Supplementary Materials for

Deciphering Protein Kinase Specificity Through Large-Scale Analysis of Yeast Phosphorylation Site Motifs

Janine Mok, Philip M. Kim, Hugo Y. K. Lam, Stacy Piccirillo, Xiuqiong Zhou, Grace R. Jeschke, Douglas L. Sheridan, Sirlester A. Parker, Ved Desai, Miri Jwa, Elisabetta Cameroni, Hengyao Niu, Matthew Good, Attila Remenyi, Jia-Lin Nianhan Ma, Yi-Jun Sheu, Holly E. Sassi, Richelle Sopko, Clarence S. M. Chan, Claudio De Virgilio, Nancy M. Hollingsworth, Wendell A. Lim, David F. Stern, Bruce Stillman, Brenda J. Andrews, Mark B. Gerstein, Michael Snyder,* Benjamin E. Turk*

*To whom correspondence should be addressed. E-mail: ben.turk@yale.edu (B.E.T.); mpsnyder@stanford.edu (M.S.).

Data set S3. MUSCLE alignment of all predicted *S. cerevisiae* ORFs with orthologs from 12 other yeast species (clustal alignment files).
Data set S4. Alignment of yeast kinases analyzed in this study (clustal alignment file).

Materials and Methods

Yeast strains

pDEST-*PRK1KD* was generated from pDEST-*PRK1WT* (*1*) by mutating Asp176 to Ala by the QuikChange method using the following primers: PRK1KDUP: 5'-TGTATAAAGTTTGTGCTTTTGGTTCCGTTTC-3', PRK1KDDN: 5'-GAAACGGAACCAAAAGCACAAACTTTATACA-3'. Sequence-confirmed pDEST-*PRK1KD* was then transformed into yeast strain Y258 using standard LiAc transformation methods (*2*).

prk1Δ ark1Δ strains were generated by first introducing a *prk1-Δ::KANMX6* deletion cassette by transformation using the available Bem2 and Ede1 chromosomally TAP-tagged strains (*3*). These *MAT*α *prk1-Δ::KANMX6* strains were then mated to a *MAT*a *ark1-Δ::URA3* strain that was obtained by rescuing haploids from the marker swapped *ark1-Δ::KANMX6* heterozygote diploid strain generated by the YKO consortium (pM4758) (*4, 5*). Following sporulation and tetrad dissection, *MAT*α *prk1Δ::KANMX6 ark1Δ::URA3* haploids that still contained the TAP-tagged candidate substrate were identified by growth on synthetic complete media lacking uracil (SC-ura), rich media containing geneticin (YPAD + G418), and synthetic complete media lacking histidine (SC-his) and positive mating to the a mating type tester strain.

vhs1Δ::KANMX6 Sol2-TAP::*HIS3MX6* was generated by transformation using the available Sol2 chromosomally TAP-tagged strain.

44 of the kinases were purified from yeast using either the N-terminal GST-His_6-tagged overexpression ORF collection (*6*) or the C-terminal TAP-tagged overexpression ORF collection (*1*). Yeast strains were first grown as starter cultures in 4 ml of SC-ura media, and then diluted into 400 ml of SC-ura/raffinose media and allowed to grow ~16 hr at 30˚C to an OD_{600} of 0.6-0.8. Fusion protein expression was induced by adding 200 ml of 3X YEP-Gal (3% yeast extract, 6% peptone, 6% galactose) for 6 hr. Cells were harvested by centrifugation, washed with ice-cold water, aliquoted into four tubes, and stored at -80˚C.

Preparation of crude yeast extracts

To prepare crude extracts for affinity purification, cell pellets were thawed on ice, and 500 μl of 0.5 mm acid washed glass beads (Biospec) was added to each tube. Two rounds of lysis were performed: once with lysis buffer (50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 0.1% Triton X-100, 10% glycerol, 0.5 mM DTT, 1 mM PMSF, 1 mM NaF, 12.5 mM β-glycerophosphate, 1X Complete protease inhibitors [Roche]) containing 150 mM NaCl (LB150) and once with lysis buffer containing 650 mM NaCl (LB650). 500 μl of LB150 was added to each tube, and cells were lysed by shaking for 6 min in a paint-shaker (5G-HD, Harvil) at 4˚C followed by centrifugation at 20,000 X *g* for 5 min. Supernatants were combined together in a single 15 ml conical tube. 500 μl of LB 650 was then added to each tube containing the remaining cell debris. Cells were subjected to another round of paint-shaking, and second round lysates were collected as described above and combined with the first round lysates.

Affinity purification of GST-His_6-tagged kinases

To affinity purify GST-His_6-tagged kinases from yeast, crude extracts were mixed with 500 μl of washed glutathione sepharose (GE Healthcare) resuspended in 4 ml of lysis buffer (without added NaCl) and incubated with agitation for 2 hr at 4ºC. Beads were pelleted by centrifugation at 2,500 X *g* for 5 min and washed three times with 10 ml of cold wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol). Beads were washed an additional two times with kinase buffer without $MgCl_2$ (20 mM HEPES, pH 7.4, 150 mM NaCl, 0.1% Tween 20, 25% glycerol, 1 mM DTT), and bound kinase was recovered by two rounds of elution with 200 μl of kinase buffer containing 30 mM reduced GSH (15 min, 4ºC with agitation). Eluate was separated from beads by centrifugation through a 3 μm polycarbonate filter plate (5 min at 1,900 X *g*). The eluted kinase was snap frozen and stored at -80ºC.

Affinity purification of TAP-tagged kinases

To affinity purify TAP-tagged kinases from yeast, crude extracts were mixed with 500 μl of washed IgG sepharose (GE Healthcare) resuspended in 4 ml of lysis buffer (without added NaCl) and incubated with agitation for 2 hr at 4ºC. Beads were pelleted by centrifugation at 210 X *g* for 5 min and washed three times with 10 ml of cold wash buffer (above). Beads were washed an additional two times with kinase buffer without $MgCl_2$ (above). To elute bound kinase, beads resuspended in 400 μl of kinase buffer were incubated with 15 μl of GST-3C protease (expressed and purified as described (*1*)) on a nutator overnight at 4ºC. Cleaved kinase was separated from beads by centrifugation through a 3 μm polycarbonate filter plate as described above. GST-3C protease was removed by mixing the flow-through with 250 μl of washed glutathione sepharose (1 hr, 4ºC). Pure kinase was recovered by a second round of centrifugation through fresh wells of a filter plate. The purified kinase was snap frozen and stored at -80ºC.

Expression and purification of kinases from mammalian cells

Coding sequences for Cak1, Cdc15 (residues 1 – 303), Gcn2 (residues 590 – 994), Prr1, Skm1 (residues 339 – 655), Vhs1, and Ykl171w (residues 436 – 913) were PCR amplified from yeast genomic DNA and ligated into the mammalian expression vector pEBG2, which produces proteins as N-terminal GST fusions. Kinases were expressed by transient transfection of HEK293T cells using Lipofectamine Plus (Invitrogen). Between 40 and 44 hr after the start of transfection, cells were washed with ice-cold PBS, and extracted into 293 lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM DTT, 1 mM PMSF, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 10 μg/ml leupeptin, 2 μg/ml pepstatin A, 10 μg/ml aprotinin), 250 μl per 6 cm plate. Lysates were cleared by centrifugation (10 min at 16,000 X *g*, 4ºC), and incubated with glutathione sepharose (8 μl per plate) for 2 hr at 4ºC. Beads were pelleted and washed twice with 293 lysis buffer, twice with GSH wash buffer (50 mM HEPES, pH 7.4, 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM $MgCl_2$), and then eluted with two rounds GSH wash buffer containing 20 mM reduced GSH and 10% glycerol (10 μl per plate each round for 30 min on ice). Eluted kinases were snap frozen in aliquots and stored at -80ºC.

Production of remaining kinases

The following kinases were prepared in active form as described: Cdc28-Cln1 (*7*), Cdc7 (*8*), Fus3 (*9*), Hog1 (*10*), Ipl1-Sli15 (*11*), Mek1 (*12*), Pho85-Pcl1/Pcl2/Pho80 (*13*), Rad53 (*14*), and Rim15 (*15*).

Full length Kss1 coding sequence was cloned by PCR into the bacterial His_6 tag expression vector pET28. To produce active Kss1, BL21 *E. coli* were co-transformed with pET28-Kss1 and pGEX4T-Ste7EE, which produces a constitutively active mutant of Ste7 that has its two activation loop phosphorylation sites mutated to Glu. Phosphorylated Kss1 was expressed and purified as described for mammalian MAPKs (*16*).

Rck2 purified from yeast was activated by incubation with Hog1 (15 µg/ml) in 20 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP for 30 min at 30ºC immediately prior to peptide screening. Residual Hog1 activity was blocked during assay of Rck2 by including 50 µM SB203580 in the reactions.

Characterization of yeast kinase preparations

Purified kinases were analyzed by SDS-PAGE followed by Coomassie staining and immunoblotting against the GST or HA tag (anti-GST antibody, 1:1,000, Sigma or anti-HA16B12 antibody, 1:1,000, Covance) as appropriate. Kinase preparations deemed to be sufficiently pure (>90%) and of the correct fusion size were tested for kinase activity by radiolabel assay using a cocktail of myelin basic protein (MBP, Upstate) and histone H1 (Upstate) as substrates. Purified kinase (7 µl) was incubated for 1 hr at 30ºC with 0.8 µg each of MBP and histone H1 in the presence of 100 µM ATP, 0.25 µCi γ-[^{33}P]-ATP (Amersham), and 10 mM $MgCl_2$ in a total reaction volume of 10 µl. The reactions were stopped by adding 3X SDS-PAGE sample loading buffer (5 µl) followed by heating for 4 min at 95ºC. Proteins were resolved by SDS-PAGE, and gels were dried and exposed to autoradiography film. Activity of each kinase preparation was assessed by analyzing the extent of both autophosphorylation and phosphorylation of the exogenous substrates as indicated by the amount of incorporated radiolabel.

Assay of individual peptide substrates

Optimized peptide substrates and individual variants were synthesized using standard Fmoc chemistry and purified by reversed phase HPLC. Kinase assays were performed at 10 µM peptide concentration in kinase buffer with 50 µM γ-[^{33}P]-ATP (1 µCi per 25 µl reaction) (10 - 15 min, 30ºC). At 5 min time points, reactions were spotted onto P81 phosphocellulose membrane, which was washed extensively with 0.42% phosphoric acid, immersed briefly in acetone, and air-dried. Peptide phosphorylation signals were quantified by scintillation counting.

In vitro kinase assays of protein substrates

Prk1 WT kinase, Prk1 KD kinase, and candidate substrates were purified from the C-terminal TAP-tagged overexpression ORF collection (*1*) on IgG sepharose beads as described above. Purified kinase (2.5 µl) was mixed with each purified candidate substrate (5 µl) and incubated for 1 hr at 30ºC in the presence of 100 µM ATP, 0.25 µCi

γ-[^{33}P]-ATP (Amersham), and 10 mM $MgCl_2$. The total volume of each reaction was 10 µl. Reactions were stopped by adding 3X SDS-PAGE sample loading buffer (5 µl) followed by heating for 4 min at 95ºC. Proteins were fractionated by SDS-PAGE, and gels were dried and exposed to autoradiography film.

Electrophoretic mobility shift analyses

1.8 x 10^7 cells grown in YPAD to OD_{600} of 0.6 were harvested by centrifugation and washed with ice-cold water. TAP-tagged Prk1/Ark1 substrates were purified on IgG sepharose from yeast cell lysates as described above, except that proteins were not eluted from the beads. Beads were washed twice with λ protein phosphatase (λ-PPase) reaction buffer [New England Biolabs (NEB)], and resuspended in the same buffer (30 µl). Samples were phosphatase treated where indicated by incubating each sample with 1.5 units each of calf intestinal phosphatase (NEB), protein phosphatase 1 (NEB), and λ-PPase (NEB) for 30 min at 30ºC. Substrates were eluted from the beads in 1X SDS loading buffer by heating for 4 min at 95˚C and resolved by SDS-PAGE, followed by immunoblotting with anti-TAP antibody (Open Biosystems, 1:3000).

Electrophoretic mobility of Sol2 was determined from crude lysates. Cell pellets from WT Sol2-TAP and *vhs1Δ* Sol2-TAP strains were lysed directly in 250 µl 1X SDS loading buffer (NEB) using the FastPrep®-24 System (6 m/s for 20 sec, MP Biomedicals) with 250 µl of 0.5 mm acid washed glass beads (Biospec). The resulting crude extracts were heated for 4 min at 95ºC and resolved on SDS-PAGE gels containing 25 mM Phos-tag (*17*) (NARD Institute, Ltd.) followed by immunoblotting with anti-TAP antibody as described above.

Supplemental Datasets

Dataset S1. Tab delimited files containing average PWMs for each of the 61 kinases assayed. PWM format follows spot array design where each row represents which position relative to the targeted phosphoacceptor site is fixed and each column represents which amino acid is fixed at that position. Spots intensities from the peptide arrays were quantified and normalized so that the total value within a single row (corresponding to a single position relative to the phosphorylation site), not including pThr and pTyr, was given a value of 20. Values greater than 1.0 thus represent positively selected residues, and values less than 1.0 are negatively selected.

Dataset S2. Tab delimited files containing MOTIPS output for each of the 61 kinases assayed. Phosphorylation sites in yeast proteins were ranked by match to the PWM and scored for predicted accessibility, probability that it falls in a disordered region, and sequence conservation across 13 yeast proteomes. An overall likelihood score is given that is the output of the Bayesian classifier of these four features. Separate columns in each file indicate whether the kinase and predicted substrate share a common GO cellular compartment, whether the substrate was identified through proteome chip screening by Ptacek, *et al.* (*18*), and whether a physical or genetic interaction has been reported (according to Biogrid). If the site appeared in a MS-based phosphoproteomic screen, the identified phosphopeptide is given.

Dataset S3. Clustal files containing multiple sequence alignments of every *S. cerevisiae* protein sequence with its orthologs from 12 other yeasts.

