Figure S1

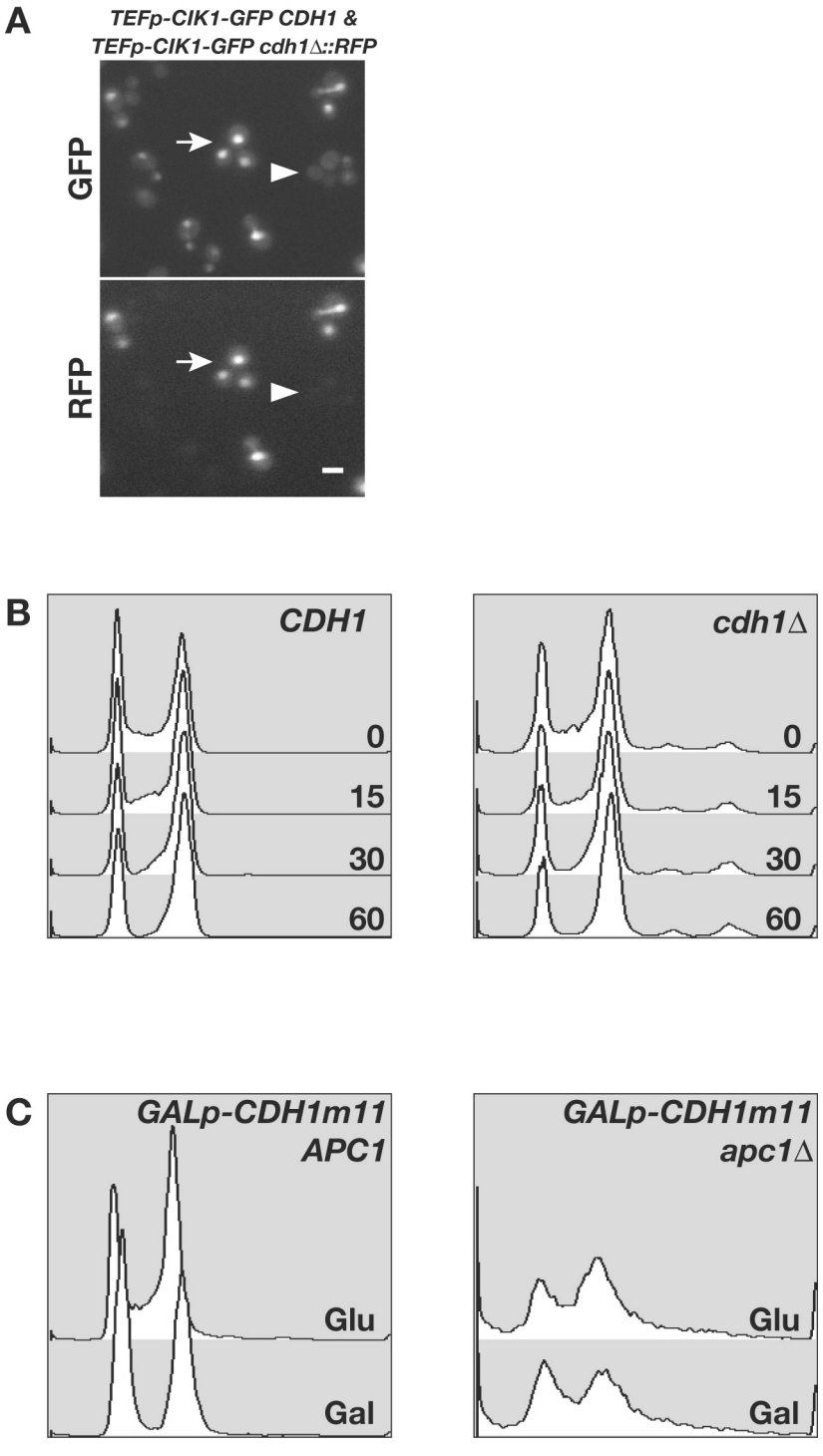

A
TEFp-CIK1-GFP CDH1 &
TEFp-CIK1-GFP cdh1Δ::RFP
GFP
RFP
B
CDH1
0
15
30
60
cdh1Δ
0
15
30
60
C
GALp-CDH1m11
APC1
Glu
Gal
GALp-CDH1m11
apc1Δ
Glu
Gal

