

## 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'-GAAACGGAACCAAAAGCACAACTTTATACA-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 *MATα prk1-Δ::KANMX6* strains were then mated to a *MATa 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, *MATα 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<sub>6</sub>-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<sub>600</sub> 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<sub>6</sub>-tagged kinases*

To affinity purify GST-His<sub>6</sub>-tagged kinases from yeast, crude extracts were mixed with 500  $\mu$ l of washed glutathione sepharose (GE Healthcare) resuspended in 4 ml of lysis buffer (without added NaCl) and incubated with agitation for 2 hr at 4°C. 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<sub>2</sub> (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  $\mu$ l of kinase buffer containing 30 mM reduced GSH (15 min, 4°C with agitation). Eluate was separated from beads by centrifugation through a 3  $\mu$ m polycarbonate filter plate (5 min at 1,900 X g). The eluted kinase was snap frozen and stored at -80°C.

#### *Affinity purification of TAP-tagged kinases*

To affinity purify TAP-tagged kinases from yeast, crude extracts were mixed with 500  $\mu$ l of washed IgG sepharose (GE Healthcare) resuspended in 4 ml of lysis buffer (without added NaCl) and incubated with agitation for 2 hr at 4°C. 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<sub>2</sub> (above). To elute bound kinase, beads resuspended in 400  $\mu$ l of kinase buffer were incubated with 15  $\mu$ l of GST-3C protease (expressed and purified as described (1)) on a nutator overnight at 4°C. Cleaved kinase was separated from beads by centrifugation through a 3  $\mu$ m polycarbonate filter plate as described above. GST-3C protease was removed by mixing the flow-through with 250  $\mu$ l of washed glutathione sepharose (1 hr, 4°C). 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 -80°C.

#### *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  $\beta$ -glycerophosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 10  $\mu$ g/ml leupeptin, 2  $\mu$ g/ml pepstatin A, 10  $\mu$ g/ml aprotinin), 250  $\mu$ l per 6 cm plate. Lysates were cleared by centrifugation (10 min at 16,000 X g, 4°C), and incubated with glutathione sepharose (8  $\mu$ l per plate) for 2 hr at 4°C. Beads were pelleted and washed twice with 293 lysis buffer, twice with GSH wash buffer (50 mM HEPES, pH 7.4, 5 mM  $\beta$ -glycerophosphate, 2 mM DTT, 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, 10 mM MgCl<sub>2</sub>), and then eluted with two rounds GSH wash buffer containing 20 mM reduced GSH and 10% glycerol (10  $\mu$ l per plate each round for 30 min on ice). Eluted kinases were snap frozen in aliquots and stored at -80°C.

### *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<sub>6</sub> 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<sub>2</sub>, 1 mM DTT, 1 mM ATP for 30 min at 30°C 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 30°C with 0.8 µg each of MBP and histone H1 in the presence of 100 µM ATP, 0.25 µCi γ-[<sup>33</sup>P]-ATP (Amersham), and 10 mM MgCl<sub>2</sub> 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 95°C. 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-[<sup>33</sup>P]-ATP (1 µCi per 25 µl reaction) (10 - 15 min, 30°C). 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 30°C in the presence of 100 µM ATP, 0.25 µCi

$\gamma$ -[<sup>33</sup>P]-ATP (Amersham), and 10 mM MgCl<sub>2</sub>. The total volume of each reaction was 10  $\mu$ l. Reactions were stopped by adding 3X SDS-PAGE sample loading buffer (5  $\mu$ l) followed by heating for 4 min at 95°C. Proteins were fractionated by SDS-PAGE, and gels were dried and exposed to autoradiography film.

#### *Electrophoretic mobility shift analyses*

1.8 x 10<sup>7</sup> cells grown in YPAD to OD<sub>600</sub> 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  $\lambda$  protein phosphatase ( $\lambda$ -PPase) reaction buffer [New England Biolabs (NEB)], and resuspended in the same buffer (30  $\mu$ l). 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  $\lambda$ -PPase (NEB) for 30 min at 30°C. 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).

