild type *NET1* control is shown in (Fig. 1A).

(C) Mutant *cdc15-2* cells carrying *net1-3Ax* (an allele in which the 3 non-Cdk sites S169, S231, and S259 from *net1-6m* were mutated to Alanine) were synchronized with／ factor at 25°C and released in Yeast-Peptone 2% glucose at 37°C. Cells collected at 70 to 110 min after／ factor release were double-labeled with anti-Cdc14 and anti-tubulin antibodies. Release of Cdc14 from the nucleolus was determined to be either complete (black boxes) or partial (white boxes; see legend to Fig. 1A) and was plotted against spindle length. Over 350 cells were counted for each panel. The wild type *NET1* control is shown in (Fig. 1A).

Supplementary fig. 3. Net1 phosphosite mutants are defective in Cdc14 release in early anaphase.

(A) Defective release of Cdc14 from the nucleolus in early anaphase in Net1 mutants. Mutant *cdc15-2* cells carrying either *NET1* (WT) (RJD2617), *net1-3Cdk* (*3Cdk*; RJD2613), or *net1-6Cdk* (*6Cdk*; RJD2614) alleles were synchronized with／ factor at 25°C and transferred to Yeast-Peptone media containing 2% glucose at 37°C. Cells were collected at 10 to 15 min time intervals and stained with DAPI, or antibodies to Cdc14 and tubulin to determine nuclear position, Cdc14 localization, and microtubule spindle length, respectively. Cell outlines are indicated.

(B) Enhanced temperature-sensitive growth phenotype of *dbf2-2* and *cdc15-2* combined with *net1-3Cdk* and *net1-6Cdk*, respectively. Starting with 3000 cells, serially diluted with 3 volumes of Yeast-Peptone media containing 2% glucose. Strains of *dbf2-2* (RJD2625), *dbf2-2* carrying a *net1-3Cdk* allele (RJD2626), *cdc15-2* (RJD2610), and *cdc15-2* carrying a *net1-6Cdk* allele

(RJD2614) were spotted on YPD plates from right to left, and incubated at the indicated temperature for 2-3 days. Two independent isolates of each strain were used. The first two isolates in the 33.5°C panel were compiled from different sections of the same plate.

Supplementary fig. 4. Cdk sites are conserved in Net1 orthologs from different yeast species.

Sequence alignment of *S. cerevisiae* Net1 and its orthologs from other budding yeasts reveals that Cdk phosphorylation sites mapped *in vivo* are highly conserved. Percent identities refers to exact matches in amino acid alignment for both sequences being compared divided by the total sequence length of *S. cerevisiae* Net1. Percent positives refers to matches where an amino acid difference exists between the two aligned sequences but both amino acids belong to the same family (acidic, basic, uncharged polar, nonpolar) divided by the total sequence length of *S. cerevisiae* Net1. Percent gaps refers to the number of spaces introduced into an alignment to compensate for insertions and deletions in one sequence relative to another divided by the total sequence length of *S. cerevisiae* Net1. Each parameter is shown for each species compared to Net1 from *Saccharomyces cerevisiae*.

Supplementary fig. 5. Loading controls and immuno-fluorescence analysis of *clb2*Δ and *clb1*Δ *clb2*Δ *GAL1p-CLB2* cells.

(A) Phospho-specific antibodies to Net1. Phospho-specific antibodies were raised against three peptides containing phosphorylated Serine166 (Anti-PP-A), Threonine 212 (Anti-PP-B), or Serine 252 (Anti-PP-C). Crude extracts from *cdc14-1* strains carrying either a wild type (+; RJD2615) or a mutant (3Cdk; RJD2616) *NET1-myc9* allele in which the three sites were

converted to alanine were fractionated by SDS-PAGE and immunoblotted with the different antibodies. Anti-myc (9E10) detection of Net1-myc9 was used as a loading control.

(B) Clb2–Cdk phosphorylates Net1 on physiological sites. Same as in (Fig.2B) except immunoblotted with antibodies to PP-C or myc.

(C) Loading controls using (anti-Cdc28) and Clb2 amounts (anti-Clb2) for (Fig. 2C).

(D) Loading controls using (anti-Cdc28) for (Fig. 2D).

(E) Samples of *clb2*Δ cells from (Fig. 2C) were subjected to indirect immuno-fluorescence with anti-Cdc14 and anti-tubulin antibodies as previously described for the indicated time points to monitor Cdc14 localization and spindle length, respectively.

(F) Samples of *clb1*Δ *clb2*Δ *GAL1p-CLB2* cells from (Fig. 2D) were subjected to indirect immuno-fluorescence with anti-Cdc14 and anti-tubulin antibodies as previously described for the indicated time points.

Supplementary fig. 6. Mutant *clb2O*cells display FEAR defects.