Figure S2

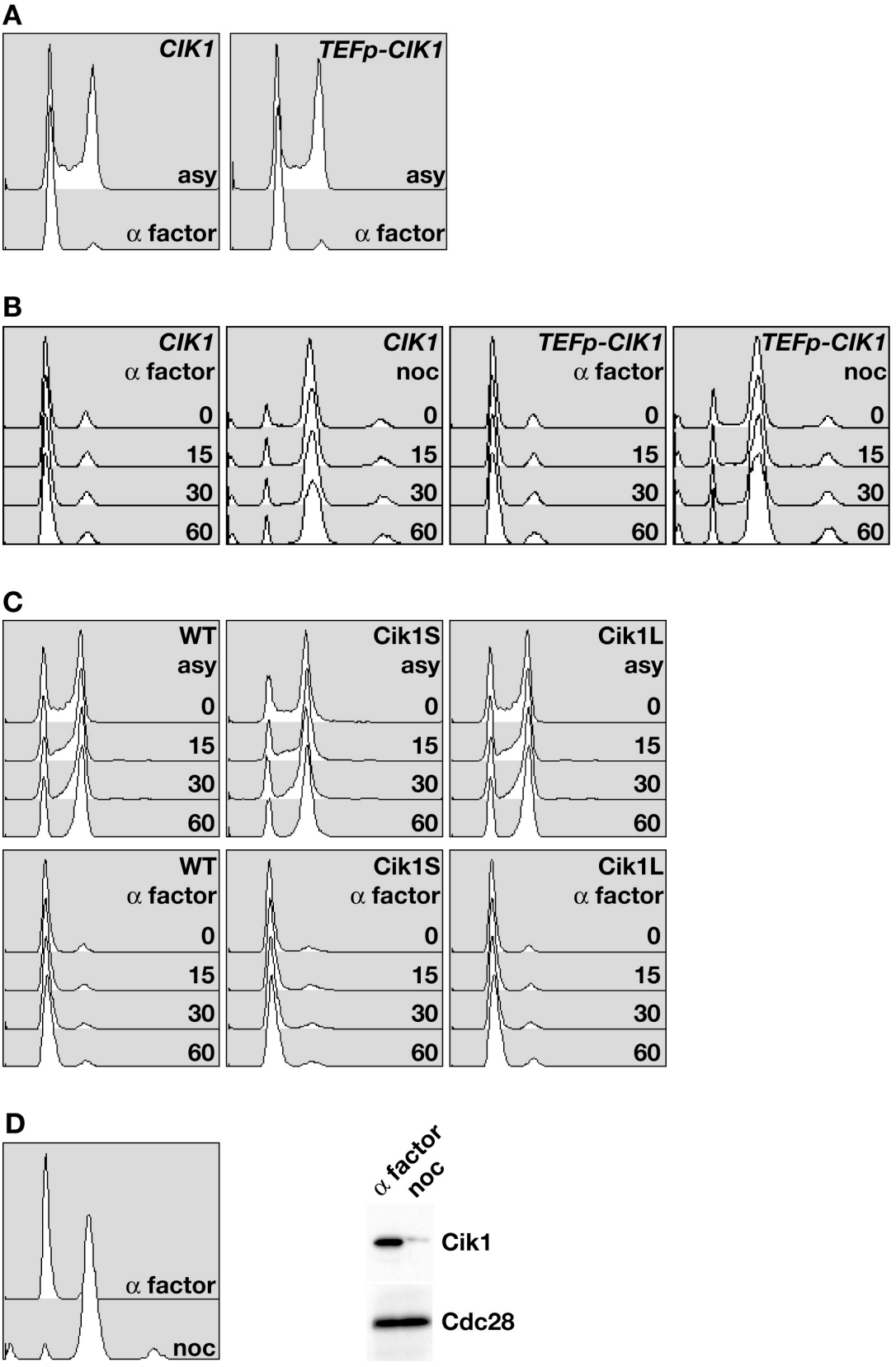

A
CIK1
asy
α factor
TEFp-CIK1
asy
α factor
B
CIK1
α factor
0
15
30
60
CIK1
noc
0
15
30
60
TEFp-CIK1
α factor
0
15
30
60
TEFp-CIK1
noc
0
15
30
60
C
WT
asy
0
15
30
60
Cik1S
asy
0
15
30
60
Cik1L
asy
0
15
30
60
WT
α factor
0
15
30
60
Cik1S
α factor
0
15
30
60
Cik1L
α factor
0
15
30
60
D
α factor
noc
α factor
noc
Cik1
Cdc28