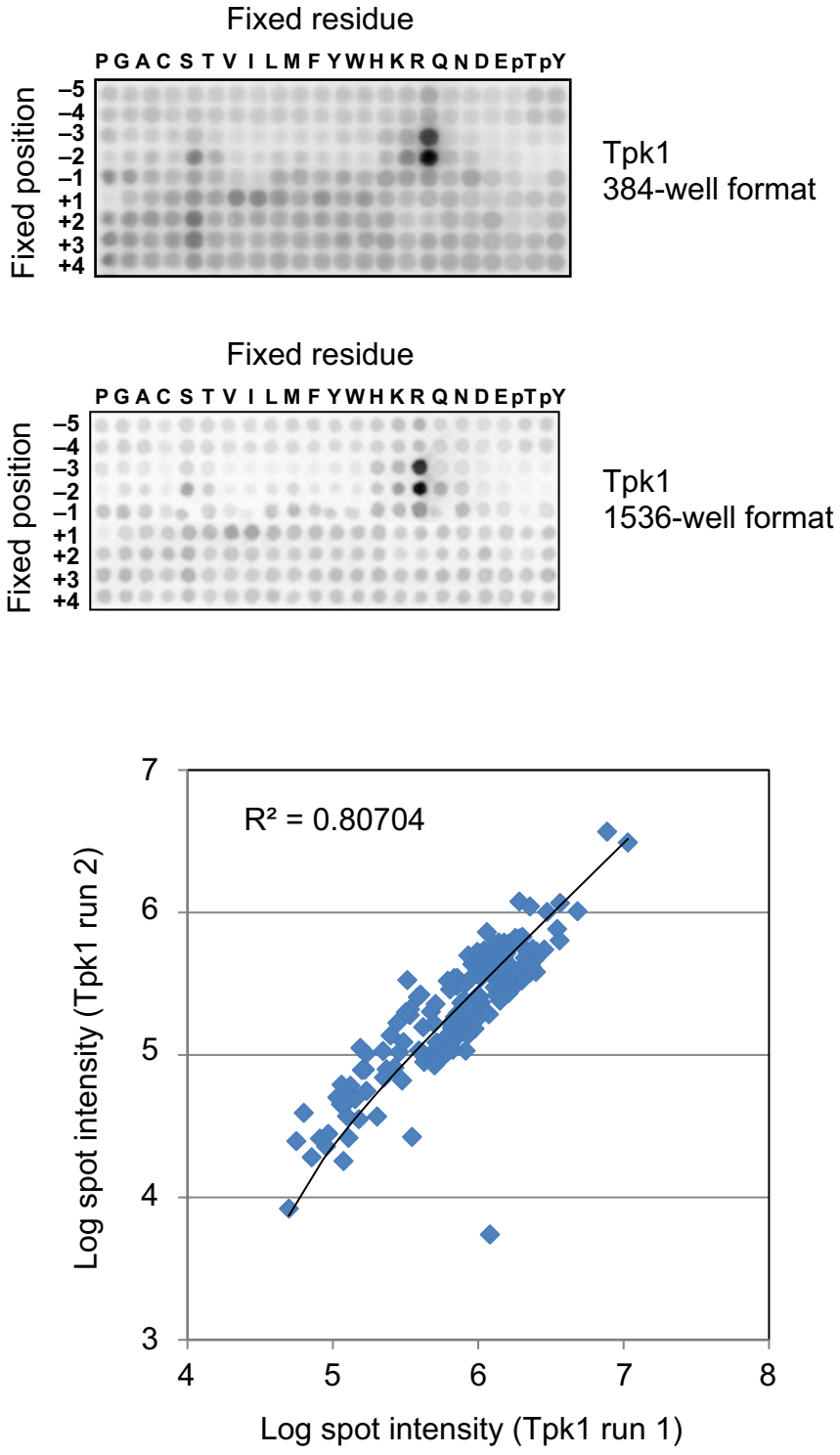

Figure S1. Assay reproducibility. Tpk1 was assayed using both the 384-well plate format originally reported and the miniaturized 1536-well format. The graph at bottom shows the correlation between spot intensities corresponding to each peptide for two runs with Tpk1 using the miniaturized 1536-well format.

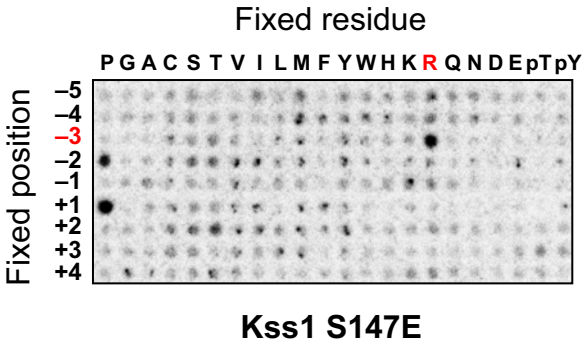

Figure S2. Representative peptide array screening results for the Kss1 S147E mutant.

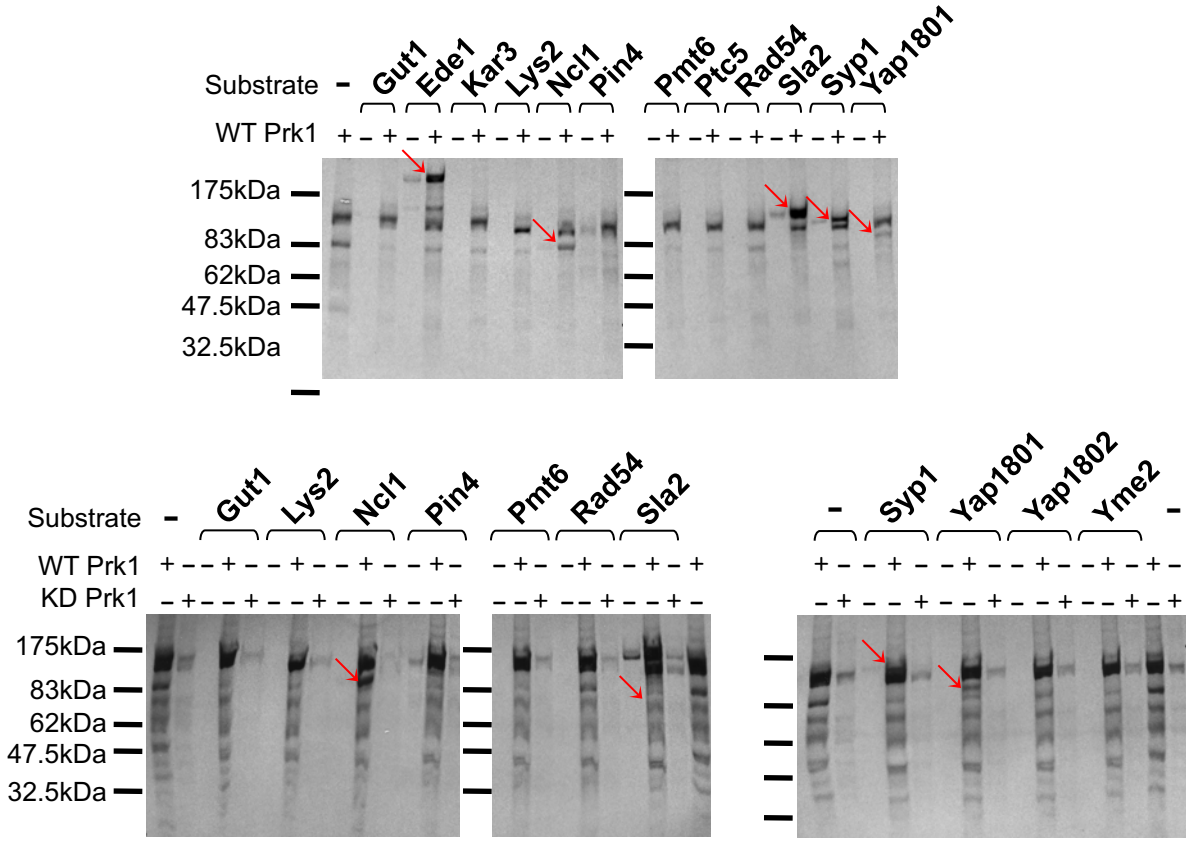

Figure S3. Representative Prk1 in vitro assays. Candidate substrates were purified and assayed in the presence of either wildtype Prk1 (WT Prk1) or a Prk1 kinase inactive mutant (KD Prk1, D176A), or in the absence of any kinase in 10 μl reactions. Red arrows point to validated substrates.

Table S1. Representative peptide array screening results and sequence logos for each of the 61 kinases assayed. Hsl1 was assayed in the original 384-well format which lacked the zero position control mixtures, and thus no information regarding its selectivity for Ser or Thr residues as the targeted phosphoacceptor site was obtained.

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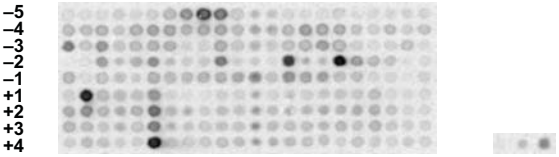
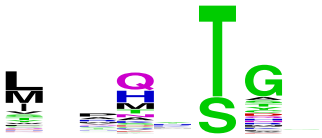
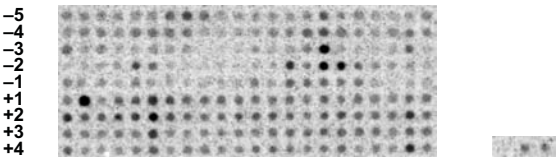
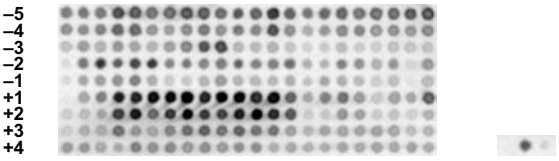
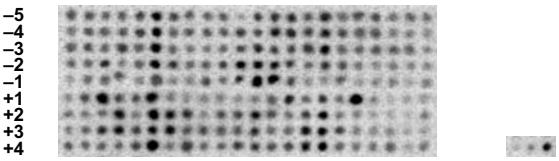
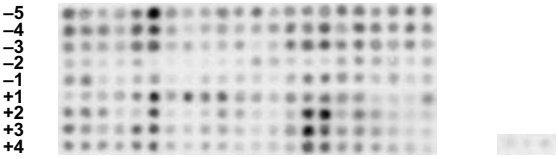
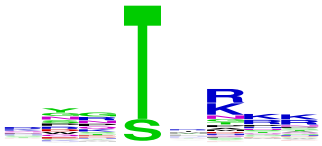
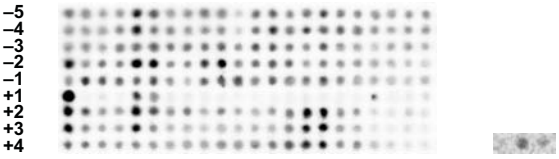
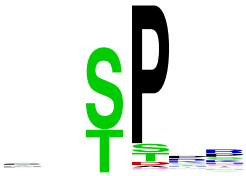
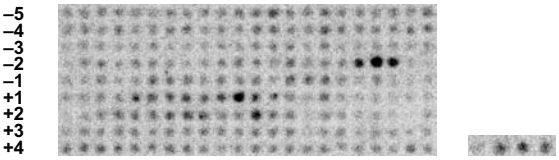
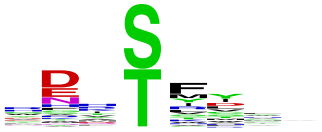
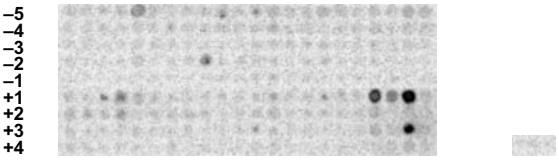
AKL1	*YBR059C*		
ARK1	*YNL020C*		
ATG1	*YGL180W*		
CAK1	*YFL029C*		
CDC15	*YAR019C*		
CDC28	*YBR160W*		
CDC5	*YMR001C*		
CDC7	*YDL017W*		

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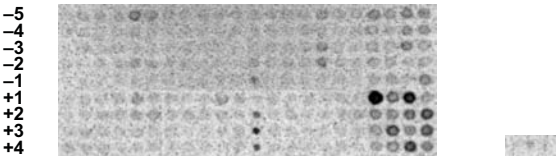
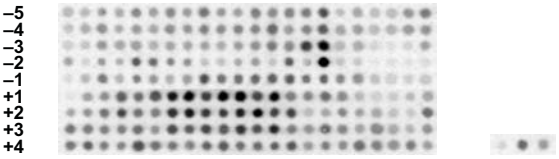
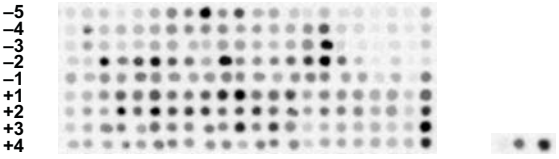
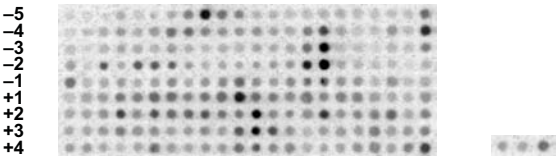
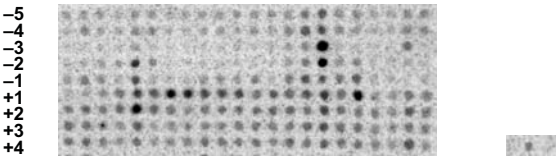
CKA1	*YIL035C*		
CLA4	*YNL298W*		
CMK1	*YFR014C*		
CMK2	*YOL016C*		
FMP48	*YGR052W*		
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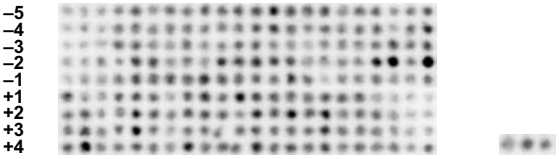
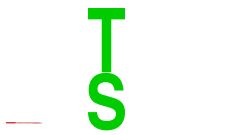
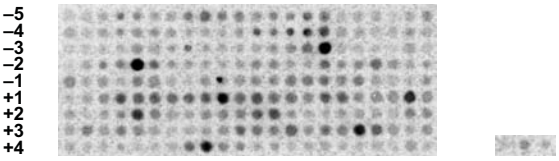
GCN2	*YDR283C*		
GIN4	*YDR507C*		

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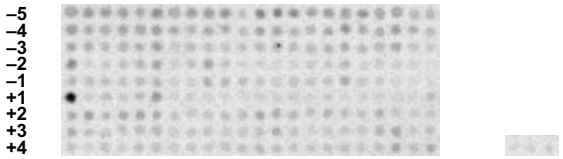
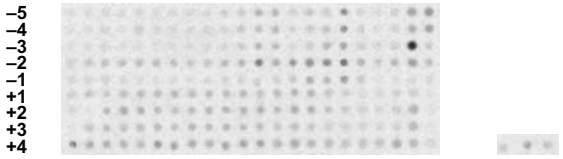
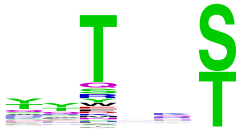
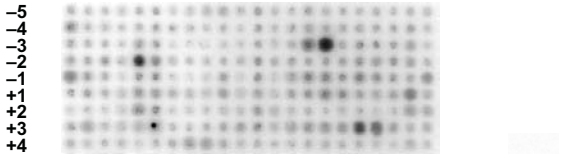
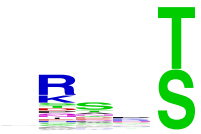
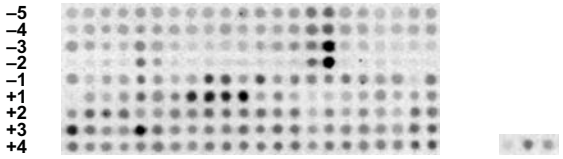
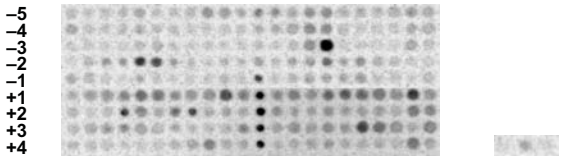
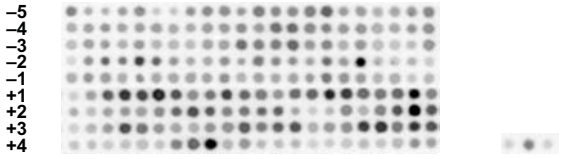
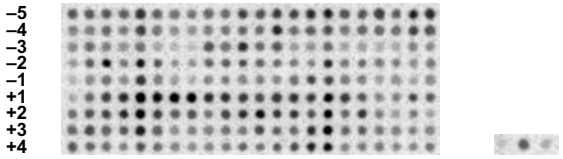
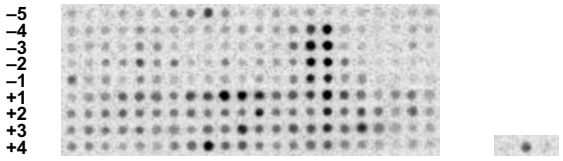
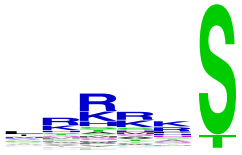
HOG1	*YLR113W*		
HRR25	*YPL204W*		
HSL1	*YKL101W*		
IPL1	*YPL209C*		
KCC4	*YCL024W*		
KIN1	*YDR122W*		
KIN3	*YAR018C*		
KIN4	*YOR233W*		