(A) Impaired release of Cdc14 from the nucleolus in early anaphase in *clb1*Δ *clb2*Δ and *cdc15-2 clb2*Δ mutants. Samples of *clb1*Δ *clb2*Δ *GAL1p-CLB2* (RJD2624) and *cdc15-2 clb2*Δ cells (RJD 2622) were collected after elutriation/*GAL*-shutoff (RJD2624) or／ factor block/release (RJD 2622) and stained with DAPI, anti-Cdc14, and anti-tubulin antibodies. Two panels are shown for each mutant cell line, representing early anaphase (1st panel) and late anaphase (2nd panel). Cell outlines are indicated.

(B) Mutant *clb2*Δ exacerbates the temperature-sensitive growth phenotype of MEN mutants. Starting with 3000 cells, three-fold serial dilutions of *cdc15-2* (RJD602) and *cdc15-2 clb2*Δ cells

(RJD2622) were spotted on Yeast-Peptone plates containing 2% glucose as described in the legend of (fig. S3B). Three independent isolates of each strain were used.

(C) Mutant *cdc15-2 clb2*Δ cells arrest in late anaphase. Mutant *cdc15-2* cells (open squares; RJD2630), *cdc15-2 clb2*Δ cells (closed diamonds; RJD2631), and *esp1-1* cells (open circles; RJD2629) containing a *tetO112* array at the *URA3* locus and expressing 3tetR-GFP from the *HIS3* locus were synchronized in G1 phase with／ factor and released at 37°C. Samples were taken at the indicated time points to determine the percentage of cells with unsegregated (one GFP dot) or segregated (two GFP dots) chromosomes.

Supplementary fig. 7. Cdc28 loading controls and cell cycle analysis of Cdc14 release and spindle length for (Fig. 4, A and B).

(A) Cdc28 levels (anti-Cdc28) were used as a loading controls for the indicated time points and strains for (Fig. 4A).

(B) Cdc28 levels (anti-Cdc28) were used as a loading controls for the indicated time points for (Fig. 4B). Roman numerals refer to the following strain genotypes: (i) wild type (RJD2617), (ii) *cdc15-2* (RJD2610), (iii) *cdc15-2 spo12*Δ (RJD2620), and (iv) *cdc15-2 slk19*Δ (RJD2621).

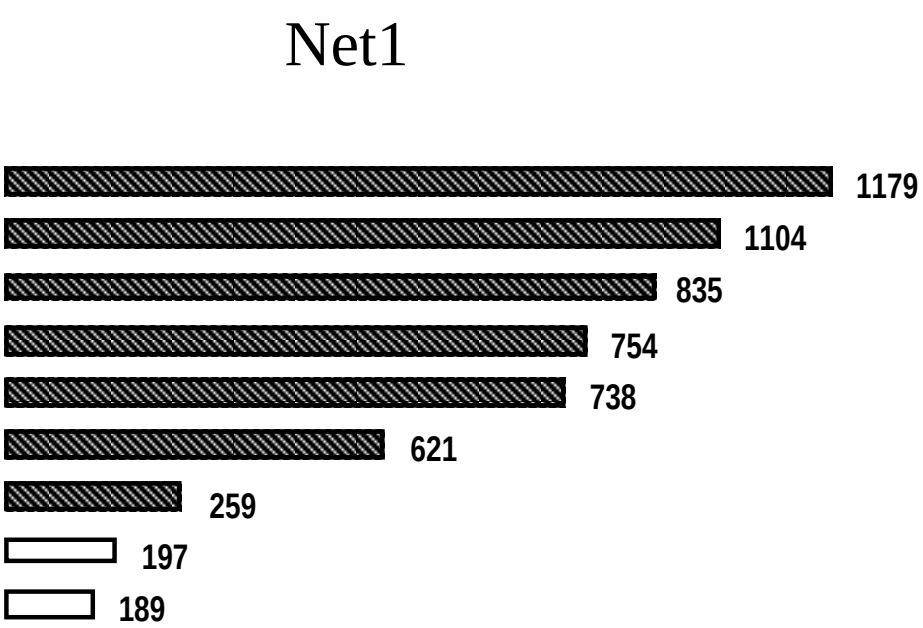
(C) Cdc15 along with either Spo12 or Slk19 is required for proper release of Cdc14 from the nucleolus during early anaphase. The same aliquots collected for (Fig. 4B) were also analyzed by indirect immuno-fluorescence with anti-Cdc14 and anti-tubulin antibodies as previously described.

(D) Levels of Clb2 (anti-Clb2) and loading control (anti-Cdc28) for (Fig. 4C)

(E) Levels of Cdc5(ΔDB) (anti-HA) and amount of Clb2 (anti-Clb2) for (Fig. 4D).