Figure S3

A

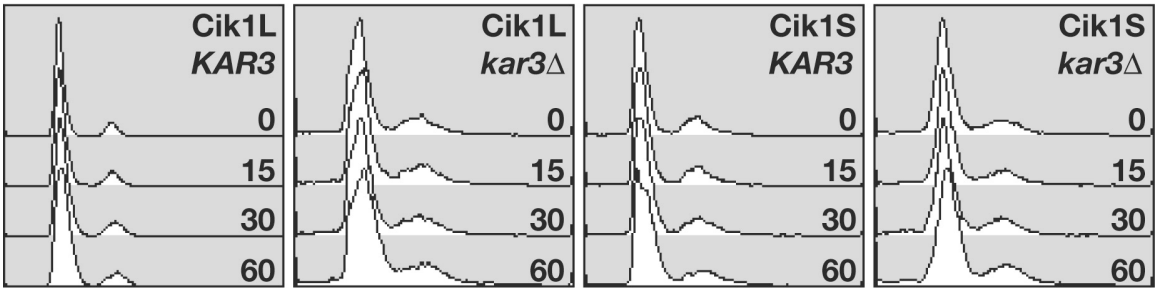

Cik1L
KAR3
0
15
30
60
Cik1L
kar3Δ
0
15
30
60
Cik1S
KAR3
0
15
30
60
Cik1S
kar3Δ
0
15
30
60