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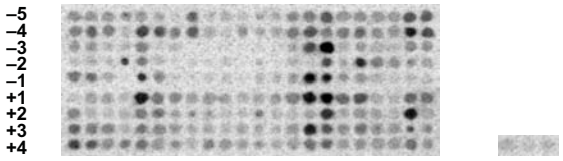
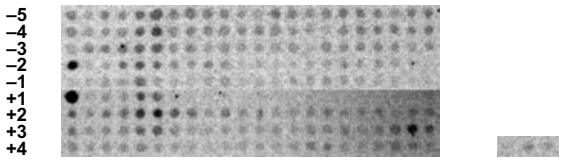
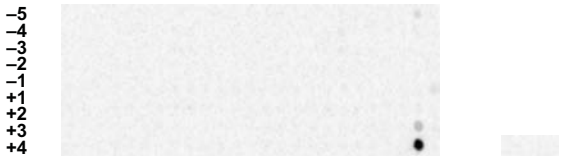
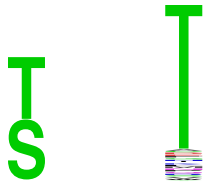
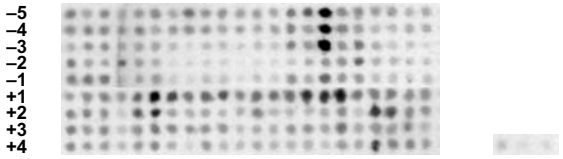
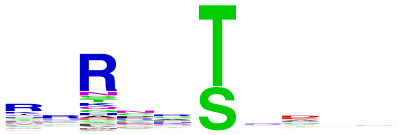
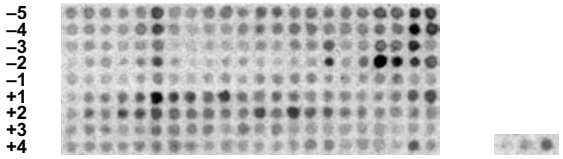
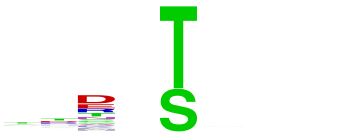
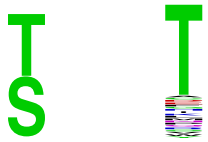
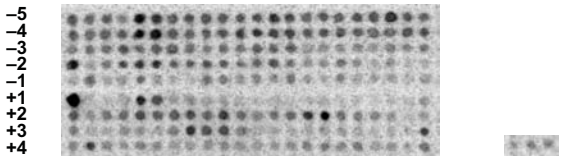
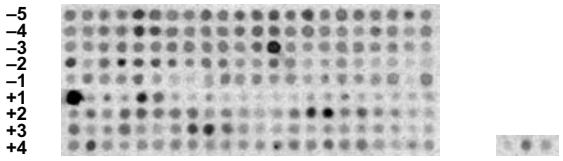
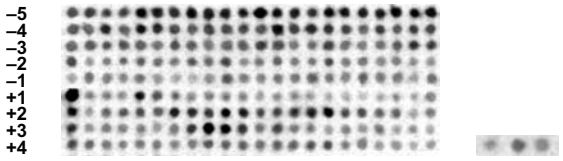
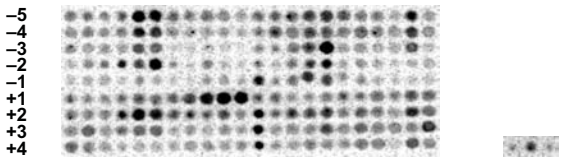
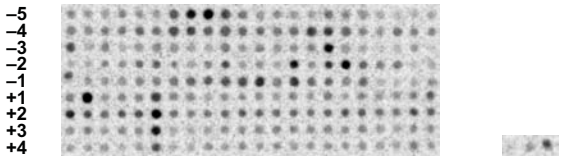
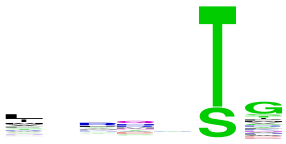
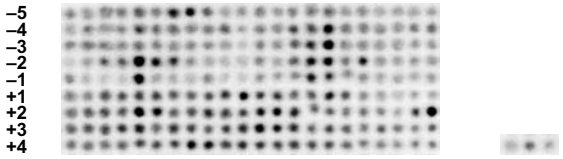
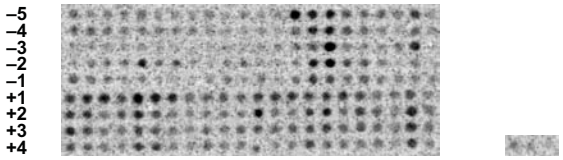
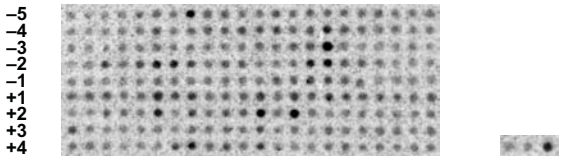
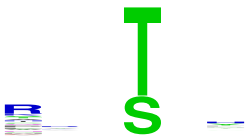
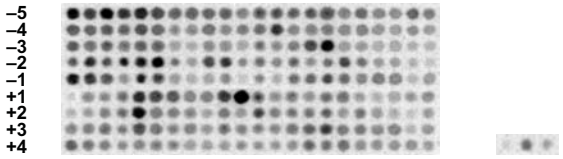
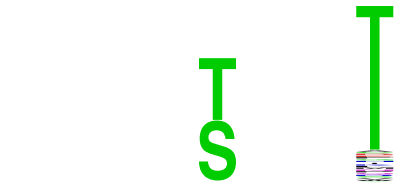
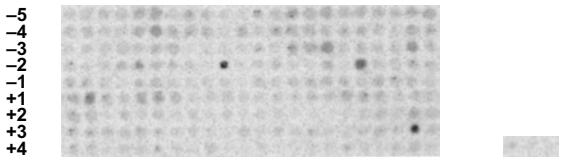
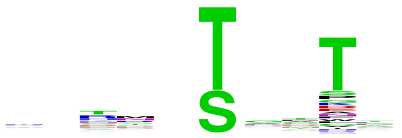
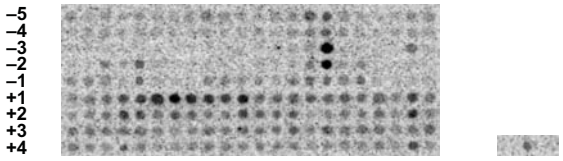
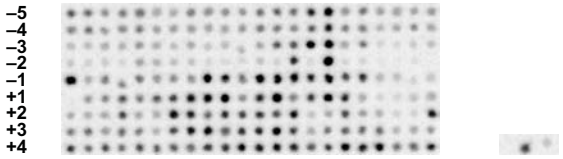
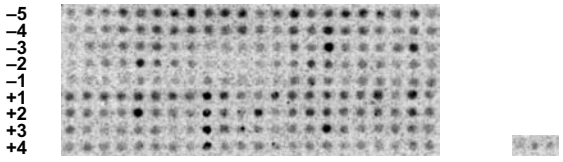
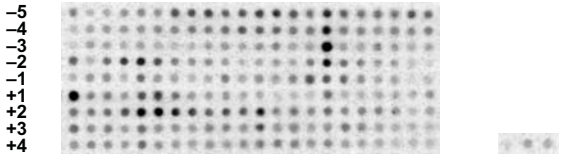
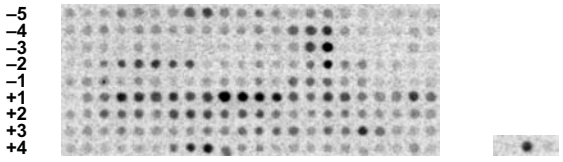
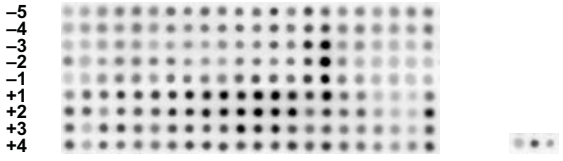
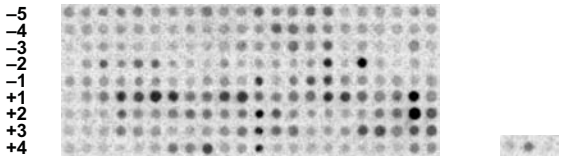
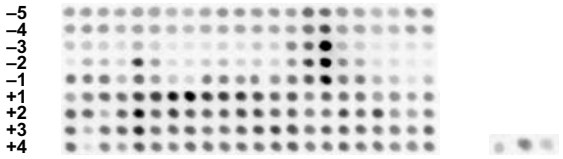
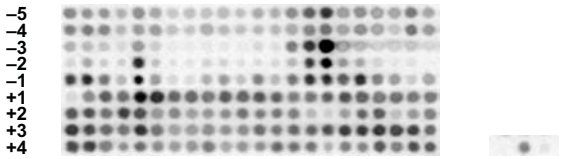
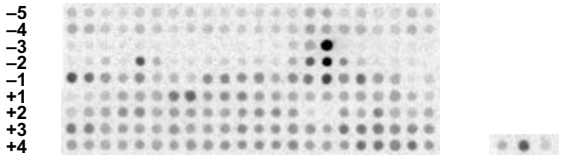
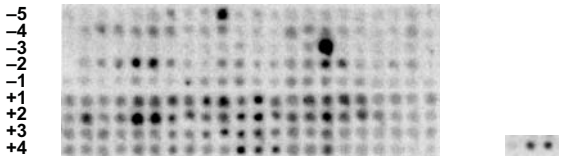
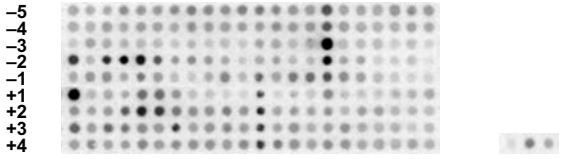
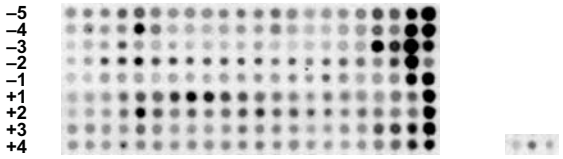
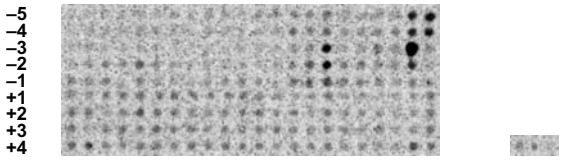
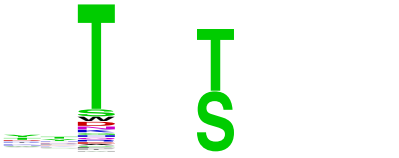
KSP1	*YHR082C*		
KSS1	*YGR040W*		
MCK1	*YNL307C*		
MEK1	*YOR351C*		
MPS1	*YDL028C*		
MRK1	*YDL079C*		
PHO85-PCL1	*YPL031C-YNL289W*		
PHO85-PCL2	*YPL031C-YDL127W*		

Gene Name	ORF Name	Array Image P G A C S T V I L M F Y W H K R Q N D E pT pY 0: - S T	Logo
PHO85-PHO80	*YPL031C-YOL001W*		
PKH2	*YOL100W*		
PRK1	*YIL095W*		
PRR1	*YKL116C*		
PSK2	*YOL045W*		
PTK2	*YJR059W*		
RAD53	*YPL153C*		
RCK2	*YLR248W*		

Gene Name	ORF Name	Array Image P G A C S T V I L M F Y W H K R Q N D E pT pY 0:- S T	Logo
RIM11	*YMR139W*		
RIM15	*YFL033C*		
SCH9	*YHR205W*		
SKM1	*YOL113W*		
SKY1	*YMR216C*		
SLT2	*YHR030C*		
SNF1	*YDR477W*		
STE20	*YHL007C*		

Gene Name	ORF Name	Array Image P G A C S T V I L M F Y W H K R Q N D E pT pY 0:- S T	Logo
TOS3	*YGL179C*		
TPK1	*YJL164C*		
TPK2	*YPL203W*		
TPK3	*YKL166C*		
VHS1	*YDR247W*		
YAK1	*YJL141C*		
YCK1	*YHR135C*		
YCK2	*YNL154C*		

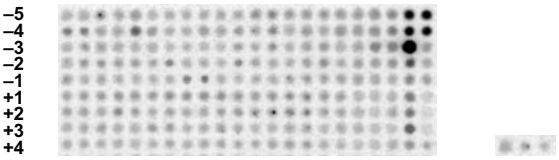
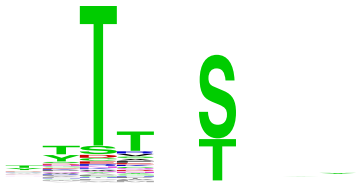
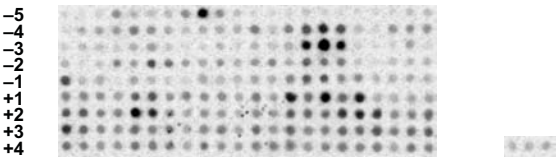
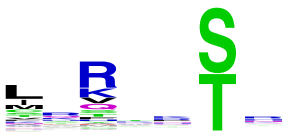
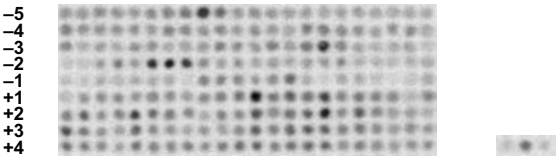
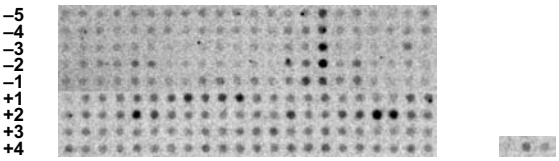
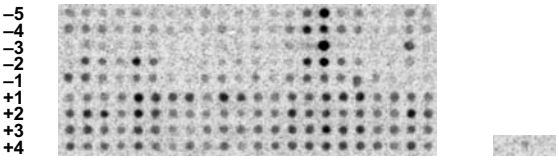
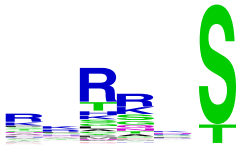
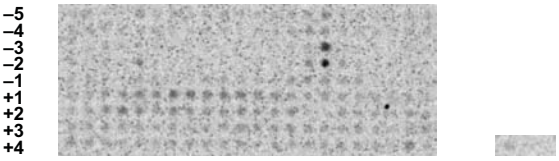
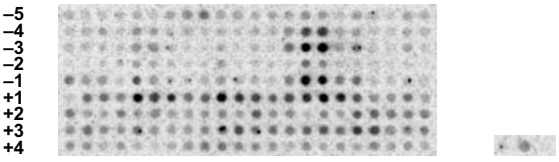
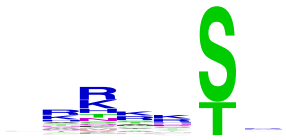
Gene Name	ORF Name	Array Image P G A C S T V I L M F Y W H K R Q N D E pT pY 0:- S T	Logo
YCK3	*YER123W*		
YDL025C	*YDL025C*		
YKL171W	*YKL171W*		
YNR047W	*YNR047W*		
YPK1	*YKL126W*		
YPK2	*YMR104C*		
YPL141C	*YPL141C*		

Table S2. Protein kinases analyzed in this study. Mammalian orthologs are indicated where known. References for reported phosphorylation motifs are listed where either the yeast kinase itself or the mammalian ortholog has been previously characterized.