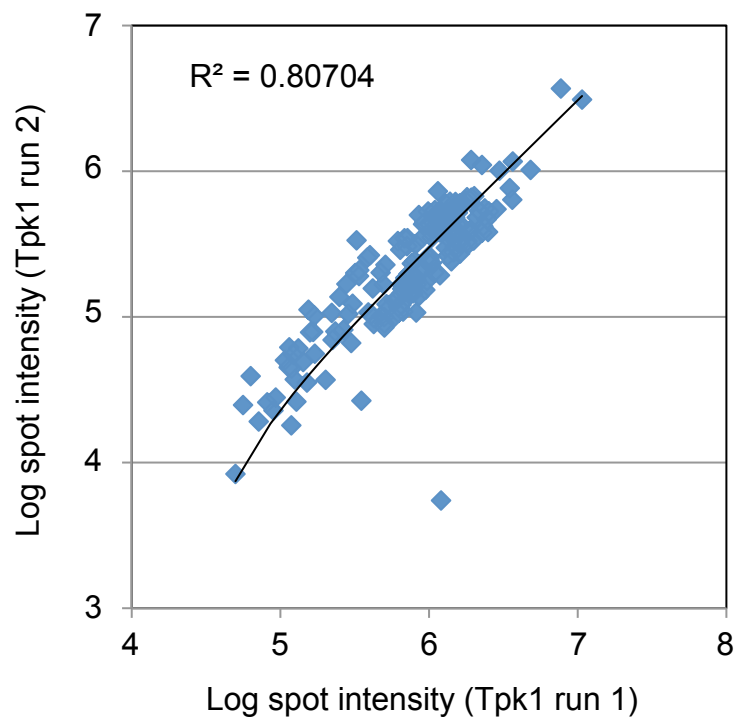
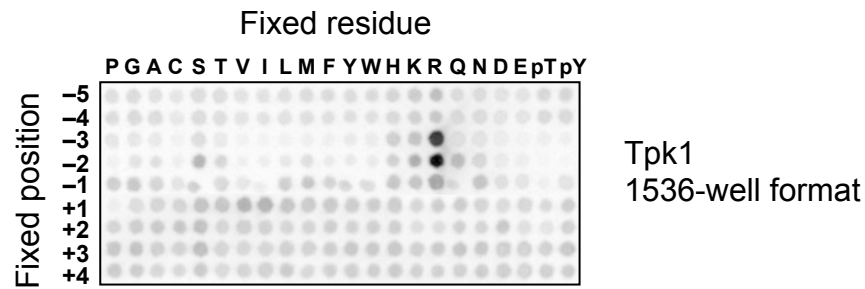
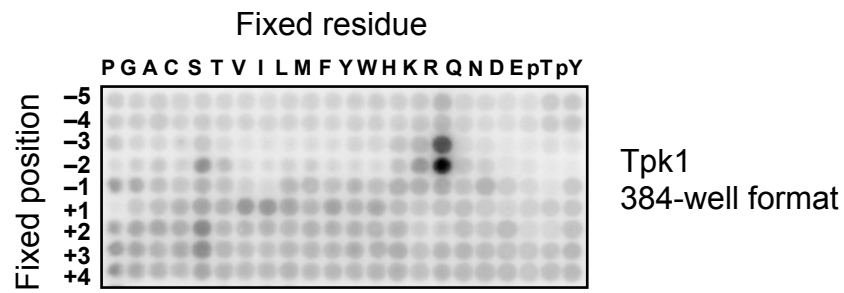
Electrophoretic mobility of Sol2 was determined from crude lysates. Cell pellets from WT Sol2-TAP and *vhs1* $\Delta$  Sol2-TAP strains were lysed directly in 250  $\mu$ l 1X SDS loading buffer (NEB) using the FastPrep®-24 System (6 m/s for 20 sec, MP Biomedicals) with 250  $\mu$ l of 0.5 mm acid washed glass beads (Biospec). The resulting crude extracts were heated for 4 min at 95°C 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**

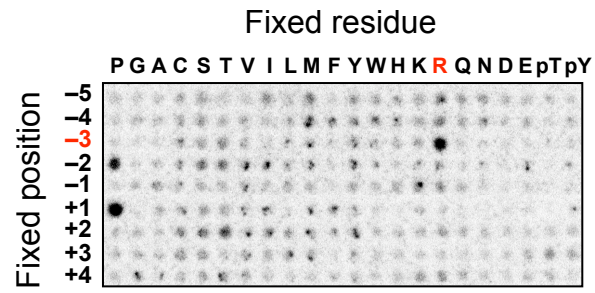
Dataset S1. Tab delimited files containing average PWMs for each of the 61 kinases assayed. PWM format follows spot array design where each row represents which position relative to the targeted phosphoacceptor site is fixed and each column represents which amino acid is fixed at that position. Spots 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.