Figure S4

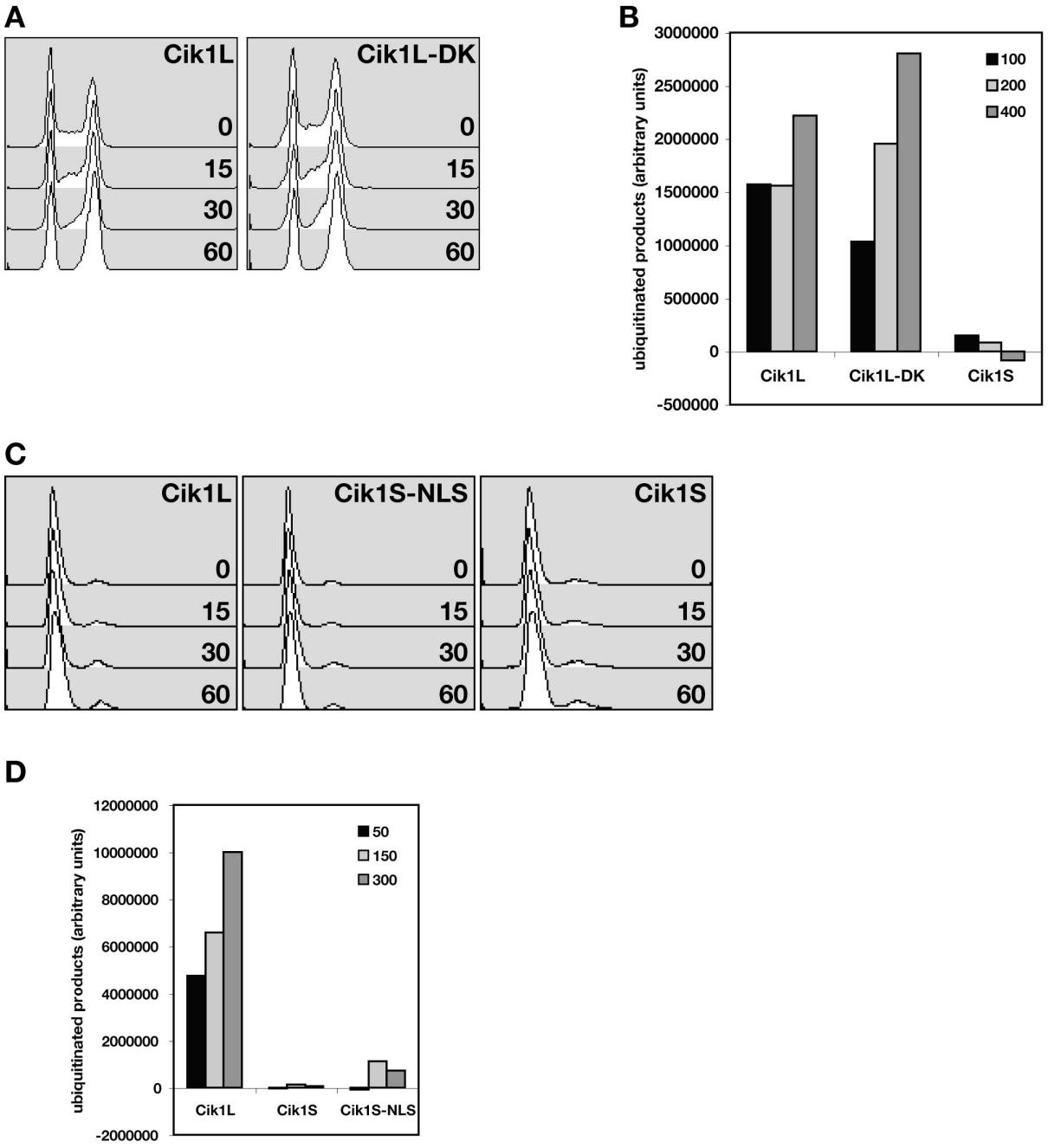
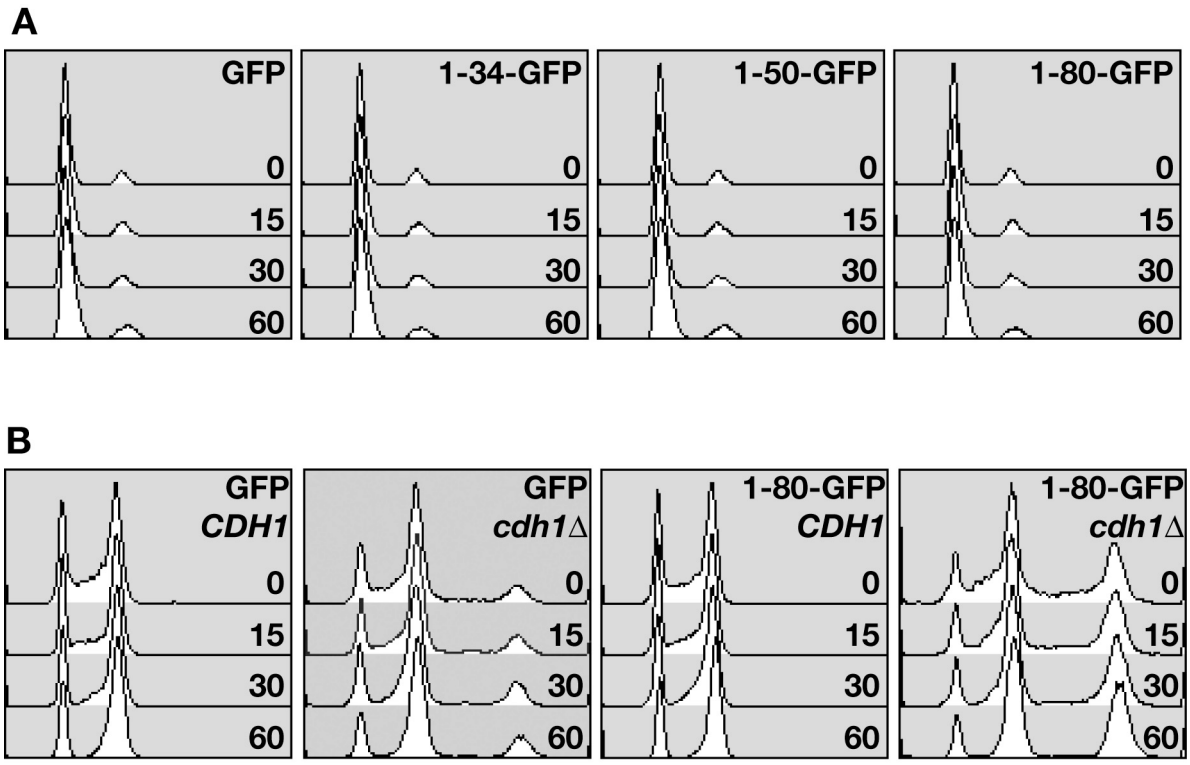