Kinase	ORF	Category	Ortholog	Reported motif
Akl1	YBR059C	Other	BiKE	None
Ark1	YNL020C	Other	BiKE	None
Atg1	YGL180W	Other	ULK2	None
Cak1	YFL029C	Other	None	None
Cdc15	YAR019C	Other	None	(*19*)
Cdc28	YBR160W	Proline	CDK2	(*20*)
Cdc5	YMR001C	Acidophilic	PLK1	(*21*)
Cdc7	YDL017W	Acidophilic	CDC7	None
Cka1	YIL035C	Acidophilic	CK2	(*22*)
Cla4	YNL298W	Basophilic	PAK1	(*23*)
Cmk1	YFR014C	Basophilic	CAMK1	(*24*)
Cmk2	YOL016C	Basophilic	CAMK1	(*24*)
Fmp48	YGR052W	Basophilic	None	None
Fus3	YBL016W	Proline	ERK	(*22*)
Gcn2	YDR283C	Acidophilic	GCN2	None
Gin4	YDR507C	Basophilic	BRSK1	None
Hog1	YLR113W	Proline	p38-α	(*25*)
Hrr25	YPL204W	Acidophilic	CK1-δ	(*22*)
Hsl1	YKL101W	Basophilic	BRSK1	None
Ipl1	YPL209C	Basophilic	Aurora kinase C	(*26*)
Kcc4	YCL024W	Basophilic	BRSK1	None
Kin1	YDR122W	Other	MARK2/Par1b	None
Kin3	YAR018C	Other	NEK2	(*27*)
Kin4	YOR233W	Basophilic	None	None
Ksp1	YHR082C	Basophilic	None	None
Kss1	YGR040W	Proline	ERK	(*22*)
Mck1	YNL307C	Acidophilic	GSK3	(*28*)
Mek1	YOR351C	Basophilic	None	None
Mps1	YDL028C	Acidophilic	TTK/MPS1	None
Mrk1	YDL079C	Acidophilic	GSK3	(*28*)
Pho85	YPL031C	Proline	None	None
Pkh2	YOL100W	Basophilic	PDK1	None
Prk1	YIL095W	Other	AAK1	None
Prr1	YKL116C	Basophilic	None	None
Psk2	YOL045W	Basophilic	PASK	None
Ptk2	YJR059W	Basophilic	None	None
Rad53	YPL153C	Basophilic	None	None
Rck2	YLR248W	Basophilic	MK2	(*25*)

Table S2, continued

Rim11	YMR139W	Acidophilic	GSK3	(*28*)
Rim15	YFL033C	Other	None	None
Sch9	YHR205W	Basophilic	Akt/PKB	(*29*)
Skm1	YOL113W	Basophilic	PAK1	(*23*)
Sky1	YMR216C	Basophilic	SRPK2	None
Slt2	YHR030C	Proline	None	None
Snf1	YDR477W	Basophilic	AMPK	(*30*)
Ste20	YHL007C	Basophilic	PAK1	(*23*)
Tos3	YGL179C	Other	LKB1	(*31*)*
Tpk1	YJL164C	Basophilic	PKA	(*20*)
Tpk2	YPL203W	Basophilic	PKA	(*20*)
Tpk3	YKL166C	Basophilic	PKA	(*20*)
Vhs1	YDR247W	Basophilic	None	None
Yak1	YJL141C	Proline	DYRK4	(*32*)
Yck1	YHR135C	Acidophilic	CK1-γ2	(*22*)
Yck2	YNL154C	Acidophilic	CK1-γ2	(*22*)
Yck3	YER123W	Acidophilic	CK1-γ3	(*22*)
Ydl025c	YDL025C	Basophilic	None	None
Ykl171w	YKL171W	Basophilic	None	None
Ynr047w	YNR047W	Basophilic	None	None
Ypk1	YKL126W	Basophilic	SGK2	None
Ypk2	YMR104C	Basophilic	SGK2	None
Ypl141c	YPL141C	Basophilic	None	None

*Our observed motif for Tos3 differs from that reported for its human ortholog LKB1.

Table S3. Pairwise correlation coefficients for each of four genomic features and the Scansite match score. The PRK1 PWM was run on MOTIPS to obtain the top 2,000 predicted binding sites with values for all the five features: hits per protein, match score, disorder score, accessibility score, and conservation score. Multiple hits on a single open reading frame (ORF) were combined and the best set of scores was chosen, resulting in a list of 1,583 hits with no redundant protein targets. Pairwise Spearman correlation was then performed for all the features using the statistical software R.

	Hits per ORF	Scansite score	Accessibility score	Disorder score	Conservation score
Hits per ORF	1.00	-0.25	0.06	0.17	-0.02
Scansite score	-0.25	1.00	-0.01	-0.03	-0.01
Accessibility score	0.06	-0.01	1.00	0.57	-0.42
Disorder score	0.17	-0.03	0.57	1.00	-0.27
Conservation score	-0.02	-0.01	-0.42	-0.27	1.00

Table S4. Alignment of yeast kinases analyzed in this study. Dataset S4 contains this alignment in clustal format.

```
                          1        10        20        30        40        50
TOS3_Scer/1-295           FEILATLGNGQYGKVKLARDLGT--------------------GALVAIKILNR-----
TPK3_Scer/1-255           FQILRTLGTGSFGRVHLIRSNHN--------------------GRFYALKTLKKHTIVK
TPK1_Scer/1-255           FQILRTLGTGSFGRVHLIRSRHN--------------------GRYYAMKVLKKEIVVR
TPK2_Scer/1-255           FQIMRTLGTGSFGRVHLVRSVHN--------------------GRYYAIKVLKKQQVVK
YPK1_Scer/1-256           FDLLKVIGKGSFGKVMQVRKKDT--------------------QKVYALKAIRKSYIVS
YPK2_Scer/1-256           FDLLKVIGKGSFGKVMQVRKKDT--------------------QKIYALKALRKAYIVS
SCH9_Scer/1-260           FEVLRLLGKGTFGQVYQVKKKDT--------------------QRIYAMKVLSKKVIVK
YNR047W_Scer/1-282        FEKIRLLGQGDVGKVFLVREKKT--------------------NRVYALKVLSKDEMIK
RIM15_Scer/1-269          YDILKPISKGAYGSVYLARKKLT--------------------GDYFAIKVLRKSDMIA
IPL1_Scer/1-252           FELGKKLGKGKFGKVYCVRHRST--------------------GYICALKVMEKEEIIK
PKH2_Scer/1-265           FKFGSVIGDGAYSTVMLATSIDT--------------------KKRYAAKVLNKEYLIR
KCC4_Scer/1-265           WKLGETLGFGSTGKVQLAQHERT--------------------GHRTAVKVISKSIFNN
GIN4_Scer/1-271           WKLGETLGLGSTGKVQLARNGST--------------------GQEAAVKVISKAVFNT
HSL1_Scer/1-289           WKLGKTLGKGSSGRVRLAKNMET--------------------GQLAAIKIVPKKKAFV
SNF1_Scer/1-252           YQIVKTLGEGSFGKVKLAYHTTT--------------------GQKVALKIINKKVLAK
KIN1_Scer/1-279           WEFVETVGAGSMGKVKLAKHRYT--------------------NEVCAVKIVNRATKAF
KIN4_Scer/1-268           YIIGSTLGEGEFGKVKLGWTKASSSNE----------------VPKQVAIKLIRRDTIKK
YPL141C_Scer/1-273        YILGSTLGEGEFGKVKLGWPKNFSNSSNS-----------TFDFPKQVAIKLIKRDSISN
CMK1_Scer/1-263           YVFGKTLGAGTFGVVRQAKNTET--------------------GEDVAVKILIKKALKG
CMK2_Scer/1-263           YIFGRTLGAGSFGVVRQARKLST--------------------NEDVAIKILLKKALQG
RAD53_Scer/1-269          SIIDEVVGQGAFATVKKAIERTT--------------------KTFAVKIISKRKVIG
RCK2_Scer/1-316           YKLINKIGEGAFSKVFRAIPAKNSSNEFL------------TKNYKAVAIKVIKKADLSS
APG1_Scer/1-302           YTAEKEIGKGSFATVYRGHLTSDK--------------------SQHVAIKEVSRAKLKN
MEK1_Scer/1-284           EITNRIVGNGTFGHVLITHNSKERDEDVCYH-------------PENYAVKIIKLK----
YKL171W_Scer/1-283        HRLGKIIGFGAWGIIRECFDIET--------------------GVGRVIKIVKFKGHQN
CDC5_Scer/1-256           YHRGHFLGEGGFARCFQ-IKDDS--------------------GEIFAAKTVAKASIKS
PSK2_Scer/1-259           FTILQVMGEGAYGKVNLCIHN----------------------REHYIVVIKMIFKERILV
YDL025C_Scer/1-274        GIPGRKLGEGASGSVSVVER----------------------TDGKLFACKMFRKPHLNN
SKY1_Scer/1-312           YILVRKLGWGHFSTVWLAKDMVNN--------------------THVAMKIVRGDKVYT
YAK1_Scer/1-257           YLVLDILGQGTFGQVVKCQNLLTK--------------------EILAVKVVKSRTEYL
PTK2_Scer/1-305           DTDNKPIGSGGSSEVRKVKSS----------------------YRQKDVYALKKLN--MIYH
PHO85_Scer/1-291          FKQLEKLGNGTYATVYKGLNKTT--------------------GVYVALKEVKLDSE--
CDC28_Scer/1-288          YKRLEKVGEGTYGVVYKALDLRPGQG-----------------QRVVALKKIRLESED-
SLT2_Scer/1-296           FQLIKEIGHGAYGIVCSARFAEAAE------------------DTTVAIKKVTNVFSKT
KSS1_Scer/1-301           YKLVDLIGEGAYGTVCSAIHKPS--------------------GIKVAIKKIQ-PFSKK
FUS3_Scer/1-297           FQLKSLLGEGAYGVVCSATHKPT--------------------GEIVAIKKIE-PFDKP
HOG1_Scer/1-280           YNDLNPVGMGAFGLVCSATDTLT--------------------SQPVAIKKIMKPFSTA
RIM11_Scer/1-284          FPTTEVVGHGSFGVVFATVIQET--------------------EKVAIKKVLQDKRFK
MRK1_Scer/1-284           YPTTEVVGHGSFGVVVTTVIIET--------------------NQKVAIKKVLQDRRYK
MCK1_Scer/1-293           VKEYRKIGRGAFGTVVQAYLTQDKKNWL----------------GPFAIKKVPAHTEYK
CKA1_Scer/1-324           YEIENKVGRGKYSEVFQGVKLDS--------------------KVKIVIKMLKPVKKK
CDC7_Scer/1-272           YKLIDKIGEGTFSSVYKAKDITGKITKKFASHFW-------NYGSNYVALKKIYVTSSPQ
YCK1_Scer/1-261           YKIGKKIGEGSFGVLFEGTNMIN--------------------GVPVAIKFEPRKTEAP
YCK2_Scer/1-261           YKIGKKIGEGSFGVLFEGTNMIN--------------------GLPVAIKFEPRKTEAP
YCK3_Scer/1-296           YAVGPKIGEGSFGVIFEGENILHSCQ----AQTGSKRDSSIIMANEPVAIKFEPRHSDAP
HRR25_Scer/1-259          FRIGRKIGSGSFGDIYHGTNLIS--------------------GEEVAIKLESIRSRHP
PRR1_Scer/1-317           WKKVRPIGSGNFSTVLLYELMDQSN----------------PKLKQVAVKRLKYPEELS
MPS1_Scer/1-277           YEKIELLGRGGSSRVYKVKGSGN--------------------RVYALKRVSFDAFDD
GCN2_Scer/1-268           FEEIAVLGQGAFGQVVKARNALD--------------------SRYYAIKKIRHTEEK-
KIN3_Scer/1-319           YQVLEEIGRGSFGSVRKVIHIPT--------------------KKLLVRKDIKYGHMNS
PRK1_Scer/1-277           AKIIKYLTSGGFAQVYTA----------------EISPPDPYSNANIACLKRVIVPHKQG
ARK1_Scer/1-277           VEIIKYLTSGGFAQVYSA----------------LINPPDPHSNSSVACLKRVIVPDKPS
AKL1_Scer/1-285           VEVVNYLAEGGFAQIYVVKFL------EYLNEF-DNTASVPLKIGDVACLKRVLVQDENG
VHS1_Scer/1-321           YLITSQIGEGAYGLVYRALDIRTD--------------------RQYAIKAVVQSYGVS
SKM1_Scer/1-280           FQLVEKAGQGASGAVYLSKRIKLP--QENDPRF-LKSHCHR-VVGERVAIKQIRLSEQPK
CLA4_Scer/1-280           FKVIEKAGQGASGSVYLAERTHIP--TESNMIELINNDIDEPHVGDKVAIKQMVLSKQPR
STE20_Scer/1-252          YANLVKIGQGASGGVYTAYEIGT--------------------NVSVAIKQMNLEKQPK
CDC15_Scer/1-248          YHLKQVIGRGSYGVVYKAINKHT--------------------DQVVAIKEVVYENDEE
FMP48_Scer/1-285          YTKLRSIQSGTFSTVYKAWSTTHN--------------------RYVALKITPKYKTSE
KSP1_Scer/1-260           YQKIEDISEGSYGYVSLAKDVREK--------------------RLVAVKYIFKLEDLE
CAK1_Scer/1-304           -MKLDSIDITHCQLVKSTRTARIYR------------------SDTYAIKCLALDFDIP
```