Supplementary Table 1. Net1 *in vivo* phosphorylation sites.

Supplementary Table 2. Strains used in this study.



Cdc14 Localization

G1	Anaphase
nucleolus	N+C
nucleolus	N+C
nucleolus	N+C
nucleolus	N+C
nucleolus	N+C
nucleolus	N+C
N+C	N+C
N+C	N+C
N+C	N+C

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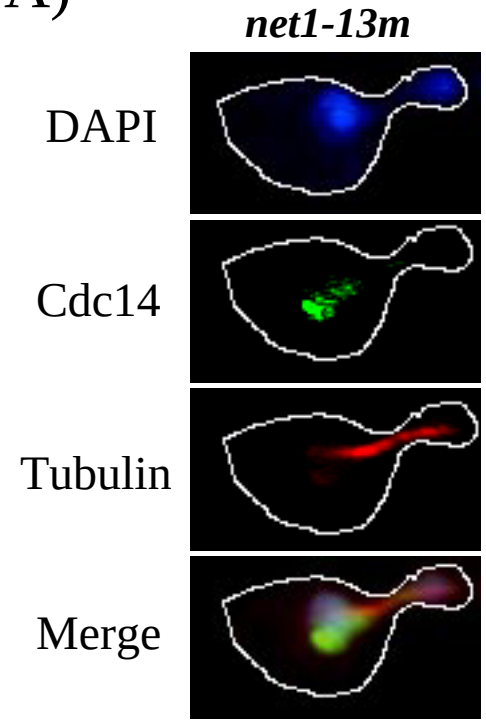
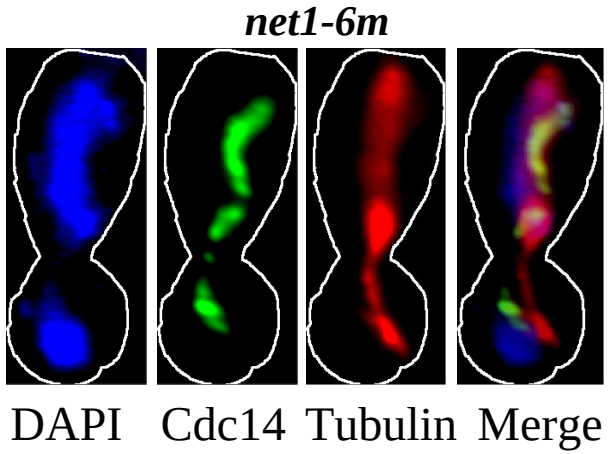
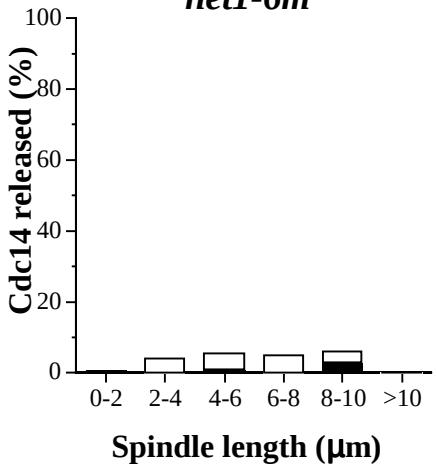
fig. S1

A)





B)



C)