A
Cik1L
Cik1L-DK
0
15
30
60
B
ubiquitinated products (arbitrary units)
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
100
200
400
Cik1L
Cik1L-DK
Cik1S
C
Cik1L
Cik1S-NLS
Cik1S
0
15
30
60
D
ubiquitinated products (arbitrary units)
12000000
10000000
8000000
6000000
4000000
2000000
0
-2000000
50
150
300
Cik1L
Cik1S
Cik1S-NLS

Figure S5


A
GFP
1-34-GFP
1-50-GFP
1-80-GFP
0
15
30
60
B
GFP
CDH1
GFP
cdh1Δ
1-80-GFP
CDH1
1-80-GFP
cdh1Δ
0
15
30
60
C
ubiquitinated products (arbitrary units)
6000000
5000000
4000000
3000000
2000000
1000000
0
-1000000
50
150
300
GFP
1-34-GFP
1-50-GFP
1-80-GFP

Supplemental Table S1. Strains List

Strain	Genotype	Source
YJB201	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-CIK1-GFP-HIS3	this study
YJB237	MATa his3Δ1 leu2Δ0 ura3Δ0 lyp1Δ can1Δ :STE2p-LEU2 Hyg-TEF1p-CIK1-GFP-HIS3 cdh1Δ ::ADHp-NLS-mRFP-URA3	this study
YJB329	MATa ade2-1 his3-11 leu2-3,112 ura3-1 can1-100 trp1-1::(SIC1-TRP)10X clb5Δ::HIS3 pds1Δ::LEU2 GAL-CDH1m11::ADE2 CIK1-3FLAG-Hyg	this study
YJB333	MATa ade2-1 his3-11 leu2-3,112 ura3-1 can1-100 trp1-1::(SIC1-TRP)10X clb5Δ::HIS3 pds1Δ::LEU2 GAL-CDH1m11::ADE2 apc1Δ::KanMX CIK1-3FLAG-Hyg	this study
YJB244	MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 cdc28-13 CIK1-GFP-HIS3	this study
CIK1-GFP	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 CIK1-GFP-HIS3	GFP library
YJB320	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-ΔATG-CIK1-GFP-HIS3	this study
YJB345	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 CIK1-GFP-HIS3 tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB346	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEFp-CIK1-GFP-HIS3 tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB350	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEFp-CIK1-GFP-HIS3 kar3Δ::KanMX tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB349	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEFp-ΔATG-CIK1-GFP-HIS3 tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB348	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEFp-ΔATG-CIK1-GFP-HIS3 kar3Δ::KanMX tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB332	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 kar3Δ::KanMX Hyg-TEFp-CIK1-GFP-HIS3	this study
YJB328	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 kar3Δ::KanMX Hyg-TEFp-ΔATG-CIK1-GFP-HIS3	this study
YJB318	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 HYG-TEFp-cik1db1db2db3ken-GFP-HIS3	this study
cik1Δ	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 cik1Δ::KanMX	deletion collection
kar3Δ	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 kar3Δ::KanMX	deletion collection