Table S4, continued

```
                    61        70        80        90        100       110
TOS3_Scer/1-295     --------------------------------FEKRSG----YSLQLKV-ENPRVNQEIE
TPK3_Scer/1-255     ------------------------------------------------LKQVEHTNDERR
TPK1_Scer/1-255     ------------------------------------------------LKQVEHTNDERL
TPK2_Scer/1-255     ------------------------------------------------MKQVEHTNDERR
YPK1_Scer/1-256     ------------------------------------------------KSEVTHTLAERT
YPK2_Scer/1-256     ------------------------------------------------KCEVTHTLAERT
SCH9_Scer/1-260     ------------------------------------------------KNEIAHTIGERN
YNR047W_Scer/1-282  ------------------------------------------------RNKIKRVLTEQE
RIM15_Scer/1-269    ------------------------------------------------KNQVTNVKSERA
IPL1_Scer/1-252     ------------------------------------------------YNLQKQFRREVE
PKH2_Scer/1-265     ------------------------------------------------QKKVKYVSIEKT
KCC4_Scer/1-265     NGN-------------------------------------HSNDDSVLPYNIEREIV
GIN4_Scer/1-271     GNVSGTSI--------------------------------VGSTTPDALPYGIEREII
HSL1_Scer/1-289     HCSNNGTVPNSYSSSMVTSNVSSPSIA-------------SREHSNHSQTNPYGIEREIV
SNF1_Scer/1-252     SDMQG-----------------------------------------------RIEREIS
KIN1_Scer/1-279     LHKEQMLPPPKNEQDVLER---------------------QKKLEKEISRDKRTIREAS
KIN4_Scer/1-268     D-----------------------------------------------ADKEIKIYREIN
YPL141C_Scer/1-273  D-----------------------------------------------YRKEVKIYREIN
CMK1_Scer/1-263     NKVQL-------------------------------------------------EALYDELD
CMK2_Scer/1-263     NNVQL-------------------------------------------------QMLYEELS
RAD53_Scer/1-269    N-------------------------------------------------MDGVTRELE
RCK2_Scer/1-316     INGDHRKKDK--------------------------------GKDSTKTSSRDQVLKEVA
APG1_Scer/1-302     KKLL--------------------------------------------------ENLEIEIA
MEK1_Scer/1-284     ------------------------------------------------PNKFDKEAR
YKL171W_Scer/1-283  ----------------------------------------------IKKHVLREVA
CDC5_Scer/1-256     EKTR--------------------------------------------KKLLSEIQ
PSK2_Scer/1-259     DT----------------------------------------WVRDRKLGTIPSEIQ
YDL025C_Scer/1-274  EGTN------------------------------------QSQLANYSKKVTTEFC
SKY1_Scer/1-312     ------------------------------------------------EAAEDEIK
YAK1_Scer/1-257     ------------------------------------------------TQSITEAK
PTK2_Scer/1-305     E-----------------------------------------SPEKFYKRCSKEFI
PHO85_Scer/1-291    ------------------------------------------------EGTPSTAIREIS
CDC28_Scer/1-288    ------------------------------------------------EGVPSTAIREIS
SLT2_Scer/1-296     ----------------------------------------------LLCKRSLRELK
KSS1_Scer/1-301     ----------------------------------------------LFVTRTIREIK
FUS3_Scer/1-297     ----------------------------------------------LFALRTLREIK
HOG1_Scer/1-280     ----------------------------------------------VLAKRTYRELK
RIM11_Scer/1-284    ------------------------------------------------------NRELE
MRK1_Scer/1-284     ------------------------------------------------------NRELE
MCK1_Scer/1-293     -------------------------------------------------------SRELQ
CKA1_Scer/1-324     ----------------------------------------------------IKREIK
CDC7_Scer/1-272     ----------------------------------------------------RIYNELN
YCK1_Scer/1-261     ----------------------------------------------------QLRDEYK
YCK2_Scer/1-261     ----------------------------------------------------QLKDEYR
YCK3_Scer/1-296     ----------------------------------------------------QLRDEFR
HRR25_Scer/1-259    ----------------------------------------------------QLDYESR
PRR1_Scer/1-317     NVEQIN--------------------------------TSLRYKETLSRLENSLTRELQ
MPS1_Scer/1-277     S-----------------------------------------------SIDGFKGEIE
GCN2_Scer/1-268     ----------------------------------------------LSTILSEVM
KIN3_Scer/1-319     KE----------------------------------------------RQQLIAECS
PRK1_Scer/1-277     ----------------------------------------------LNTLRAEVD
ARK1_Scer/1-277     ----------------------------------------------LNTLRAEVD
AKL1_Scer/1-285     ----------------------------------------------LNEMRNEVE
VHS1_Scer/1-321     KEADMGNDKIHKNSVKLQKKLAKLFKESKNVVRVPSIDLESIENMSEEDFKKLPHYKEIS
SKM1_Scer/1-280     ----------------------------------------------KQLIMNELL
CLA4_Scer/1-280     ----------------------------------------------KELIVNEIL
STE20_Scer/1-252    ----------------------------------------------KELIINEIL
CDC15_Scer/1-248    ----------------------------------------------LNDIMAEIS
FMP48_Scer/1-285    ----------------------------------------------ANMKNEYD
KSP1_Scer/1-260     ----------------------------------------------AMYEVD
CAK1_Scer/1-304     ----------------------------------------------PHNAKFEVS
```

Table S4, continued

```
                    121       130       140       150       160       170
TOS3_Scer/1-295     VM--------------------------------------KRCHHENVVELYEILNDPE
TPK3_Scer/1-255     MLSIVS----------------------------------------HPFIIRMWGTFQ---
TPK1_Scer/1-255     MLSIVT----------------------------------------HPFIIRMWGTFQ---
TPK2_Scer/1-255     MLKLVE----------------------------------------HPFLIRMWGTFQ---
YPK1_Scer/1-256     VLARVD----------------------------------------CPFIVPLKFSFQ---
YPK2_Scer/1-256     VLARVD----------------------------------------CPFIVPLKFSFQ---
SCH9_Scer/1-260     ILVTTASKS-------------------------------------SPFIVGLKFSFQ---
YNR047W_Scer/1-282  ILATSN--------------------------------------HPFIVTLYHSFQ---
RIM15_Scer/1-269    IMMVQSD---------------------------------------KPYVARLFASFQ---
IPL1_Scer/1-252     IQTSLN----------------------------------------HPNLTKSYGYFH---
PKH2_Scer/1-265     ALQKLNNS--------------------------------------PSVVRLFSTFQ---
KCC4_Scer/1-265     IMKLLS----------------------------------------HPNVLSLYDVWE---
GIN4_Scer/1-271     IMKLLN----------------------------------------HPNVLRLYDVWE---
HSL1_Scer/1-289     IMKLIS----------------------------------------HTNVMALFEVWE---
SNF1_Scer/1-252     YLRLLR----------------------------------------HPHIIKLYDVIK---
KIN1_Scer/1-279     LGQILY----------------------------------------HPHICRLFEMCT---
KIN4_Scer/1-268     ALKHLT----------------------------------------HPNIIYLEEVLQ---
YPL141C_Scer/1-273  ALKHLS----------------------------------------HPNIVKLEEVLQ---
CMK1_Scer/1-263     ILQRLH----------------------------------------HPNIVAFKDWFE---
CMK2_Scer/1-263     ILQKLS----------------------------------------HPNIVSFKDWFE---
RAD53_Scer/1-269    VLQKLN----------------------------------------HPRIVRLKGFYE---
RCK2_Scer/1-316     LHKTVSAG--------------------------------------CSQIVAFIDFQE---
APG1_Scer/1-302     ILKKIK----------------------------------------HPHIVGLIDCER---
MEK1_Scer/1-284     ILLRLD----------------------------------------HPNIIKVYHTFCD--
YKL171W_Scer/1-283  IWRTLK--------------------------------------HNRILPLLDWKLDDN
CDC5_Scer/1-256     IHKSMS----------------------------------------HPNIVQFIDCFE---
PSK2_Scer/1-259     IMATLNKN--------------------------------------SQENILKLLDFFEDDD
YDL025C_Scer/1-274  IGSTLH----------------------------------------HENIVETLDMLTEG-
SKY1_Scer/1-312     LLQRVNDADN----------------------------TKEDSMGANHILKLLDHFNHKG
YAK1_Scer/1-257     ILELLNQK----------------------------------IDPTNKHHFLRMYDSFV
PTK2_Scer/1-305     IAKHLSHN----------------------------------VHITNTFYLLKVPTTTY
PHO85_Scer/1-291    LMKELK----------------------------------------HENIVRLYDVIHTEN
CDC28_Scer/1-288    LLKELK----------------------------------------DDNIVRLYDIVHSDA
SLT2_Scer/1-296     LLRHFR----------------------------------------GHKNITCLYDMDIVFY
KSS1_Scer/1-301     LLRYFH----------------------------------------EHENIISILDKVRPVS
FUS3_Scer/1-297     ILKHFK----------------------------------------HENIITIFNIQRPDS
HOG1_Scer/1-280     LLKHLR----------------------------------------HENLICLQDIFLS--
RIM11_Scer/1-284    IMKMLS----------------------------------------HINIIDLKYFFY---
MRK1_Scer/1-284     TMKMLC----------------------------------------HPNTVGLQYYFY---
MCK1_Scer/1-293     ILRIAD----------------------------------------HPNIVKLQYFFT-HL
CKA1_Scer/1-324     ILTDLSNEKVPPTTLPFQKDQYYTNQKEDVLKFIRPYIFDQPHNGHANIIHLFDIIKDPI
CDC7_Scer/1-272     LLYIMT----------------------------------------GSSRVAPLCDAKRVR-
YCK1_Scer/1-261     TYKIL-----------------------------------------NGTPNIPYAYYFGQEG
YCK2_Scer/1-261     TYKIL-----------------------------------------AGTPGIPQEYYFGQEG
YCK3_Scer/1-296     AYRIL-----------------------------------------NGCVGIPHAYYFGQEG
HRR25_Scer/1-259    VYRYL-----------------------------------------SGGVGIPFIRWFGREG
PRR1_Scer/1-317     VLKSLN----------------------------------------HPCIVKLLGINNPIF
MPS1_Scer/1-277     LLEKLK----------------------------------------DQKRVIQLLDYEMGDG
GCN2_Scer/1-268     LLASLN----------------------------------------HQYVVRYYAAWLEED
KIN3_Scer/1-319     ILSQL-----------------------------------------KHENIVEFYNWDFDEQ
PRK1_Scer/1-277     AMKLLRNN--------------------------------------KHVVSYIDSHAARS
ARK1_Scer/1-277     AMRLLKNN--------------------------------------RYVVSYIDSHAAKA
AKL1_Scer/1-285     VMKKLKGA--------------------------------------PNIVQYFDSNASRR
VHS1_Scer/1-321     LHLRVHH---------------------------------------HKNIVTIHEVLQS--
SKM1_Scer/1-280     VMNDS-----------------------------------------RQENIVNFLEAYIIDD
CLA4_Scer/1-280     VMKDS-----------------------------------------RHKNIVNFLEAYLRTD
STE20_Scer/1-252    VMKGS-----------------------------------------KHPNIVNFIDSYVLKG
CDC15_Scer/1-248    LLKNL-----------------------------------------NHN-NIVKYHGFIRKS
FMP48_Scer/1-285    VMKILSSCN-------------------------------------PHPNICSMLDFYTDDS
KSP1_Scer/1-260     IQTKIG----------------------------------------RHQNIAALLDFFDS--
CAK1_Scer/1-304     ILNKLGN---------------------------------------KCKHILPLLESKATDN
```