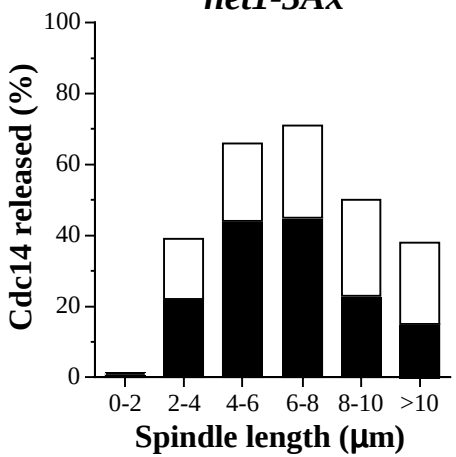


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fig. S2

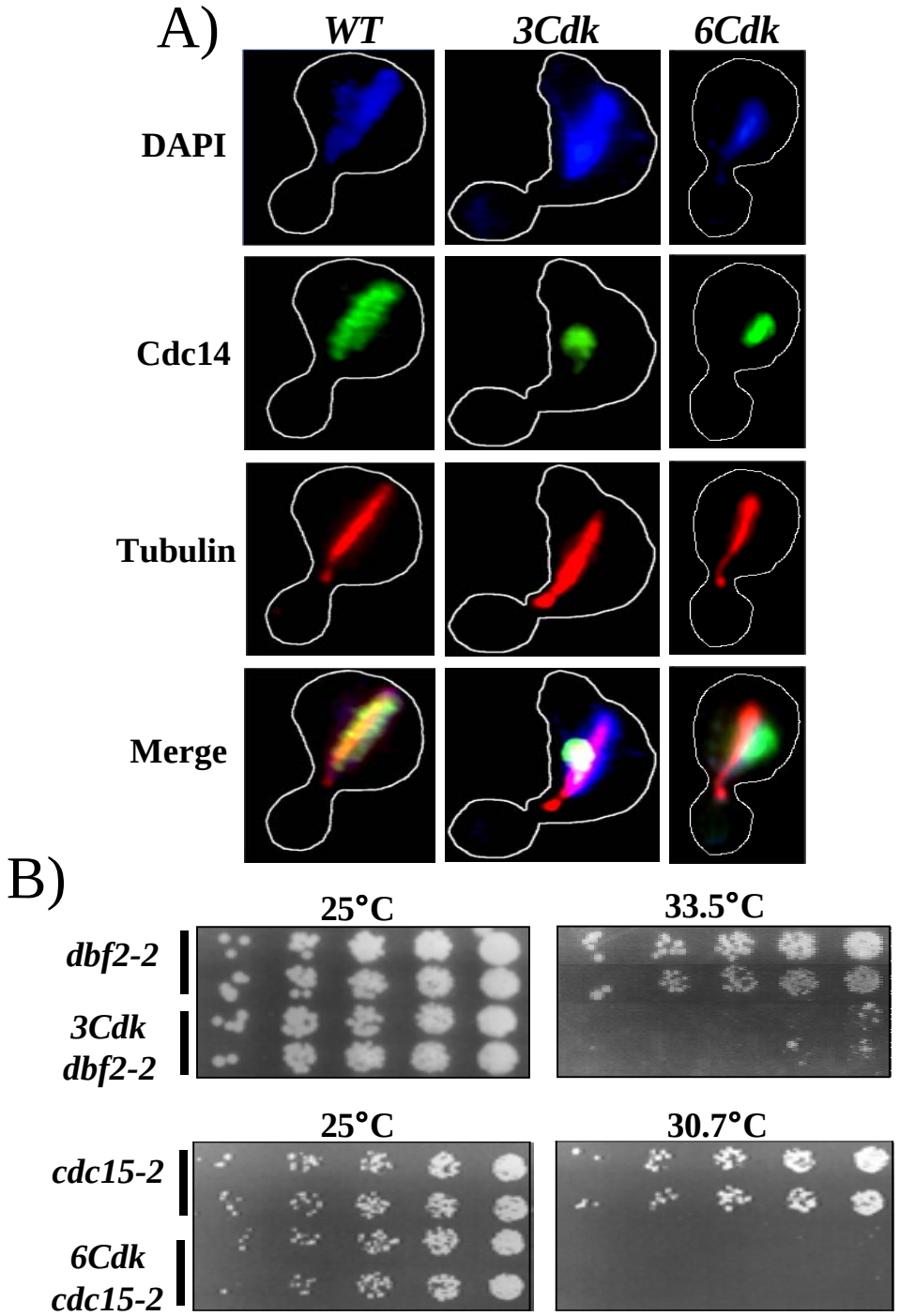

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fig. S3

S. cerevisiae	T62P	S166P	T212P	S252P	S297P	T304P	%identities	%positives	%Gaps
S. bayanus	+	+	+	+	+	+	56	63	4
S. mikatae	+	+	+	+	+	+	60	66	2
S. paradoxus	+	+	+	+	+	+	66	69	1
S. castellii	-	-	+	+	+	+	29	42	20
S. kudriavzevii	+	+	+	+	+	+	60	65	2
Candida glabrata	+	-	+	+	+	+	36	50	12
Kluyveromyces lactis	-	-	+	+	+	+	31	44	19