18-20	matΔ cyh2 can1 lys5 ade2 ade3::GalHO trp1 his3 ura3 leu2 +chromosome VII [aro2 adh2::URA3]	V. Zakian
YDPT180-3	matΔ cyh2 can1 lys5 ade2 ade3::GalHO trp1 his3 ura3 leu2 cik1Δ::KanMX +chromosome VII [aro2 adh2::URA3]	this study
YDPT182-1	matΔ cyh2 can1 lys5 ade2 ade3::GalHO trp1 his3 ura3 leu2 Hyg-TEF1p- Δ ATG-CIK1 +chromosome VII [aro2 adh2::URA3]	this study
YDPT183-1	matΔ cyh2 can1 lys5 ade2 ade3::GalHO trp1 his3 ura3 leu2 Hyg-TEF1p-CIK1 +chromosome VII [aro2 adh2::URA3]	this study
YJB323	MATα his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 CIK1-GFP-HIS3	this study
YJB324	MATα his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-CIK1-GFP-HIS3	this study
YJB325	MATα his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-ΔATG-CIK1-GFP-HIS3	this study
YJB326	MATα his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 cik1Δ::KanMX	this study
YJB354	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-ΔATG-CIK1-NLS-GFP-HIS3	this study
YJB356	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 Hyg-TEF1p-ΔATG-CIK1-NLS-GFP-HIS3 tub1::[TUB1-Clb1/2trm-URA3-HIS3p-mCherry- TUB1]	this study
YJB358	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-CIK1-1-34-GFP-HIS3	this study
YJB359	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-CIK1-1-50-GFP-HIS3	this study
YJB360	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-CIK1-1-80-GFP-HIS3	this study
YJB361	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-GFP-HIS3	this study
YJB362	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-GFP-HIS3 cdh1Δ ::ADHp-NLS-mRFP-URA3	this study
YJB363	MATa his3Δ1 ura3Δ0 leu2Δ0 met15Δ0 ybr071wΔ::Hyg-TEFp-CIK1-1-80-GFP-HIS3 cdh1Δ ::ADHp-NLS-mRFP-URA3	this study

Supplemental Figure Legends

Figure S1. (A) Cik1 expression is regulated by Cdh1. Wild type and *cdh1Δ* strains, each expressing GFP-tagged Cik1 from the *TEF1* promoter, were mixed together and imaged on a high-throughput microscope. Levels of Cik1-GFP were compared between wild type (arrowhead) and *cdh1Δ* (arrow) cells (top panel). *cdh1Δ* cells are marked by RFP expression in the nucleus (lower panel). Scale bar represents 5μm. (B) Cell cycle profiles of *CDH1* and *cdh1Δ* cells after the addition of cycloheximide for the indicated number of minutes (control for Figure 1A). (C) Cell cycle profiles of *APC1* and *apc1Δ* strains before (Glu) and after (Gal) switching cells to media containing 2% galactose in order to induce expression of Cdh1-m11 (control for Figure 1B).

Figure S2. (A) Cell cycle profiles of *CIK1* and *TEFp-CIK1* cells growing asynchronously (asy) or arrested with α factor (control for Figure 2A). (B) Cell cycle profiles of *CIK1* or *TEFp-CIK1* cells arrested with α factor or nocodazole, then treated with cycloheximide for the indicated number of minutes (control for figure 2B). (C) Cell cycle profiles of wild type (WT), Cik1S (*TEFp-ΔATG-CIK1*), or Cik1L (*TEFp-CIK1*) cells growing asynchronously (asy) or arrested with α factor, then treated with cycloheximide for the indicated number of minutes (control for Figure 2C). Note that in asynchronous cells at the zero time point, a greater fraction of Cik1S cells are in mitosis, indicative of a mitotic delay. (D) Cell cycle profiles and Western blot of GFP and Cdc28 from *CIK1-GFP* cells that were used for 5'RACE and RT-qPCR analysis in Figure 2E and 2F.

Figure S3. Cell cycle profiles of strains expressing Cik1L (*TEFp-CIK1*) or Cik1S (*TEFp-ΔATG-CIK1*), in a *KAR3* or *kar3Δ* background, after addition of cycloheximide for the indicated number of minutes (control for Figure 3C).

Figure S4. (A) Cell cycle profiles of asynchronous Cik1L (*TEFp-CIK1*) and Cik1L-DK (*TEFp-CIK1-DK*) strains after the addition of cycloheximide for the indicated number of minutes (control for Figure 4A). (B) Quantitation of ubiquitinated products shown in Figure 4B, as detailed in the experimental procedures section. Different colored bars represent the concentration (in nM) of Cdh1 added to each reaction. Approximately equivalent amounts of substrates were added to each reaction. (C) Cell cycle profiles of Cik1L (*TEFp-CIK1*), Cik1S-NLS (*TEFp-ΔATG-CIK1-NLS*), and Cik1S (*TEFp-ΔATG-CIK1*) strains following α factor arrest and addition of cycloheximide for the indicated number of minutes (control for Figure 4D). (D) Quantitation of ubiquitinated products shown in Figure 4E, as detailed in the experimental procedures section. Different colored bars represent the concentration (in nM) of Cdh1 added to each reaction. In this experiment approximately one quarter the amount of Cik1S protein was used, as compared to Cik1L and Cik1S-NLS.