Table S4, continued

```
                    181       190       200       210       220       230
TOS3_Scer/1-295     S-----------------TKVYLVLEYCSRGPVKWCPENK-----------------MEI
TPK3_Scer/1-255     ----------------DSQQVFMVMDYIEGGELFSLLRK---------------------
TPK1_Scer/1-255     ----------------DAQQIFMIMDYIEGGELFSLLRK---------------------
TPK2_Scer/1-255     ----------------DARNIFMVMDYIEGGELFSLLRK---------------------
YPK1_Scer/1-256     ----------------SPEKLYFVLAFINGGELFYHLQK---------------------
YPK2_Scer/1-256     ----------------SPEKLYLVLAFINGGELFYHLQH---------------------
SCH9_Scer/1-260     ----------------TPTDLYLVTDYMSGGELFWHLQK---------------------
YNR047W_Scer/1-282 ----------------SEDYLYLCMEYCMGGEFFRALQTR-------------------K
RIM15_Scer/1-269    ----------------NKDNLFLVMEYLPGGDLATLIKM---------------------
IPL1_Scer/1-252     ----------------DEKRVYLLMEYLVNGEMYKLLRL---------------------
PKH2_Scer/1-265     ----------------DESSLYFLLEYAPNGDFLSLMKK---------------------
KCC4_Scer/1-265     ----------------TNNNLYLILEYAEKGELFNLLVD---------------------
GIN4_Scer/1-271     ----------------TNTDLYLVLEYAEKGELFNLLVE---------------------
HSL1_Scer/1-289     ----------------NKSELYLVLEYVDGGELFDYLVS---------------------
SNF1_Scer/1-252     ----------------SKDEIIMVIEYA-GNELFDYIVQ---------------------
KIN1_Scer/1-279     ----------------LSNHFYMLFEYVSGGQLLDYIIQ---------------------
KIN4_Scer/1-268     ----------------NSKYIGIVLEFVSGGEFYKYIQR---------------------
YPL141C_Scer/1-273 ----------------NSRYIGIVLEYACGGEFYKYIQK---------------------
CMK1_Scer/1-263     ----------------SKDKFYIITQLAKGGELFDRILK---------------------
CMK2_Scer/1-263     ----------------SKDKFYIVTQLATGGELFDRILS---------------------
RAD53_Scer/1-269    ----------------DTESYYMVMEFVSGGDLMDFVAA---------------------
RCK2_Scer/1-316     ----------------TDSYYYIIQELLTGGEIFGEIVR---------------------
APG1_Scer/1-302     ----------------TSTDFYLIMEYCALGDLTFLLKR---------------------
MEK1_Scer/1-284     ----------------RNNHLYIFQDLIPGGDLFSYLAKG------------------DC
YKL171W_Scer/1-283 ------------------YAMYCLTERINDGTLYDLVISW---------------------
CDC5_Scer/1-256     ----------------DDSNVYILLEICPNGSLMELLKR---------------------
PSK2_Scer/1-259     YYY----------------IETPVHGETGSIDLFDVIEF---------------------
YDL025C_Scer/1-274 --D----------------TYLLVMEYAPY-DFFNLVMS---------------------
SKY1_Scer/1-312     P---------------NGVHVVMVFEVLGENLLALIKKY---------------------
YAK1_Scer/1-257     HKN----------------HLCLVFELLSNNLYELLKQ---------------------
PTK2_Scer/1-305     TTR----------------GWGFIMEL-GVKDLFQLMER---------------------
PHO85_Scer/1-291    -------------------KLTLVFEF-MDNDLKKYMDS---------------------
CDC28_Scer/1-288    H-------------------KLYLVFEF-LDLDLKRYMEG---------------------
SLT2_Scer/1-296     PDG-------------SINGLYLYEEL-MECDMHQIIKSGQ--------------------
KSS1_Scer/1-301     ID--------------KLNAVYLVEEL-METDLQKVINNQNS-------------------
FUS3_Scer/1-297     FE--------------NFNEVYIIQEL-MQTDLHRVISTQ---------------------
HOG1_Scer/1-280     ----------------PLEDIYFVTEL-QGTDLHRLLQTR---------------------
RIM11_Scer/1-284    ERDSQ-----------DEIYLNLILEY-MPQSLYQRLRHF-VHQRT--------------
MRK1_Scer/1-284     EKDEE-----------DEVYLNLVLDY-MPQSLYQRLRHF-VNLKM--------------
MCK1_Scer/1-293     SPQDN------------KVYQHLAMEC-LPETLQIEINRY-VTNKL--------------
CKA1_Scer/1-324     S-----------------KTPALVFEYVDNVD-FRILYP----------------------
CDC7_Scer/1-272     ------------------DQVIAVLPYYPHEE-FRTFYR----------------------
YCK1_Scer/1-261     L-------------------HNILVIDLLGP-SLEDLFDW---------------------
YCK2_Scer/1-261     L-------------------HNILVIDLLGP-SLEDLFDW---------------------
YCK3_Scer/1-296     M-------------------HNILIIDLLGP-SLEDLFEW---------------------
HRR25_Scer/1-259    E-------------------YNAMVIDLLGP-SLEDLFNY---------------------
PRR1_Scer/1-317     VTSKKPLCDLIIKTPRALPPCDMIMSYCPAGDLLAAVMAR---------------------
MPS1_Scer/1-277     --------------------LLYLIMECGDHDLSQILNQ----------------------
GCN2_Scer/1-268     S------------------MLFIQMEYCENRTLYDLIHS----------------------
KIN3_Scer/1-319     K-----------------EVLYLYMEYCSRGDLSQMIKH--------------YKQEHKY
PRK1_Scer/1-277     VNG-------------IAYEVFVLMEFCERGGLIDFMNT----------------RLQNR
ARK1_Scer/1-277     MLHN------------GSYEVFVLMEYCERGGLIDFMNT----------------RLQNR
AKL1_Scer/1-285     RDGV------------QGFEVLLLMELCPNKSLLDYMNQ----------------RLSTK
VHS1_Scer/1-321     -----------------AVCTFIVMDYYPTDLFTSIVDN----------------------
SKM1_Scer/1-280     E-------------------ELWVIMEYMEGGCLTDILDA--------------VARSNTG
CLA4_Scer/1-280     D-------------------DLWVVMEFMEGGSLTDIIEN--------------SPTNDN
STE20_Scer/1-252    --------------------DLWVIMEYMEGGSLTDVVTH----------------------
CDC15_Scer/1-248    Y-------------------ELYILLEYCANGSLRRLISR----------------------
FMP48_Scer/1-285    --------------------YYIMVLEYCECGDLYDFLDIAK--------------SQGS
KSP1_Scer/1-260     ---------------------YIIMEYCSGGDLYEAIKAD----------------------
CAK1_Scer/1-304     N-------------------DLLLLFPFEEMNLYEFMQM--------HYKRDRRKKNPYYD
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Table S4, continued

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                    241       250       260       270       280       290
TOS3_Scer/1-295     KA--VGP--------------------SILTFQQSRKVVLDVVSGLEYLHSQG-------
TPK3_Scer/1-255     SQ--RFP------------------------NPVAKFYAAEVCLALEYLHSKD-------
TPK1_Scer/1-255     SQ--RFP------------------------NPVAKFYAAEVCLALEYLHSKD-------
TPK2_Scer/1-255     SQ--RFP------------------------NPVAKFYAAEVILALEYLHAHN-------
YPK1_Scer/1-256     EG--RFD------------------------LSRARFYTAELLCALDNLHKLD-------
YPK2_Scer/1-256     EG--RFS------------------------LARSRFYIAELLCALDSLHKLD-------
SCH9_Scer/1-260     EG--RFS------------------------EDRAKFYIAELVLALEHLHDND-------
YNR047W_Scer/1-282  TK--CIC------------------------EDDARFYASEVTAALEYLHLLG-------
RIM15_Scer/1-269    MG--YLP------------------------DQWAKQYLTEIVVGVNDMHQNG-------
IPL1_Scer/1-252     HG--PFN------------------------DILASDYIYQIANALDYMHKKN-------
PKH2_Scer/1-265     YG--SLD------------------------ETCARYYAAQIIDAIDYLHSNG-------
KCC4_Scer/1-265     HG--PLP------------------------EREAINCFRQIIIGISYCHALG-------
GIN4_Scer/1-271     RG--PLP------------------------EHEAIRFFRQIIIGVSYCHALG-------
HSL1_Scer/1-289     KG--KLP------------------------EREAIHYFKQIVEGVSYCHSFN-------
SNF1_Scer/1-252     RD--KMS------------------------EQEARRFFQQIISAVEYCHRHK-------
KIN1_Scer/1-279     HG--SIR------------------------EHQARKFARGIASALIYLHANN-------
KIN4_Scer/1-268     KR--RLK------------------------ESSACRLFAQLISGVNYMHYKG-------
YPL141C_Scer/1-273  KR--RLK------------------------EMNACRLFSQLISGVHYIHSKG-------
CMK1_Scer/1-263     KG--KFT------------------------EEDAVRILVEILSAVKYMHSQN-------
CMK2_Scer/1-263     RG--KFT------------------------EVDAVEIIVQILGAVEYMHSKN-------
RAD53_Scer/1-269    HG--AVG------------------------EDAGREISRQILTAIKYIHSMG-------
RCK2_Scer/1-316     LT--YFS------------------------EDLSRHVIKQLALAVKHMHSLG-------
APG1_Scer/1-302     RK--ELMENHPLLRTVFEKYPPPSENHNGLHRAFVLSYLQQLASALKFLRSKN-------
MEK1_Scer/1-284     LT--SMS------------------------ETESLLIVFQILQALNYLHDQD-------
YKL171W_Scer/1-283  DEFKRSKIPFAER--------------------CRLTIFLSLQLLSALKYMHSKT-------
CDC5_Scer/1-256     RK--VLT------------------------EPEVRFFTTQICGAIKYMHSRR-------
PSK2_Scer/1-259     KK--DMV------------------------EHEAKLVFKQVVASIKHLHDQG-------
YDL025C_Scer/1-274  -N--LMT------------------------QDEVNCYFKQLCHGVNYLHSMG-------
SKY1_Scer/1-312     EH--RG-------------------------IPLIYVKQISKQLLLGLDYMHRRCG------
YAK1_Scer/1-257     NKF-HG-------------------------LSIQLIRTFTTQILDSLCVLKESK-------
PTK2_Scer/1-305     TG--WKN------------------------VPFNEKYCLFKQVAQGIKFCHDNG-------
PHO85_Scer/1-291    -----RTVGN---------------TPRGLELNLVKYFQWQLLQGLAFCHENK-------
CDC28_Scer/1-288    -----IPKDQ--------------------PLGADIVKKFMMQLCKGIAYCHSHR-------
SLT2_Scer/1-296     ----------------------------PLTDAHYQSFTYQILCGLKYIHSAD-------
KSS1_Scer/1-301     -------------------------GFSTLSDDHVQYFTYQILRALKSIHSAQ-------
FUS3_Scer/1-297     -----------------------------MLSDDHIQYFIYQTLRAVKVLHGSN-------
HOG1_Scer/1-280     -----------------------------PLEKQFVQYFLYQILRGLKYVHSAG-------
RIM11_Scer/1-284    -----------------------------PMSRLEIKYYMFQLFKSLNYLHHFAN------
MRK1_Scer/1-284     -----------------------------QMPRVEIKFYAYQLFKALNYLHNVPR------
MCK1_Scer/1-293     -----------------------------EMPLKHIRLYTYQIARGMLYLHGLG-------
CKA1_Scer/1-324     -----------------------------KLTDLEIRFYMFELLKALDYCHSMG-------
CDC7_Scer/1-272     -----------------------------DLPIKGIKKYIWELLRALKFVHSKG-------
YCK1_Scer/1-261     CG--R------------------------KFSVKTVVQVAVQMITLIEDLHAHD-------
YCK2_Scer/1-261     CG--R------------------------RFSVKTVVQVAVQMITLIEDLHAHD-------
YCK3_Scer/1-296     CG--R------------------------KFSVKTTCMVAKQMIDRVRAIHDHD-------
HRR25_Scer/1-259    CH--R------------------------RFSFKTVIMLALQMFCRIQYIHGRS-------
PRR1_Scer/1-317     NG--RLE------------------------AWLIQRIFTEVVLAVKYLHENS-------
MPS1_Scer/1-277     RSGMPLD------------------------FNFVRFYTKEMLLCIKVVHDAG-------
GCN2_Scer/1-268     ENLNQQ-------------------------RDEYWRLFRQILEALSYIHSQG-------
KIN3_Scer/1-319     -----IP------------------------EKIVWGILAQLLTALYKCHYGVELPTLTT
PRK1_Scer/1-277     -----LQ------------------------ESEILEIMSQTVQGITAMHALQP------
ARK1_Scer/1-277     -----LH------------------------EFEILQIMSQVTQGVAAMHALQP------
AKL1_Scer/1-285     -----LT------------------------EAEIVKIMYDVALSISQMHYLPV------
VHS1_Scer/1-321     -R--HFVTN----------------------GLLVKKVFLQICSALNYCHEHG-------
SKM1_Scer/1-280     EHSSPLN------------------------ENQMAYIVKETCQGLKFLHNK--------
CLA4_Scer/1-280     SH-SPLT------------------------EPQIAYIVRETCQGLKFLHDK--------
STE20_Scer/1-252    CI---LT------------------------EGQIGAVCRETLSGLEFLHSK--------
CDC15_Scer/1-248    -SSTGLS------------------------ENESKTYVTQTLLGLKYLHGE--------
FMP48_Scer/1-285    PSSPSLI------------------------QIDMQKIIKQLCSAISFAHSLG-------
KSP1_Scer/1-260     ----AVPKK----------------------TKSITHIITQIMDAIEYVHNKG-------
CAK1_Scer/1-304     LL--NPS-IPIV-----ADPPVQKYTNQLDVNRYSLSFFRQMVEGIAFLHENK-------
```

Table S4, continued

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                    301       310       320       330       340       350
TOS3_Scer/1-295     --------------ITHRDIKPSNLLI-------------SSNG----------------
TPK3_Scer/1-255     --------------IIYRDLKPENILL-------------DKNG----------------
TPK1_Scer/1-255     --------------IIYRDLKPENILL-------------DKNG----------------
TPK2_Scer/1-255     --------------IIYRDLKPENILL-------------DRNG----------------
YPK1_Scer/1-256     --------------VVYRDLKPENILL-------------DYQG----------------
YPK2_Scer/1-256     --------------VIYRDLKPENILL-------------DYQG----------------
SCH9_Scer/1-260     --------------IVYRDLKPENILL-------------DANG----------------
YNR047W_Scer/1-282  --------------FIYRDLKPENILL-------------HQSG----------------
RIM15_Scer/1-269    --------------IIHHDLKPENLLI-------------DNAG----------------
IPL1_Scer/1-252     --------------IIHRDIKPENILI-------------GFNN----------------
PKH2_Scer/1-265     --------------IIHRDIKPENILL-------------DGEM----------------
KCC4_Scer/1-265     --------------IVHRDLKPENLLL-------------DSFY----------------
GIN4_Scer/1-271     --------------IVHRDLKPENLLL-------------DHKY----------------
HSL1_Scer/1-289     --------------ICHRDLKPENLLL-------------DKKN----------------
SNF1_Scer/1-252     --------------IVHRDLKPENLLL-------------DEHL----------------
KIN1_Scer/1-279     --------------IVHRDLKIENIMI-------------SDSS----------------
KIN4_Scer/1-268     --------------LVHRDLKLENLLL-------------DKHE----------------
YPL141C_Scer/1-273  --------------LVHRDLKLENLLL-------------DKNE----------------
CMK1_Scer/1-263     --------------IVHRDLKPENLLY------------IDKSDES--------------
CMK2_Scer/1-263     --------------VVHRDLKPENVLY------------VDKSENS--------------
RAD53_Scer/1-269    --------------ISHRDLKPDNILI-------------EQDDP---------------
RCK2_Scer/1-316     --------------VVHRDIKPENLLFEPIEFTRSIKPKLRKSDD---------------
APG1_Scer/1-302     --------------LVHRDIKPQNLLL-------------STPLIGY-------------
MEK1_Scer/1-284     --------------IVHRDLKLDNILL-------------CTPEP---------------
YKL171W_Scer/1-283  --------------IVHGDIKLENCLL-------------QKEGKKS-------------
CDC5_Scer/1-256     --------------VIHRDLKLGNIFF-------------DSNY----------------
PSK2_Scer/1-259     --------------IVHRDIKDENVIV-------------DSHG----------------
YDL025C_Scer/1-274  --------------LAHRDLKLDNCVV-------------TKDG----------------
SKY1_Scer/1-312     --------------IIHTDIKPENVLM-------------EENLI---------------
YAK1_Scer/1-257     --------------LIHCDLKPENILL-------------CAP-----------------
PTK2_Scer/1-305     --------------IAHRDLKPENVLI-------------SKEG----------------
PHO85_Scer/1-291    --------------ILHRDLKPQNLLI-------------NKR-----------------
CDC28_Scer/1-288    --------------ILHRDLKPQNLLI-------------NKD-----------------
SLT2_Scer/1-296     --------------VLHRDLKPGNLLV-------------NAD-----------------
KSS1_Scer/1-301     --------------VIHRDIKPSNLLL-------------NSN-----------------
FUS3_Scer/1-297     --------------VIHRDLKPSNLLI-------------NSN-----------------
HOG1_Scer/1-280     --------------VIHRDLKPSNILI-------------NEN-----------------
RIM11_Scer/1-284    --------------VCHRDIKPQNLLV-------------DPET----------------
MRK1_Scer/1-284     --------------ICHRDIKPQNLLV-------------DPTT----------------
MCK1_Scer/1-293     --------------VCHRDIKPSNVLV-------------DPET----------------
CKA1_Scer/1-324     --------------IMHRDVKPHNVMI-------------DHKN----------------
CDC7_Scer/1-272     --------------IIHRDIKPTNFLF-------------NLEL----------------
YCK1_Scer/1-261     --------------LIYRDIKPDNFLI-------------GRPG----------------
YCK2_Scer/1-261     --------------LIYRDIKPDNFLI-------------GRPG----------------
YCK3_Scer/1-296     --------------LIYRDIKPDNFLI-------------SQYQRISPEGKVIKSCA---
HRR25_Scer/1-259    --------------FIHRDIKPDNFLM-------------GVGR----------------
PRR1_Scer/1-317     --------------IIHRDLKLENILL-------------KYSFDDIN------------
MPS1_Scer/1-277     --------------IVHSDLKPANFVL-------------VKG-----------------
GCN2_Scer/1-268     --------------IIHRDLKPMNIFI-------------DESR----------------
KIN3_Scer/1-319     IYDRMKPPVKGKNIVIHRDLKPGNIFLSYDDS-D-----YNINEQVDGHEE---VNSNYY
PRK1_Scer/1-277     -------------PLIHRDIKIENVLI-------------SHDG----------------
ARK1_Scer/1-277     -------------PLIHRDIKIENVLI-------------SANN----------------
AKL1_Scer/1-285     -------------SLIHRDIKIENVLV-------------DAKN----------------
VHS1_Scer/1-321     --------------IYHCDIKPENLLL-------------DTED----------------
SKM1_Scer/1-280     -------------KIIHRDIKSDNILL-------------NSQG----------------
CLA4_Scer/1-280     -------------HIIHRDIKSDNVLL-------------DTRA----------------
STE20_Scer/1-252    -------------GVLHRDIKSDNILL-------------SMEG----------------
CDC15_Scer/1-248    -------------GVIHRDIKAANILL-------------SADN----------------
FMP48_Scer/1-285    --------------IAHRDIKPENILL-------------TING----------------
KSP1_Scer/1-260     --------------IYHRDIKPENILI-------------SGIDW---------------
CAK1_Scer/1-304     --------------IIHRDIKPQNIML-------------TNNTSTVS------------
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Table S4, continued