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fig. S4

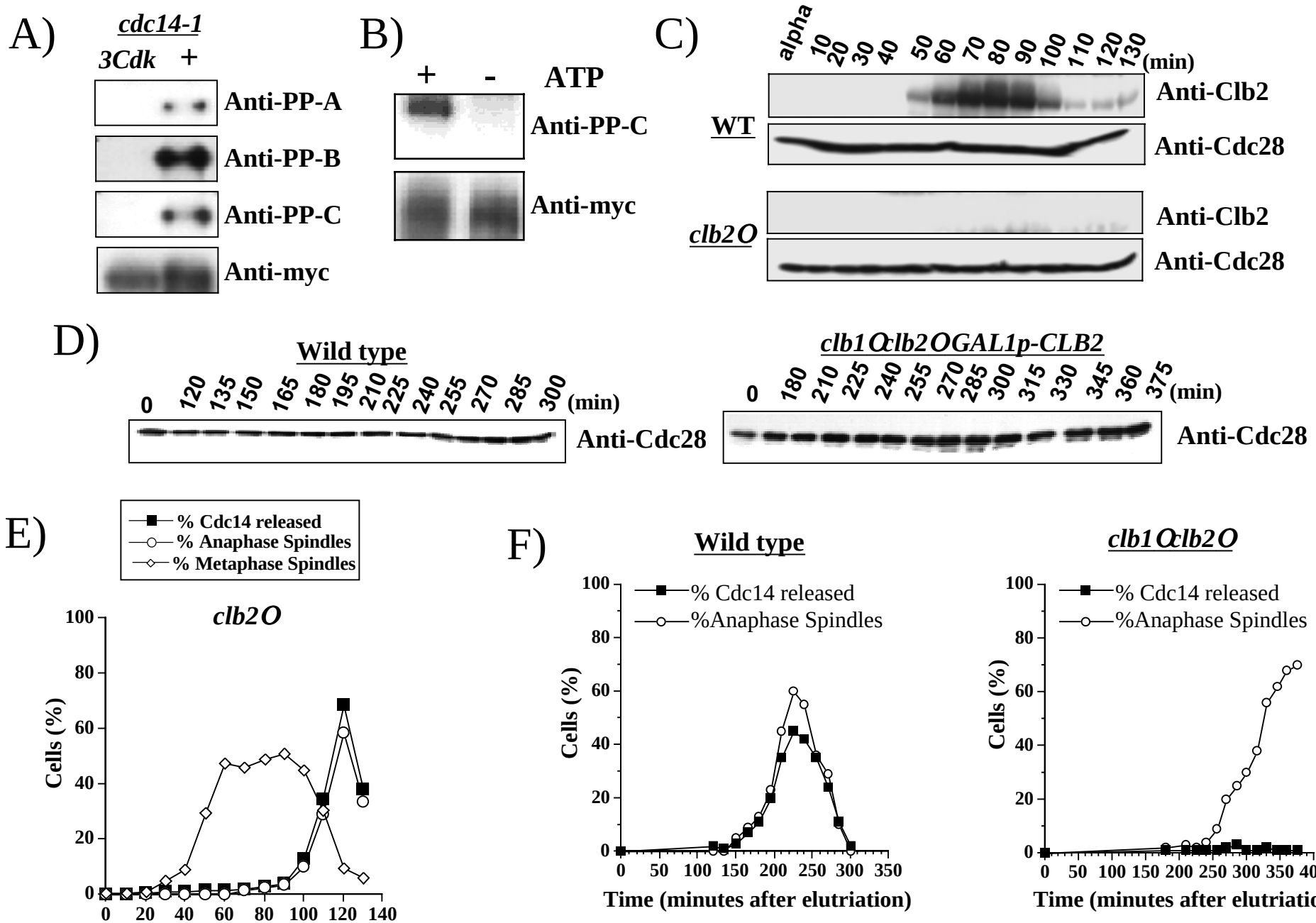

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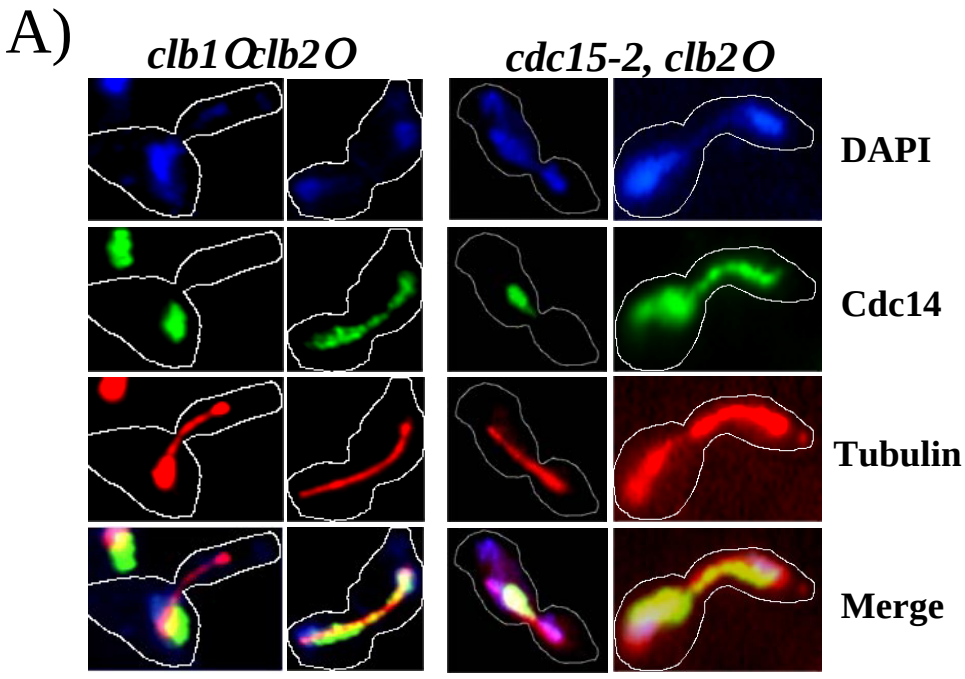
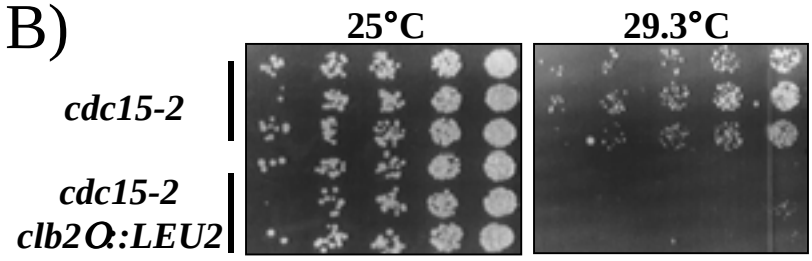
fig. S5