Figure S5. (A) Cell cycle profiles of strains expressing GFP or Cik1-GFP fusion proteins after α treatment and addition of cycloheximide for the indicated number of minutes (control for Figure 5B). (B) Cell cycle profiles of asynchronous strains expressing GFP

or 1-80-GFP, in a *CDH1* or *cdh1Δ* background, after the addition of cycloheximide for the indicated number of minutes (control for Figure 5C). (C) Quantitation of ubiquitinated products shown in Figure 5D, as detailed in the experimental procedures section. Different colored bars represent the concentration (in nM) of Cdh1 added to each reaction.

Supplemental Experimental Procedures

High-throughput microscopy screening. High-throughput microscopy screening was carried out as previously described (Benanti et al., 2007), except that cells were imaged on a Molecular Devices ImageXpress Micro high-throughput microscope using a 40X air Plan-Apo, 0.95 NA objective. Data were analyzed using the Multiwavelength Cell Scoring module in the MetaXpress software, also from Molecular Devices.

Construction of strains and plasmids. All MATa yeast strains expressing GFP-tagged Cik1 are in the S288c background and were constructed by sequential transformation into the CIK1-GFP strain from the GFP library (Huh et al., 2003). All strains designated Cik1L and Cik1S express Cik1 from the *TEF1* promoter, as indicated in the figure legends. MATα strains used in karyogamy assays were generated by crosses to a wild type S288c haploid strain. Strains in the APC/C suppressor background (YJB329, YJB333) and the *cdc28-13* (YJB244) strain are in the W303 background and were constructed by direct tagging of *CIK1*. Strains for chromosome loss assays (YDPT180-

3, YDPT182-1, YDPT183-1) are in the LS background and were generated by transformation of 18-20 (Sandell and Zakian, 1993).

A tagged copy of mCherry-tagged tubulin was introduced into strains by transformation of HindIII digested pRS406-mCherry-TUB1 plasmid (provided by Steve Reed), in order to integrate *mCherry-TUB1* at the *TUB1* locus. To introduce D-box/KEN box mutations into CIK1, the CIK1-GFP-HIS3 sequence was first cloned into the pGEMT-Easy vector (Promega) and mutagenized by site directed mutagenesis. For D-box mutations, RxxL sequences were changed to AxxA, and for the KEN box mutation, KEN was changed to AAA. The Cik1-DK-GFP-HIS3 sequence was then cut out of the vector and transformed into a strain expressing *CIK1* from the *TEF1* promoter. In order to relocalize Cik1S to the nucleus, a sequence encoding the SV40 NLS-GFP was used to tag Cik1 in a *TEFp-ΔATG-CIK1* strain. To introduce Cik1-GFP fusion proteins into cells, *YDR071W* (an uncharacterized, non-essential gene) was replaced in the YDR071W-GFP strain from the GFP library with increasing amounts of *Hyg-TEFp-CIK1* sequence to generate *TEFp*-Cik1-GFP fusion proteins.

For *in vitro* transcription and translation of Cik1 and GFP proteins, the open reading frames were cloned into the pGEMT-Easy vector in the SP6 orientation. Because *in vitro* translation of the full length Cik1L protein in rabbit reticulocyte lysates preferentially initiated at the second ATG codon (data not shown), methionine residues at positions 35 and 37 were mutated to alanine residues. These changes were also incorporated into the 1-50-GFP and 1-80-GFP fusion protein sequences.

Supplemental References

Benanti, J.A., Cheung, S.K., Brady, M.C., and Toczyski, D.P. (2007). A proteomic screen reveals SCFGrr1 targets that regulate the glycolytic-gluconeogenic switch. Nat Cell Biol *9*, 1184-1191.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. (2003). Global analysis of protein localization in budding yeast. Nature *425*, 686-691.

Sandell, L.L., and Zakian, V.A. (1993). Loss of a yeast telomere: arrest, recovery, and chromosome loss. Cell *75*, 729-739.