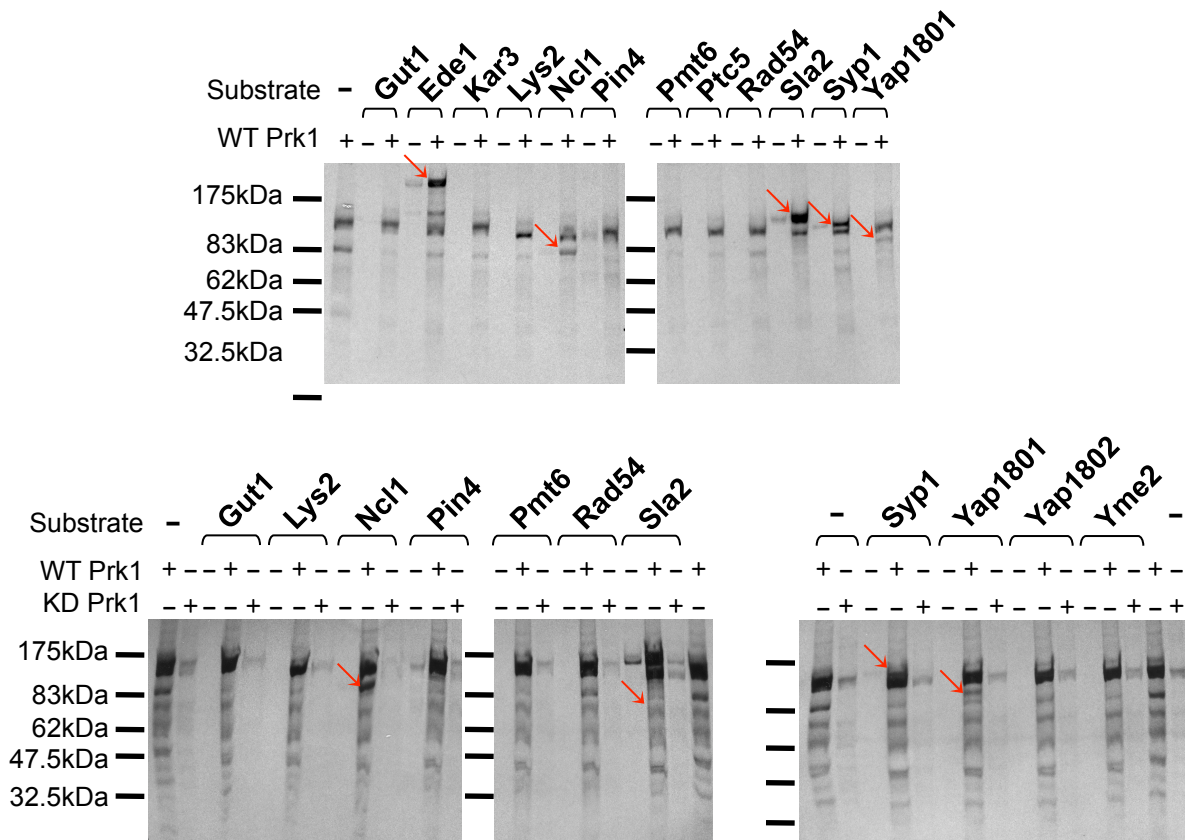


**Figure S1.** Assay reproducibility. Tpk1 was assayed using both the 384-well plate format originally reported and the miniaturized 1536-well format. The graph at bottom shows the correlation between spot intensities corresponding to each peptide for two runs with Tpk1 using the miniaturized 1536-well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