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                   361       370       380       390       400       410
TOS3_Scer/1-295    ----------------------TVKISDFGVAMST-ATGSTNIQ-----------SSHE
TPK3_Scer/1-255    ----------------------HIKITDFGFAKYVP----------------------
TPK1_Scer/1-255    ----------------------HIKITDFGFAKYVP----------------------
TPK2_Scer/1-255    ----------------------HIKITDFGFAKEVQ----------------------
YPK1_Scer/1-256    ----------------------HIALCDFGLCKLNM--------------------KD
YPK2_Scer/1-256    ----------------------HIALCDFGLCKLNM--------------------KD
SCH9_Scer/1-260    ----------------------NIALCDFGLSKADL--------------------KD
YNR047W_Scer/1-282 ----------------------HIMLSDFDLSIQAKDSKVPVVKGSAQSTLVDTKICSD
RIM15_Scer/1-269   ----------------------HVKLTDFGLSRAGLI------------------RRH
IPL1_Scer/1-252    ----------------------VIKLTDFGWSIINP----------------------P
PKH2_Scer/1-265    ----------------------KIKLTDFGTAKLLN--PTNNSVSK--------PEYDL
KCC4_Scer/1-265    ----------------------NIKIADFGMAALQT----------------------D
GIN4_Scer/1-271    ----------------------NIKIADFGMAALET----------------------E
HSL1_Scer/1-289    ----------------------RRIKIADFGMAALEL----------------------P
SNF1_Scer/1-252    ----------------------NVKIADFGLSNIMT----------------------D
KIN1_Scer/1-279    ----------------------EIKIIDFGLSNIYD----------------------S
KIN4_Scer/1-268    ----------------------NLVITDFGFVNEFF--------------------ED
YPL141C_Scer/1-273 ----------------------NLVITDFGFVNEFC--------------------SR
CMK1_Scer/1-263    ----------------------PLVVADFGIAKRLKS--------------------D
CMK2_Scer/1-263    ----------------------PLVIADFGIAKQLKG--------------------E
RAD53_Scer/1-269   ----------------------VLVKITDFGLAKVQG--------------------N
RCK2_Scer/1-316    ---PQTKADEGIFTPGVGGGGIGIVKLADFGLSKQIFS----------------------
APG1_Scer/1-302    -----HDSKSFHELGFVGIYNLPILKIADFGFARFLP--------------------N
MEK1_Scer/1-284    ---------------------CTRIVLADFGIAKDLN--------------------SN
YKL171W_Scer/1-283 ---------------------DWKVFLCDFGMSCHFD--------------------EKH
CDC5_Scer/1-256    ----------------------NLKIGDFGLAAVLA--------------------NE
PSK2_Scer/1-259    ----------------------FVKLIDFGSAAYIKSG----------------------
YDL025C_Scer/1-274 ----------------------ILKLIDFGSAVVFQYP----------------YEDT
SKY1_Scer/1-312    ----------------------QIKIADLGNACWYD----------------------
YAK1_Scer/1-257    --------------------DKPELKIIDFGSSCEEA----------------------
PTK2_Scer/1-305    ----------------------ICKLTDFGISDWYHVIPH--------------DYTSP
PHO85_Scer/1-291   ----------------------GQLKLGDFGLARAFG--------------------IP
CDC28_Scer/1-288   ----------------------GNLKLGDFGLARAFG--------------------VP
SLT2_Scer/1-296    ----------------------CQLKICDFGLARGYSENPVENSQ---------------
KSS1_Scer/1-301    ----------------------CDLKVCDFGLARCLA-SSSDSRE-------------TL
FUS3_Scer/1-297    ----------------------CDLKVCDFGLARIIDESAADNSE-----------PTGQ
HOG1_Scer/1-280    ----------------------CDLKICDFGLARIQD----------------------
RIM11_Scer/1-284   ----------------------WSLKLCDFGSAKQLKP----------------------
MRK1_Scer/1-284    ----------------------FSFKICDFGSAKCLKP----------------------
MCK1_Scer/1-293    ----------------------GVLKICDFGSAKKLEH----------------------
CKA1_Scer/1-324    ----------------------KKLRLIDWGLAEFYHV----------------------
CDC7_Scer/1-272    ----------------------GRGVLVDFGLAEAQMDY-----------------KSTR
YCK1_Scer/1-261    -------------------QPDANNIHLIDFGMAKQYRDPK--------------TKQHIP
YCK2_Scer/1-261    -------------------QPDANKVHLIDFGMAKQYRDPK--------------TKQHIP
YCK3_Scer/1-296    ----------------SSSNNDPNLIYMVDFGMAKQYRDPR--------------TKQHIP
HRR25_Scer/1-259   --------------------RGSTVHVIDFGLSKKYRDFN--------------THRHIP
PRR1_Scer/1-317    -----------SFRDSPIYCKQNFIELADFGLCKKIE--------------------N
MPS1_Scer/1-277    ----------------------ILKIIDFGIANAVP--------------------EHTV
GCN2_Scer/1-268    ----------------------NVKIGDFGLAKNVHRSLDILK-------LDSQNLPGS
KIN3_Scer/1-319    RDHRVNSGKRGSPMDYSQV----VVKLGDFGLAKSLE--------------------TS
PRK1_Scer/1-277    ----------------------LYKVCDFGSVSGVIRPPR------------NTQEFNY
ARK1_Scer/1-277    ----------------------EYKLCDFGSVCGIIRPPR------------NSQELSY
AKL1_Scer/1-285    ----------------------NFKLADFGSTSTCFPIVT------------THQDIAL
VHS1_Scer/1-321    ----------------------NVFLCDFGLSTTST----------------------
SKM1_Scer/1-280    ----------------------LVKITDFGFCVELT--------------------EK
CLA4_Scer/1-280    ----------------------RVKITDFGFCARLT--------------------DK
STE20_Scer/1-252   ----------------------DIKLTDFGFCAQIN--------------------EL
CDC15_Scer/1-248   ----------------------TVKLADFGVSTIVN----------------------
FMP48_Scer/1-285   ----------------------DIKLADWGHAIQSP----------------------
KSP1_Scer/1-260    ----------------------TIKLTDWGLATTDK----------------------
CAK1_Scer/1-304    ----------------------PKLYIIDFGISYDMANN-------------SQTSAEP
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Table S4, continued

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                    421       430       440       450       460       470
TOS3_Scer/1-295     QLLKSRALGTPAFFAPELCSTEKEYS--------------CSSAIDIWSLGVTIYCLLF-
TPK3_Scer/1-255     -DVTYTLCGTPDYIAPEVVST-----------------KPYNKSVDWWSFGVLIYEMLA-
TPK1_Scer/1-255     -DVTYTLCGTPDYIAPEVVST-----------------KPYNKSIDWWSFGILIYEMLA-
TPK2_Scer/1-255     -TVTWTLCGTPDYIAPEVITT-----------------KPYNKSVDWWSLGVLIYEMLA-
YPK1_Scer/1-256     DDKTDTFCGTPEYLAPELLLG-----------------LGYTKAVDWWTLGVLLYEMLT-
YPK2_Scer/1-256     NDKTDTFCGTPEYLAPEILLG-----------------QGYTKTVDWWTLGILLYEMMT-
SCH9_Scer/1-260     --RTNTFCGTTEYLAPELLLDE----------------TGYTKMVDFWSLGVLIFEMCC-
YNR047W_Scer/1-282 GFRTNSFVGTEEYIAPEVIRG----------------NGHTAAVDWWTLGILIYEMLF-
RIM15_Scer/1-269    KKQNKKFFGTPDYLAPETIEGK---------------GEDNKQCDWWSVGCIFFELLL-
IPL1_Scer/1-252     ENRRKTVCGTIDYLSPEMVES----------------REYDHTIDAWALGVLAFELLT-
PKH2_Scer/1-265     STRSKSFVGTAEYVSPELLND----------------SFTDYRCDIWAFGCILFQMIA-
KCC4_Scer/1-265     ADLLETSCGSPHYAAPEIVSG---------------LPYEGFASDVWSCGVILFALLT-
GIN4_Scer/1-271     GKLLETSCGSPHYAAPEIVSG---------------IPYQGFASDVWSCGVILFALLT-
HSL1_Scer/1-289     NKLLKTSCGSPHYASPEIVMG---------------RPYHGGPSDVWSCGIVLFALLT-
SNF1_Scer/1-252     GNFLKTSCGSPNYAAPEVISG---------------KLYAGPEVDVWSCGVILYVMLC-
KIN1_Scer/1-279     RKQLHTFCGSLYFAAPELLKA---------------NPYTGPEVDVWSFGVVLFVLVC-
KIN4_Scer/1-268     NELMKTSCGSPCYAAPELVVST--------------KAYEARKADVWSCGVILYAMLA-
YPL141C_Scer/1-273 NELMKTSCGSPCYAAPELVISA--------------EPYEARKADIWSCGVILYAILA-
CMK1_Scer/1-263     EELLYKPAGSLGYVAPEVLT----------------QDGHGKPCDIWSIGVITYTLLC-
CMK2_Scer/1-263     EDLIYKAAGSLGYVAPEVLT----------------QDGHGKPCDIWSIGVITYTLLC-
RAD53_Scer/1-269    GSFMKTFCGTLAYVAPEVIRGKDTSVSPDEYEE----RNEYSSLVDMWSMGCLVYVILT-
RCK2_Scer/1-316     -KNTKTPCGTVGYTAPEVVKD----------------EHYSMKVDMWGIGCVLYTMLC-
APG1_Scer/1-302     TSLAETLCGSPLYMAPEILN----------------YQKYNAKADLWSVGTVVFEMCC-
MEK1_Scer/1-284     KERMHTVVGTPEYCAPEVGFRANRKAYQSFSRAATLEQRGYDSKCDLWSLGVITHIMLT-
YKL171W_Scer/1-283 EPEPSKYIGSLPYASPELLDFP-------------CIVSPLGPASDIWALGVMLYTMLV-
CDC5_Scer/1-256     SERKYTICGTPNYIAPEVLMGK--------------HSGHSFEVDIWSLGVMLYALLI-
PSK2_Scer/1-259     --PFDVFVGTMDYAAPEVLGGSS----------------YKGKPQDIWALGVLLYTIIY-
YDL025C_Scer/1-274 IVKSHGIVGSDPYLAPELLKQT----------------SYDPRVADVWSIAIIFYCMVL-
SKY1_Scer/1-312     -EHYTNSIQTREYRSPEVLLG----------------APWGCGADIWSTACLIFELIT-
YAK1_Scer/1-257     -RTVYTYIQSRFYRAPEIILG----------------IPYSTSIDMWSLGCIVAELFL-
PTK2_Scer/1-305     VKTCQGMIGSPPYTPPEVMYFDAKKHYPEK-----FQKPYNPLAMDSYALGIMLITMIN-
PHO85_Scer/1-291    VNTFSSEVVTLWYRAPDVLMGS---------------RTYSTSIDIWSCGCILAEMIT-
CDC28_Scer/1-288    LRAYTHEIVTLWYRAPEVLLGG---------------KQYSTGVDTWSIGCIFAEMCN-
SLT2_Scer/1-296     --FLTEYVATRWYRAPEIMLSY---------------QGYTKAIDVWSAGCILAEFLG-
KSS1_Scer/1-301     VGFMTEYVATRWYRAPEIMLTF---------------QEYTTAMDIWSCGCILAEMVS-
FUS3_Scer/1-297     QSGMTEYVATRWYRAPEVMLTS---------------AKYSRAMDVWSCGCILAELFL-
HOG1_Scer/1-280     -PQMTGYVSTRYYRAPEIMLTW---------------QKYDVEVDIWSAGCIFAEMIE-
RIM11_Scer/1-284    TEPNVSYICSRYYRAPELIFGA---------------TNYTNQIDIWSSGCVMAELLL-
MRK1_Scer/1-284     DQPNVSYICSRYYRAPELMFGA---------------TNYSNQVDVWSSACVIAELLL-
MCK1_Scer/1-293     NQPSISYICSRFYRAPELIIGC---------------TQYTTQIDIWGLGCVMGEMLI-
CKA1_Scer/1-324     NMEYNVRVASRFFKGPELLVDY---------------RMYDYSLDLWSFGTMLASMIF-
CDC7_Scer/1-272     RIKRANRAGTRGFRAPEVLMKC---------------GAQSTKIDIWSVGVILLSLLG-
YCK1_Scer/1-261     YREKKSLSGTARYMSINTHLG----------------REQSRRDDMEALGHVFFYFLR-
YCK2_Scer/1-261     YREKKSLSGTARYMSINTHLG----------------REQSRRDDMEAMGHVFFYFLR-
YCK3_Scer/1-296     YRERKSLSGTARYMSINTHFG----------------REQSRRDDLESLGHVFFYFLR-
HRR25_Scer/1-259    YRENKSLTGTARYASVNTHLG----------------IEQSRRDDLESLGYVLIYFCK-
PRR1_Scer/1-317     NEMCTARCGSEDYVSPEILMGVP---------------YDGHLSDTWALGVILYSLFE-
MPS1_Scer/1-277     NIYRETQIGTPNYMAPEALVAMNYTQNSENQHEG--NKWKVGRPSDMWSCGCIIYQMIY-
GCN2_Scer/1-268     SDNLTSAIGTAMYVATEVLDGTG-------------HYNEKI---DMYSLGIIFFEMIY-
KIN3_Scer/1-319     IQFATTYVGTPYYMSPEVLMDQP----------------YSPLSDIWSLGCVIFEMCS-
PRK1_Scer/1-277     VQHDILTNTTAQYRSPEMIDLYR-------------GLPIDEKS-DIWALGVFLYKICY-
ARK1_Scer/1-277     VQQDILKNTTAQYRSPEMIDTFR-------------GLPIDEKS-DIWALGIFLYKLCY-
AKL1_Scer/1-285     LTQNIYVHTTPQYRSPEMIDLYR-------------CLPINEKS-DIWALGVFLYKLLF-
VHS1_Scer/1-321     YIKPNVCIGSSYYMPPERISFDGRVSSSKSGG-HKLGKVCPSCNGDLWSLGIILINLTC-
SKM1_Scer/1-280     RSKRATMVGTPYWMAPEIVN----------------QKGYDEKVDVWSLGIMLIEMIE-
CLA4_Scer/1-280     RSKRATMVGTPYWMAPEVVK----------------QREYDEKIDVWSLGIMTIEMLE-
STE20_Scer/1-252    NLKRTTMVGTPYWMAPEVVS----------------RKEYGPKVDIWSLGIMIIEMIE-
CDC15_Scer/1-248    -SSALTLAGTLNWMAPEILG----------------NRGASTLSDIWSLGATVVEMLT-
FMP48_Scer/1-285    -KSNDFQIGTDNYRAPETFSGRVSNSCFKKNFDRSSAPLYNTYQADYWSLGATIFYLMF-
KSP1_Scer/1-260     -TSMDRNVGSERYMSPELFDSNLDIKER--------KEPYDCAKVDLWAMGIVFLNIVF-
CAK1_Scer/1-304     MDSKVTDISTGIYKAPEVLFG----------------VKCYDGGVDVWSLLIIISQWFQR
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Table S4, continued