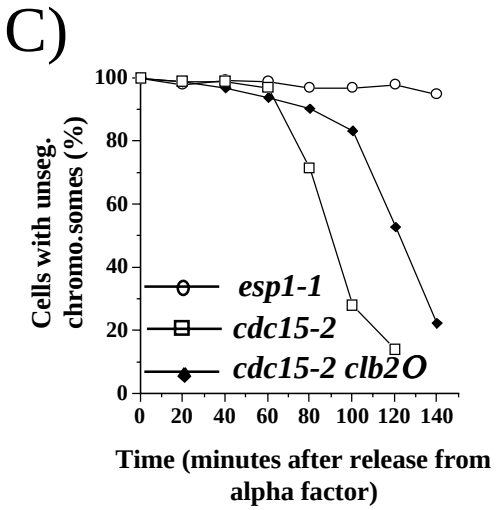


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fig. S6

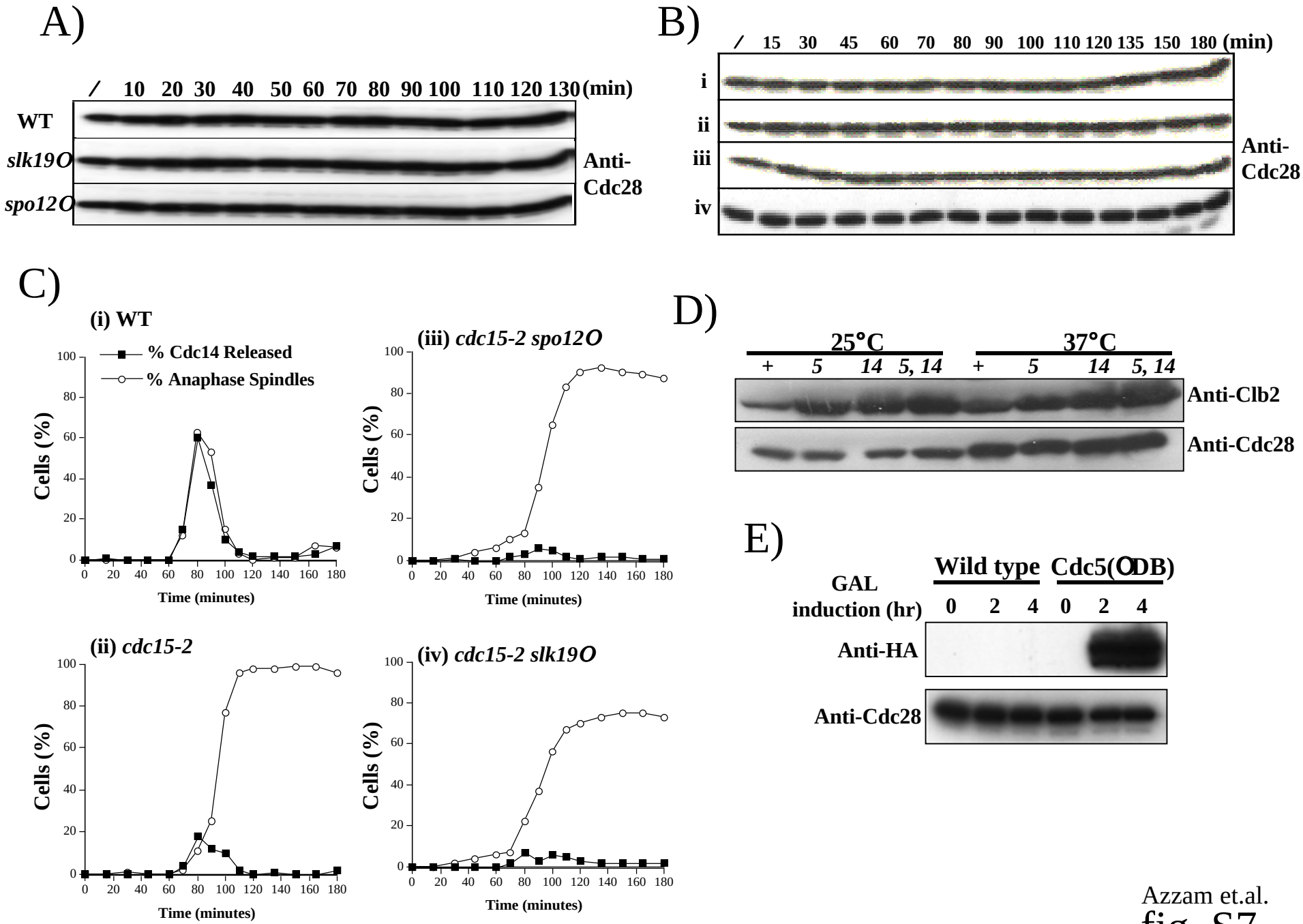

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fig. S7

Table S1. Net1 *in vivo* phosphorylation sites.

Peptide Recovered [a]	Net1 Sequence [b]	Mutated in (13m) [c]	Mutated in (6m) [c]	Mutated in (3Cdk) [c]	Mutated in (6Cdk) [c,f]
160-184 +1P	R>SKLNNG**S**PQ**S**VQPQQQIPSSSGVLR>I [d]	Y, Y	Y,Y	Y, N	Y, N
210-216 +1P	R>VS**T**PLAR>Q	Y	Y	Y	Y
226-241 +1P	K>IVSNN**S**DDEDEDIGER>S	Y	Y	N	N
242-256 +1P	R>SFLPPPTQPQ**S**PPIR>I	Y	Y	Y	Y
257-265 +1P 257-266 +1P	R>IS**S**GIDAGKK>I	Y	Y	N	N
292-307 +2P [e]	R>LLSG**T**PIMSTM**T**PNR>V	N, N	N, N	N, N	Y, Y
352-359 +1P	R>KPPV**T**TPR>I	Y	N	N	N
360-366 +1P	R>IT**S**GM(ox)LK>I	Y	N	N	N
381-393 +1P 381-393 +2P	K>EGP**SS**PASILPAK>A	Y,Y	N, N	N, N	N, N
495-505 +1P	R>KS**S**LETIVEK>K	Y	N	N	N
606-618 +1P	R>IVPQD**S**DSSSFPK>S	Y	N	N	N
670-678 +1P	R>NILPQR**T**PR>S	Y	N	N	N
742-749 +1P	K>DI**S**LHSLK>G	N	N	N	N
1013-1023 +1P	R>VVVN**T**PREPVR>S	N	N	N	N
1028-1038 +1P	K>IEAP**S**PSVNKK>I	N	N	N	N
1079-1092 +1P	K>VPR**S**LSSLSDLVSR>G	N	N	N	N

[a] '+xP' refers to the number of phosphate groups per peptide as determined by mass spectrometry.

[b] Larger-sized font indicates mapped phosphorylation sites. Carets (>) mark sites of trypsin cleavage that yielded the peptides shown. 'ox' refers to oxidized Methionine.

[c] 'Y' indicates that the codon for a given site was mutated to encode Alanine, whereas 'N' indicates that it was not mutated.

[d] Initial analysis was unable to determine which Serine was phosphorylated in the fragment spanning amino acids 160-184, but subsequent analysis with a phospho-specific antibody implicated Serine166. Phosphorylation status of Serine169 is unknown (underlined).

[e] Yielded a small amount of a species at +80 Da from the doubly-charged form at the same retention time in the HPLC analysis, but we could not detect any monophosphate in the precursor scans and it was later determined that this peptide was most likely phosphorylated on both Threonines as indicated.

[f] In the (6Cdk) mutant, Threonine 62 was also mutated to Alanine to completely eliminate all Cdk consensus sites from the first 341aa N-terminal fragment of Net1.

Table S2. Strains.