**Kss1 S147E**

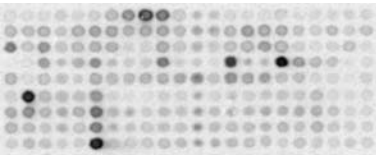


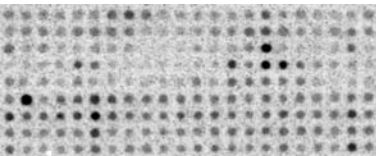


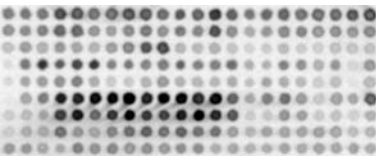


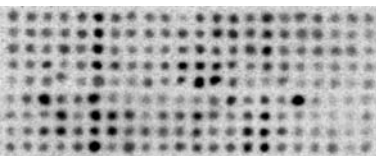

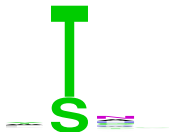
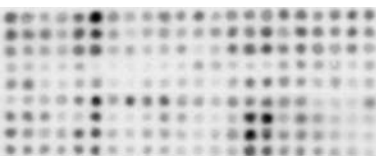


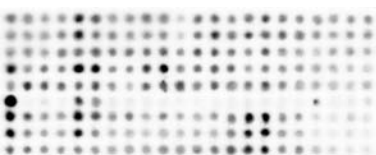

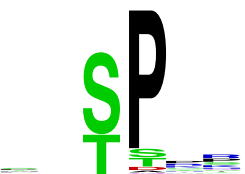
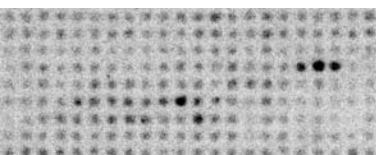


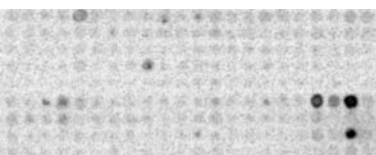


**Figure S2.** Representative peptide array screening results for the Kss1 S147E mutant.



**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  $\mu$ l reactions. Red arrows point to validated substrates.



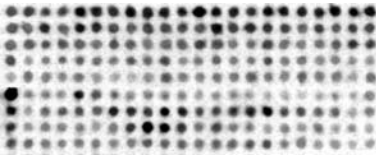


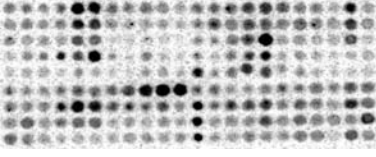


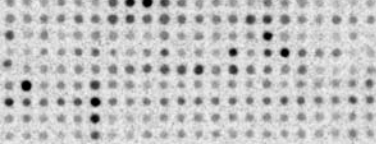





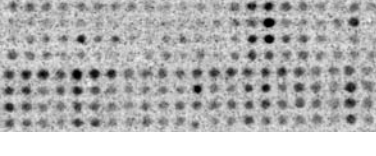


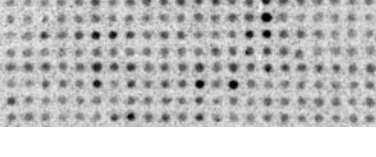

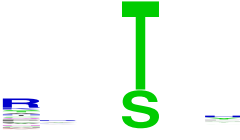
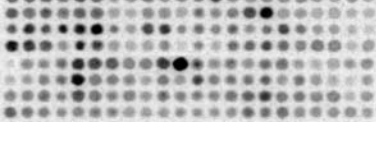


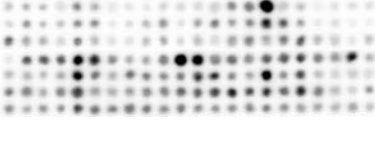


**Table S1.** Representative peptide array screening results and sequence logos for each of the 61 kinases assayed. Hsl1 was assayed in the original 384-well format which lacked the zero position control mixtures, and thus no information regarding its selectivity for Ser or Thr residues as the targeted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