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                    481       490       500       510       520       530
TOS3_Scer/1-295     -----GKLPFNANS--------GLELFDSIINKPLEFPSY--------------------
TPK3_Scer/1-255     -----GYTPFYNSN--------TMKTYENILNAELKFP----------------------
TPK1_Scer/1-255     -----GYTPFYDSN--------TMKTYEKILNAELRFP----------------------
TPK2_Scer/1-255     -----GYTPFYDTT--------PMKTYEKILQGKVVYP----------------------
YPK1_Scer/1-256     -----GLPPYYDED--------VPKMYKKILQEPLVFP----------------------
YPK2_Scer/1-256     -----GLPPYYDEN--------VPVMYKKILQQPLLFP----------------------
SCH9_Scer/1-260     -----GWSPFFAEN--------NQKMYQKIAFGKVKFPR---------------------
YNR047W_Scer/1-282 -----GFTPFKGDN--------TNETFTNILKNEVSFPNN---------------------
RIM15_Scer/1-269    -----GYPPFHAET--------PDAVFKKILSGVIQWPEFK--------------------
IPL1_Scer/1-252     -----GAPPFEEEM--------KDTTYKRIAALDIKMP----------------------
PKH2_Scer/1-265     -----GKPPFKATN--------EYLTFQKVMKVQYAFTPG--------------------
KCC4_Scer/1-265     -----GRLPFDEEN------GNVRDLLLKVQKGQF-------------------------
GIN4_Scer/1-271     -----GRLPFDEED------GNIRTLLLKVQKGEF-------------------------
HSL1_Scer/1-289     -----GHLPFNDD--------NIKKLLLKVQSGKY-------------------------
SNF1_Scer/1-252     -----RRLPFDDE--------SIPVLFKNISNGVY-------------------------
KIN1_Scer/1-279     -----GKVPFDDE--------NSSVLHEKIKQGKV-------------------------
KIN4_Scer/1-268     -----GYLPWDDDHEN-PTGDDIARLYKYITQTPL-------------------------
YPL141C_Scer/1-273 -----GYLPWDDDPNN-PEGSDIGRLYNYINSTPL-------------------------
CMK1_Scer/1-263     -----GYSAFRAER--------VQDFLDECTTGEYPV----------------------
CMK2_Scer/1-263     -----GYSPFIAES--------VEGFMEECTASRYPV----------------------
RAD53_Scer/1-269    -----GHLPFSGST--------QDQLYKQIGRGSY-------------------------
RCK2_Scer/1-316     -----GFPPFYDEK--------IDTLTEKISRGEY-------------------------
APG1_Scer/1-302     -----GTPPFRASN--------HLELFKKIKRAND-------------------------
MEK1_Scer/1-284     -----GISPFYGDG--------SERSIIQNAKIGKLN-----------------------
YKL171W_Scer/1-283 -----GKLPFNHEF--------EPRLRSLIKVGEFDRFS--------------------
CDC5_Scer/1-256     -----GKPPFQARD--------VNTIYERIKCR---------------------------
PSK2_Scer/1-259     -----KENPYYNI----------DEILEGELRFDKS------------------------
YDL025C_Scer/1-274 -----KRFPWKAP----------KKSFNSFRLFT----------------EEPEDED---
SKY1_Scer/1-312     -----GDFLFEPDEGHS--YTKDDDHIAQIIELLGELPSYLLRNGKYTR-----------
YAK1_Scer/1-257     -----GIPIFPGAS--------EYNQLTRIIDTLGYP-----------------------
PTK2_Scer/1-305     -----NIIPFIDS-----------CNTDARFREFEVSYDNFINHQNP----HFRDKGCHK
PHO85_Scer/1-291    -----GKPLFPGTN--------DEEQLKLIFDIMGTP--NESLWPSVTK-----------
CDC28_Scer/1-288    -----RKPIFSGDS--------EIDQIFKIFRVLGTP--NEAIWPDIVY-----------
SLT2_Scer/1-296     -----GKPIFKGKD--------YVNQLNQILQVLGTP-PDETLRRIGSK-----------
KSS1_Scer/1-301     -----GKPLFPGRD--------YHHQLWLILEVLGTP-SFEDFNQIKSK-----------
FUS3_Scer/1-297     -----RRPIFPGRD--------YRHQLLLIFGIIGTPHSDNDLRCIESP-----------
HOG1_Scer/1-280     -----GKPLFPGKD--------HVHQFSIITDLLGSP-PKDVINTICSE-----------
RIM11_Scer/1-284    -----GQPMFPGES--------GIDQLVEIIKILGTP-SKQEICSMNPN-----------
MRK1_Scer/1-284     -----GKPLFSGES--------GIDQLVEIIKIMGIP-TKDEISGMNPN-----------
MCK1_Scer/1-293     -----GKAIFQGQE--------PLLQLREIAKLLGPP-DKRFIFFSNPA-----------
CKA1_Scer/1-324     -----KREPFFHGTS-------NTDQLVKIVKVLGTSDFEKYLLKYEIT-----------
CDC7_Scer/1-272     -----RRFPMFQSLD-------DADSLLELCTIFGWKELRKCAALHGLGFEASGLID---
YCK1_Scer/1-261     -----GHLPWQGLKAP------NNKQKYEKIG-----------------------------
YCK2_Scer/1-261     -----GQLPWQGLKAP------NNKQKYEKIG-----------------------------
YCK3_Scer/1-296     -----GSLPWQGLKAP------NNKLKYEKIG-----------------------------
HRR25_Scer/1-259    -----GSLPWQGLKAT------TKKQKYDRIM-----------------------------
PRR1_Scer/1-317     -----DRLPFDPPPNAS-----ARQRSRATSHRIARFDWR--------------------
MPS1_Scer/1-277     -----GKPPYGSFQ--------GQNRLLAIMNPDVKIPFPEHTS----------------
GCN2_Scer/1-268     --------PFST----------GMERVNILKKLRSVSIE----------------------
KIN3_Scer/1-319     -----LHPPFQAKN--------YLELQTKIKNG----------------------------
PRK1_Scer/1-277     -----YTTPFEKS------GEAGILHARYQYPSFP--------------------------
ARK1_Scer/1-277     -----YTTPFEKG------GDLAILSGKFEFPLYP--------------------------
AKL1_Scer/1-285     -----FTTPFEMT------GQFAILHSKYEFPVN---------------------------
VHS1_Scer/1-321     -----IRNPWLKA---------DKTEDNTYYYFTKDPN-----------------------
SKM1_Scer/1-280     -----GEPPYLN--------EDPLKALYLIANN----------------------------
CLA4_Scer/1-280     -----GEPPYLN--------EDPLKALYLIATN----------------------------
STE20_Scer/1-252    -----GEPPYLN--------ETPLRALYLIATN----------------------------
CDC15_Scer/1-248    -----KNPPYHN--------LTDANIYYAVEN----------------------------
FMP48_Scer/1-285    -----GDCLFRVSKSK-------KVQHLKNFDEFEKD-----------------------
KSP1_Scer/1-260     -----HKNPFSIAN--------QSDKSFCYFAANREALFD--------------------
CAK1_Scer/1-304     ETSRMGHVPAMIDDGS--------DDMNSDGSDFRLICS---------------------
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Table S4, continued

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                    541       550       560       570       580       590
TOS3_Scer/1-295     -------------------------EEMLNGATSGIT-MEEYTDAKDLLKKLLQKDPDKR
TPK3_Scer/1-255     ------------------------------------PFFHPDAQDLLKKLITRDLSER
TPK1_Scer/1-255     ------------------------------------PFFNEDVKDLLSRLITRDLSQR
TPK2_Scer/1-255     ------------------------------------PYFHPDVVDLLSKLITADLTRR
YPK1_Scer/1-256     ------------------------------------DGFDRDAKDLLIGLLSRDPTRR
YPK2_Scer/1-256     ------------------------------------DGFDPAAKDLLIGLLSRDPSRR
SCH9_Scer/1-260     ------------------------------------DVLSQEGRSFVKGLLNRNPKHR
YNR047W_Scer/1-282  ------------------------------------NEISRTCKDLIKKLLTKNESKR
RIM15_Scer/1-269    ---------------------------------NEEEEREFLTPEAKDLIEKLLVVDPAKR
IPL1_Scer/1-252     ------------------------------------SNISQDAQDLILKLLKYDPKDR
PKH2_Scer/1-265     -----------------------------------FPLIIRDLVKKILVKNLDRR
KCC4_Scer/1-265     ---------------------------------EMPNDTEISRDAQDLIGKILVVDPRQR
GIN4_Scer/1-271     ---------------------------------EMPSDDEISREAQDLIRKILTVDPERR
HSL1_Scer/1-289     ---------------------------------QMPSN--LSSEARDLISKILVIDPEKR
SNF1_Scer/1-252     ---------------------------------TLPKF--LSPGAAGLIKRMLIVNPLNR
KIN1_Scer/1-279     ---------------------------------EYPQH--LSIEVISLLSKMLVVDPKRR
KIN4_Scer/1-268     ---------------------------------KFPEY--ITPIPRDLLRRILVPNPRRR
YPL141C_Scer/1-273  ---------------------------------KFPDY--ILPIPRDLLRRMLVSDPKKR
CMK1_Scer/1-263     --------------------------------KFHRPYWDSVSNKAKQFILKALNLDPSKR
CMK2_Scer/1-263     --------------------------------TFHMPYWDNISIDVKRFILKALRLNPADR
RAD53_Scer/1-269    --------------------------------HEGPLKDFRISEEARDFIDSLLQVDPNNR
RCK2_Scer/1-316     --------------------------------TFLKPWWDEISAGAKNAVAKLLELEPSKR
APG1_Scer/1-302     --------------------------------VITFPSYCNIEPELKELICSLLTFDPAQR
MEK1_Scer/1-284     --------------------------------FKLKQWDIVSDNAKSFVKDLLQTDVVKR
YKL171W_Scer/1-283  -------------------------LAQVCKFDRKKNEGTIGQGLYDTVIGCLTIDLDK
CDC5_Scer/1-256     ------------------------------DFSFPRDKPISDEGKILIRDILSLDPIER
PSK2_Scer/1-259     ------------------------------------EHVSEECISLIKRILTREVDKR
YDL025C_Scer/1-274  ----------DIVRG-------------------PNKILRLLPRHSRTIIGRMLALEPKQR
SKY1_Scer/1-312     ------TFFNSRGLLRNISKLKFWPLEDVLTE-KYKFSKDEAKEISDFLSPMLQLDPRKR
YAK1_Scer/1-257     ------------------------PSWMIR------------ECLIHFLGGVLNLNPLER
PTK2_Scer/1-305     PGPGSE----------------------------YSLARNFKNTDATRIAWRLADPNPATR
PHO85_Scer/1-291    --------LPKYNPNI-QQR--PPRDLRQVLQPHT--KEPLDGNLMDFLHGLLQLNPDMR
CDC28_Scer/1-288    --------LPDFKPSF-PQW--RRKDLSQVVP-------SLDPRGIDLLDKLLAYDPINR
SLT2_Scer/1-296     --------NVQDY--IHQLGFIPKVPFVNLYPN-------ANSQALDLLEQMLAFDPQKR
KSS1_Scer/1-301     --------RAKEY--IANLPMRPPLPWETVWSKT-----DLNPDMIDLLDKMLQFNPDKR
FUS3_Scer/1-297     --------RAREY--IKSLPMYPAAPLEKMFPR-------VNPKGIDLLQRMLVFDPAKR
HOG1_Scer/1-280     --------NTLKF--VTSLPHRDPIPFSERFKT-------VEPDAVDLLEKMLVFDPKKR
RIM11_Scer/1-284    --------YMEH------KFPQIKPIPLSRVFKK------EDDQTVEFLADVLKYDPLER
MRK1_Scer/1-284     --------YEDH-----VFPNIKPITLAEIFKA-------EDPDTLDLLTKTLKYHPCER
MCK1_Scer/1-293     --------YDGPLFSKPLFSGSSQQRFEKYFGH-------SGPDGIDLLMKILVYEPQQR
CKA1_Scer/1-324     --------LPREFYD---MDQYIRKPWHRFIN-DGNKHLSGNDEIIDLIDNLLRYDHQER
CDC7_Scer/1-272     --------KPNGYSNG--LKEFVYDLLNKECTIGTFPE---HYWCFQVLEQCFEMDPQKR
YCK1_Scer/1-261     ----------------------EKKRSTNVY----------DLAQG--LPVQFGRYLEIV
YCK2_Scer/1-261     ----------------------EKKRLTNVY----------DLAQG--LPIQFGRYLEIV
YCK3_Scer/1-296     ----------------------MTKQKLNPD----------DLLLNNAIPYQFATYLKYA
HRR25_Scer/1-259    ----------------------EKKLNVSVE----------TLCSG--LPLEFQEYMAYC
PRR1_Scer/1-317     ------------------------------------WYRLSDYKTNVGKQIVENTLTRK
MPS1_Scer/1-277     -----------------------------------NNEKIPKSAIELMKACLYRNPDKR
GCN2_Scer/1-268     ----------------------FPPDFDDN----------KMKVEKKIIRLLIDHDPNKR
KIN3_Scer/1-319     ----------------------KCDTVPEY----------YSRGLNAIIHSMIDVNLRTR
PRK1_Scer/1-277     ------------------------------------QYSDRLKNLIRLMLMEAPSQR
ARK1_Scer/1-277     -----------------------------------NYSEQLKGLIRDILVQDPRHR
AKL1_Scer/1-285     -----------------------------------KYSSKLINLIIIMLAENPNLR
VHS1_Scer/1-321     ---------------------------------ILKQILPLSDDFYSLLSKILQVNPKNR
SKM1_Scer/1-280     ----------------------GSPKLRHPE--------SVSKQTKQFLDACLQVNVESR
CLA4_Scer/1-280     ----------------------GTPKLKHPE--------SLSLEIKRFLSVCLCVDVRYR
STE20_Scer/1-252    ----------------------GTPKLKEPE--------NLSSSLKKFLDWCLCVEPEDR
CDC15_Scer/1-248    ------------------------DTYYPPS--------SFSEPLKDFLSKCFVKNMYKR
FMP48_Scer/1-285    --------------------------PFAFIYRKYVVPRLSCGYNDEEDLHVSLQHTR
KSP1_Scer/1-260     ---------------------------------VFSTMAYDFFQVLRYSLTIDPANRD
CAK1_Scer/1-304     -----------------------------------IFEKVKQKFINCILGMVSFSPNER
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Table S4, continued

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                    601      610       620
TOS3_Scer/1-295     IK-----LADIKVHPFM-------
TPK3_Scer/1-255     LGNLQNGSEDVKNHPWF-------
TPK1_Scer/1-255     LGNLQNGTEDVKNHPWF-------
TPK2_Scer/1-255     IGNLQSGSRDIKAHPWF-------
YPK1_Scer/1-256     LG--YNGADEIRNHPFF-------
YPK2_Scer/1-256     LG--VNGTDEIRNHPFF-------
SCH9_Scer/1-260     LGA-IDDGRELRAHPFF-------
YNR047W_Scer/1-282 LGC-KMGAADVKKHPFF-------
RIM15_Scer/1-269    LG--AKGIQEIKDHPYF-------
IPL1_Scer/1-252     MR-----LGDVKMHPWI-------
PKH2_Scer/1-265     LT-----ISQIKEHHFF-------
KCC4_Scer/1-265     IK-----IRDILSHPLL-------
GIN4_Scer/1-271     IK-----TRDILKHPLL-------
HSL1_Scer/1-289     IT-----TQEILKHPLI-------
SNF1_Scer/1-252     IS-----IHEIMQDDWF-------
KIN1_Scer/1-279     AT-----LKQVVEHHWM-------
KIN4_Scer/1-268     IN-----LQTIKRHVWL-------
YPL141C_Scer/1-273 IN-----LKQIKKHEWL-------
CMK1_Scer/1-263     PT-----AAELLEDPWI-------
CMK2_Scer/1-263     PT-----ATELLDDPWI-------
RAD53_Scer/1-269    ST-----AAKALNHPWI-------
RCK2_Scer/1-316     YD-----IDQFLDDPWL-------
APG1_Scer/1-302     IG-----FEEFFANKVV-------
MEK1_Scer/1-284     LN-----SKQGLKHIWI-------
YKL171W_Scer/1-283 RWK-----LKRIEEVL--------
CDC5_Scer/1-256     PS-----LTEIMDYVWF-------
PSK2_Scer/1-259     PT-----IDEIYEDKWL-------
YDL025C_Scer/1-274 VL-----MNDVVKDDWL-------
SKY1_Scer/1-312     AD-----AGGLVNHPWL-------
YAK1_Scer/1-257     WT-----PQQAMLHPFI-------
PTK2_Scer/1-305     YT-----MDDLFNDPFF-------
PHO85_Scer/1-291    LS-----AKQALHHPWF-------
CDC28_Scer/1-288    IS-----ARRAAIHPYF-------
SLT2_Scer/1-296     IT-----VDEALEHPYL-------
KSS1_Scer/1-301     IS-----AAEALRHPYL-------
FUS3_Scer/1-297     IT-----AKEALEHPYL-------
HOG1_Scer/1-280     IT-----AADALAHPYS-------
RIM11_Scer/1-284    FN-----ALQCLCSPYF-------
MRK1_Scer/1-284     LV----PLQCLLSSYF--------
MCK1_Scer/1-293     LS-----PRRILAHQFF-------
CKA1_Scer/1-324     LT-----AKEAMGHPWF-------
CDC7_Scer/1-272     SS-----AEDLLKTPFF-------
YCK1_Scer/1-261     RS-----LSFEECPDYEGYRKLLL
YCK2_Scer/1-261     RN-----LSFEETPDYEGYRMLLL
YCK3_Scer/1-296     RS-----LKFDEDPDYD----YLI
HRR25_Scer/1-259    KN-----LKFDEKPDYLFLARLFK
PRR1_Scer/1-317     NQR--WSINEIYESPFV-------
MPS1_Scer/1-277     WT-----VDKVLSSTFL-------
GCN2_Scer/1-268     PG-----ARTLLNSGWL-------
KIN3_Scer/1-319     PS-----TFELLQDIQI-------
PRK1_Scer/1-277     PN-----ICQVLEEVSRL------
ARK1_Scer/1-277     PN-----VYQLLKRISI-------
AKL1_Scer/1-285     PN-----IYQVLYHLCE-------
VHS1_Scer/1-321     MS-----LQELMKEVSS-------
SKM1_Scer/1-280     AS-----VRKLLTFEFL-------
CLA4_Scer/1-280     AS-----TEELLHHGFF-------
STE20_Scer/1-252    AS-----ATELLHDEYI-------
CDC15_Scer/1-248    PT-----ADQLLKHVWI-------
FMP48_Scer/1-285    QY-----IWQDLPDIYD-------
KSP1_Scer/1-260     LK--MMRTELQNLSEYT-------
CAK1_Scer/1-304     WS-----CQRILQELE--------
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