Strain name	Strain Genotype	Strain comments	Used in Figures
RJD 2603	*net1::mTn3/URA3*	clone V13E1 with insertion point at aa(1104)*	S1
RJD 2604	*net1::mTn3/URA3*	clone V39B3 with insertion point at aa(835)*	S1
RJD 2605	*net1::mTn3/URA3*	clone V130G6 with insertion point at aa(754)*	S1
RJD 2606	*net1::mTn3/URA3*	clone V30E10 with insertion point at aa(738)*	S1
RJD 2607	*net1::mTn3/URA3*	clone V148D2 with insertion point at aa(259)*	S1
RJD 2608	*net1::mTn3/URA3*	clone V66G4 with insertion point at aa(197)*	S1
RJD 2609	*net1::mTn3/URA3*	clone V109B1 with insertion point at aa(189)*	S1
RJD 1783	*net1::net1(1-621)-HA::KanMX6*	**	S1
RJD 2610	*net1Δ:his5, NET1-TEV-myc9::TRP1, cdc15-2 MATa*		4B, 1A, S3A, S3B, S8B
RJD 2611	*net1Δ:his5, net1-13m-TEV-myc9::TRP1, cdc15-2, MATa*		1A, S2A
RJD 2612	*net1Δ:his5, net1-6m-TEV-myc9::TRP1, cdc15-2, MATa*		S2A, S2B
RJD 2613	*net1Δ:his5, net1-3Cdk-TEV-myc9::TRP1, cdc15-2, MATa*		1A, S3A, S3B
RJD 2614	*net1Δ:his5, net1-6Cdk-TEV-myc9::TRP1, cdc15-2, MATa*		1A, S3A, S3B
RJD 2615	*net1Δ:his5, NET1-TEV-myc9::TRP1, cdc14-1, MATa*		S5A
RJD 2616	*net1Δ:his5, net1-3Cdk-TEV-myc9::TRP1, cdc14-1, MATa*		S5A
RJD 2617	*net1Δ:his5, NET1-TEV-myc9::TRP1, MATa*		1B, 2B, 2C, 2D, 4A, S5B,C,E,F
RJD 2618	*net1Δ:his5, NET1-TEV-Myc9::TRP1, slk19Δ:KanMX6, MATa*		4A
RJD 2619	*net1Δ:his5, NET1-TEV-Myc9::TRP1, spo12Δ:URA3, MATa*		1B, 4A
RJD 2620	*net1Δ:his5, NET1-TEV-myc9::TRP1, cdc15-2, spo12Δ:URA3, MATa*		4B
RJD 2621	*net1Δ:his5, NET1-TEV-myc9::TRP1, cdc15-2, slk19Δ:KanMX6, MATa*		4B
RJD 2622	*clb2Δ:LEU2, cdc15-2, MATa*		S7A, S7B
RJD 2623	*net1Δ:his5, NET1-TEV-myc9::TRP1, clb2Δ:LEU2, MATa*		2C, S5E
RJD 2624	*net1Δ:his5 , NET1-TEV-myc9::TRP1, clb1Δ:URA3, clb2Δ:LEU2, GAL1p-CLB2::URA3, MATa*		2D, S7A, S5D,F
RJD 2625	*net1Δ:his5, NET1-TEV-myc9::TRP1, dbf2-2, MATa*		S3B
RJD 2626	*net1Δ:his5, net1-3Cdk-myc9::TRP1, dbf2-2,MATa*		S3B
RJD 2627	*net1Δ:his5, Net1-WT-myc9::TRP1, dbf2-2, YCH111 [CLB2 (C2Dk100)HA3, URA3], MATa*		S3B
RJD 1349	*NET1-TEV-myc9::his5+, bar1::hisG, MATalpha*		3A, 3B
RJD 1417	*NET1-TEV-myc9::his5+, bar1::hisG, cdc5-1, MATa*		4C
RJD 1408	*NET1-TEV-myc9::his5+, bar1::LEU2, cdc14-1, MATalpha*		4C
RJD 2628	*NET1-TEV-myc9::his5+, cdc5-1, cdc14-1, bar1::hisG?, pep4::TRP1?, MATa*		4C
RJD 2629	*tetO112 ::URA3x3, 3tetR-GFP::HIS3, esp1-1, MATa*		S7C
RJD 2633	*tetO112 ::URA3x3, 3tetR-GFP::HIS3, cdc15-2, MATa*		S7C
RJD 2631	*tetO112 ::URA3x3, 3tetR-GFP::HIS3, cdc15-2, clb2Δ:LEU2, MATa*		S7C
RJD 602	*cdc15-2, MATa*		S7B
RJD 2632	*net1Δ:his5, NET1-TEV-myc9::TRP1, cdc28-as1, MATa*		2F, S5D
RJD 2862	*net1Δ:his5, net1-6Cdk-TEV-myc9::TRP1, MATa*		1B
RJD 2863	*myc9-NET1::LEU2, bar1::hisG, GAL1p-CDC5(ΔDB)-HA::URA3, MAT a*		4D
RJD 2864	*myc9-NET1::LEU2, pep4::TRP1, MAT a*		4D

*Y800 background (leu2-98, cry1(R), ade2-10,his3-200, ura3-52, lys2-801, can1(R), trp1-1, cyh2(R))

**BJ5459 background (ura3-52, trp1, lys2-801, leu2D1, his3D200, pep4::HIS3, prb1D1.6R can1)

Supplemental References

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