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L M F Y W H K R Q N D E p T p Y	0: - S T	
<i>AKL1</i>	<i>YBR059C</i>			
<i>ARK1</i>	<i>YNL020C</i>			
<i>ATG1</i>	<i>YGL180W</i>			
<i>CAK1</i>	<i>YFL029C</i>			
<i>CDC15</i>	<i>YAR019C</i>			
<i>CDC28</i>	<i>YBR160W</i>			
<i>CDC5</i>	<i>YMR001C</i>			
<i>CDC7</i>	<i>YDL017W</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC	STVI LMFYWHKRQNDE <sub>pT</sub> pY	
<i>CKA1</i>	<i>YIL035C</i>			
<i>CLA4</i>	<i>YNL298W</i>			
<i>CMK1</i>	<i>YFR014C</i>			
<i>CMK2</i>	<i>YOL016C</i>			
<i>FMP48</i>	<i>YGR052W</i>			
<i>FUS3</i>	<i>YBL016W</i>			
<i>GCN2</i>	<i>YDR283C</i>			
<i>GIN4</i>	<i>YDR507C</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L M F Y W H K R Q N D E p T p Y	0: - S T	
<i>HOG1</i>	<i>YLR113W</i>			
<i>HRR25</i>	<i>YPL204W</i>			
<i>HSL1</i>	<i>YKL101W</i>			
<i>IPL1</i>	<i>YPL209C</i>			
<i>KCC4</i>	<i>YCL024W</i>			
<i>KIN1</i>	<i>YDR122W</i>			
<i>KIN3</i>	<i>YAR018C</i>			
<i>KIN4</i>	<i>YOR233W</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L MFYWHKRQND E pT pY	0: - S T	
<i>KSP1</i>	<i>YHR082C</i>			
<i>KSS1</i>	<i>YGR040W</i>			
<i>MCK1</i>	<i>YNL307C</i>			
<i>MEK1</i>	<i>YOR351C</i>			
<i>MPS1</i>	<i>YDL028C</i>			
<i>MRK1</i>	<i>YDL079C</i>			
<i>PHO85-PCL1</i>	<i>YPL031C-YNL289W</i>			
<i>PHO85-PCL2</i>	<i>YPL031C-YDL127W</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L M F Y W H K R Q N D E p T p Y	0: - S T	
<i>PHO85-PHO80</i>	<i>YPL031C-YOL001W</i>			
<i>PKH2</i>	<i>YOL100W</i>			
<i>PRK1</i>	<i>YIL095W</i>			
<i>PRR1</i>	<i>YKL116C</i>			
<i>PSK2</i>	<i>YOL045W</i>			
<i>PTK2</i>	<i>YJR059W</i>			
<i>RAD53</i>	<i>YPL153C</i>			
<i>RCK2</i>	<i>YLR248W</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I LMFYWHKRQNDEpTpY	0:- S T	
<i>RIM11</i>	<i>YMR139W</i>			
<i>RIM15</i>	<i>YFL033C</i>			
<i>SCH9</i>	<i>YHR205W</i>			
<i>SKM1</i>	<i>YOL113W</i>			
<i>SKY1</i>	<i>YMR216C</i>			
<i>SLT2</i>	<i>YHR030C</i>			
<i>SNF1</i>	<i>YDR477W</i>			
<i>STE20</i>	<i>YHL007C</i>			

Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L M F Y W H K R Q N D E p T p Y	0: - S T	
<i>TOS3</i>	<i>YGL179C</i>			
<i>TPK1</i>	<i>YJL164C</i>			
<i>TPK2</i>	<i>YPL203W</i>			
<i>TPK3</i>	<i>YKL166C</i>			
<i>VHS1</i>	<i>YDR247W</i>			
<i>YAK1</i>	<i>YJL141C</i>			
<i>YCK1</i>	<i>YHR135C</i>			
<i>YCK2</i>	<i>YNL154C</i>			



Gene Name	ORF Name	Array Image		Logo
		PGAC ST V I L M F Y W H K R Q N D E p T p Y	0: - S T	
<i>YCK3</i>	<i>YER123W</i>			
<i>YDL025C</i>	<i>YDL025C</i>			
<i>YKL171W</i>	<i>YKL171W</i>			
<i>YNR047W</i>	<i>YNR047W</i>			
<i>YPK1</i>	<i>YKL126W</i>			
<i>YPK2</i>	<i>YMR104C</i>			
<i>YPL141C</i>	<i>YPL141C</i>			

**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- $\alpha$	(25)
Hrr25	YPL204W	Acidophilic	CK1- $\delta$	(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- $\gamma$ 2	(22)
Yck2	YNL154C	Acidophilic	CK1- $\gamma$ 2	(22)
Yck3	YER123W	Acidophilic	CK1- $\gamma$ 3	(22)
Ydi025c	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	FEILATL	GNGQY	GKVKL	ARDLGT	-----	GALVAIKILNR
TPK3_Scer/1-255	FQILRTL	GTGSF	GRVHL	IRSNHN	-----	GRFYALKTLKKHTIVK
TPK1_Scer/1-255	FQILRTL	GTGSF	GRVHL	IRSRHN	-----	GRYYAMKVLKKEIVVR
TPK2_Scer/1-255	FQIMRTL	GTGSF	GRVHL	VRSVHN	-----	GRYYAIKVLKKQVVV
YPK1_Scer/1-256	FDLLKVI	GKGSF	GKVMQ	VRKKDT	-----	QKVYALKAIRKSYIVS
YPK2_Scer/1-256	FDLLKVI	GKGSF	GKVMQ	VRKKDT	-----	QKIYALKALRKAYIVS
SCH9_Scer/1-260	FEVLRLL	GKGT	FGQVY	VQVKKDT	-----	QRIYAMKVLKSKVIVK
YNR047W_Scer/1-282	FEKIRLL	GQGDV	GKVFV	VREKKT	-----	NRVYALKVLSKDEMIK
RIM15_Scer/1-269	YDILKPI	SKGAY	GSVYL	ARKKLT	-----	GDYFAIKVLRKSDMIA
IPL1_Scer/1-252	FELGKKL	GKGGK	FGKVC	VRHRST	-----	GYICALKVMKEEIIK
PKH2_Scer/1-265	FKFGS	IGDGAY	STVML	ATSIDT	-----	KKRYAAKVLNKEYLIR
KCC4_Scer/1-265	WKLGETL	GFGST	GKVQL	AQHERT	-----	GHRTAVKVISKSIFNN
GIN4_Scer/1-271	WKLGETL	GLGST	GKVQL	ARNGST	-----	GQEAAVKVISKAVFNT
HSL1_Scer/1-289	WKLGLT	LGGSS	GRVRL	AKNMET	-----	GQLAAIKIVPKKAFV
SNF1_Scer/1-252	YQIVKTL	GEGSF	GKVKL	AYHTTT	-----	GQKVALKIINKKVLAK
KIN1_Scer/1-279	WEFVETV	GAGSM	GKVKL	LAKHRYT	-----	NEVCAVKIVNRATKAF
KIN4_Scer/1-268	YIIGSTL	GEGEF	GKVKL	GWTKASSNE	-----	VPKQVAIKLIRRDTIKK
YPL141C_Scer/1-273	YILGSTL	GEGEF	GKVKL	GWPKNFSNSNS	-----	TFDFPKQVAIKLIKRDISISN
CMK1_Scer/1-263	YVFGKTL	GAGTF	GVVRA	QAKNTET	-----	GEDVAVKILIKKALKG
CMK2_Scer/1-263	YIFGRTL	GAGSF	GVVRA	QARKLST	-----	NEDVAIKILLKKALQG
RAD53_Scer/1-269	SIIDEV	VQGAF	ATVKK	AIERTT	-----	KTFAVKIIISKRVIG
RCK2_Scer/1-316	YKLINKI	GEGAF	SKVFRAI	PAKNSSNEFL	-----	TKNYKAVAIKVIKKADLSS
APG1_Scer/1-302	YTAEKI	GKGSF	ATVYR	GHLTSDK	-----	SQHVAIKEVSRALKLN
MEK1_Scer/1-284	EITNRIV	GNGT	FGHVL	LITHNSKERDEDEDVCYH	-----	PENYAVKIIKLG----
YKL171W_Scer/1-283	HRLGKI	IGFGAW	GIIRE	CFDIET	-----	GVGRVIKIVKFKGHQN
CDC5_Scer/1-256	YHRGHFL	GEGGF	ARCFQ	-IKDSS	-----	GEIFAAKTVAKASIKS
PSK2_Scer/1-259	FTILQVM	GEGAY	GKVNLCI	HN	-----	REHYIVVIKMIFKERILV
YDL025C_Scer/1-274	GIPGRKL	GEGAS	GSVSV	VER	-----	TDGKLFACKMFRKPHLNN
KYL1_Scer/1-312	YILVRKL	GWGHF	STVWL	LAKDMVNN	-----	THVAMKIVRGDKVYT
YAK1_Scer/1-257	YLVLDI	LQGGT	FGQVVK	QNLTK	-----	EILAVKVVKSRT EYL
PTK2_Scer/1-305	DTDNKPI	GSGGS	SEVRK	VKSS	-----	YRQKDVYALKKLN--MIYH
PHO85_Scer/1-291	FKQLEKL	GNGT	YATVY	KLNKTT	-----	GVYVALKEVKL DSE--
CDC28_Scer/1-288	YKRLEKV	GEGTY	GVVYK	ALDRPQG	-----	QRVVALKKIRLESED-
SLT2_Scer/1-296	FQLIKEI	GHGAY	IVCSA	IFAEAAE	-----	DTTVAIKKVTNVFSKT
KSS1_Scer/1-301	YKLVDLI	GEGAY	GTVCS	AIHKPS	-----	GIKVAIKKIQ-PFSKK
FUS3_Scer/1-297	FQLKSL	LGE	GAYGV	VCSATHKPT	-----	GEIVAIKKIE-PFDKP
HOG1_Scer/1-280	YNDLNP	VGMGA	FGLVCS	ATDTLT	-----	SQPVAIKKIMKPFSTA
RIM11_Scer/1-284	FPTTEV	VGHGS	FGVVF	ATVIQET	-----	EKVAIKKVLQDRFKF
MRK1_Scer/1-284	YPTTEV	VGHGS	FGVVT	TVIIET	-----	NQKVAIKKVLQDRRYK
MCK1_Scer/1-293	VKEYRKI	GRGAF	TVYQ	AYLTQDKKNWL	-----	GPFAIKKVPAHTEYK
CKA1_Scer/1-324	YEIENKV	GRGKY	SEVFQ	GVKGLDS	-----	KVKIVIKMLKPVK KKK
CDC7_Scer/1-272	YKLIDKI	GEGTF	SSVYK	AKDITGKITKFFASHFW	-----	NYGSNYVALKKIYVTS SPO
YCK1_Scer/1-261	YKIGKKI	GEGSF	GVLF	EGTNMIN	-----	GVPVAIKFEPKTEAP
YCK2_Scer/1-261	YKIGKKI	GEGSF	GVLF	EGTNMIN	-----	GLPVAIKFEPKTEAP
YCK3_Scer/1-296	YAVGPKI	GEGSF	GVIF	EGENILHSCQ	---	AQTGSKRDSSII MANEPVAIKFEPHSDAP
HRR25_Scer/1-259	FRIGRKI	GSGS	FGDIY	HGTNLIS	-----	GEEVAIKLESIRSRHP
PRR1_Scer/1-317	WKKVRPI	GSGNF	STVLL	YELMDQSN	-----	PKLKQVAVKRLKYPEELS
MPS1_Scer/1-277	YEKIELL	GRGG	SSRVY	KVKGSGN	-----	RVYALKRVSFDAFDD
GCN2_Scer/1-268	FEEIAVL	GQGA	FGQV	VKARNALD	-----	SRYVAIKKIRHTEEK-
KIN3_Scer/1-319	YQVLEEI	GRGS	FGSVR	KVIHIPT	-----	KKLLVRKDIKYGHMNS
PRK1_Scer/1-277	AKIIKYL	TSGG	FAQV	YTA	-----	EISPPDPYSNANIACLKRVIVPHKQG
ARK1_Scer/1-277	VEIIKYL	TSGG	FAQV	YSA	-----	LINPPDPHSNSVACLKRVIVPDKPS
AKL1_Scer/1-285	VEVVNYL	AE	GGFAQI	YVVKFL	-----	EYLN EF-DNTASVPLKIGDVAQLKRVLVQDENG
VHS1_Scer/1-321	YLITSQI	GEGAY	GLVYR	ALDIRTD	-----	ROYAIKAVVQSYGVS
SKM1_Scer/1-280	FQLVEK	AGQGAS	GA	YLSKRIKLP	--	QENDPRF-LKSHCHR-VVGERVAIKQIRLSEQPK
CLA4_Scer/1-280	FKVIEK	AGQGAS	GSVYLA	ERTHIP	--	TESNMIELINNDIDEPHVGDKVAIKQMVLSKQPR
STE20_Scer/1-252	YANLVK	IGQ	ASGGV	YAYEIGT	-----	NVSVAIKQMNLEKQPK
CDC15_Scer/1-248	YHLKQVI	GRGSY	GVVYK	AINKHT	-----	DQVVVAIKEVYKND EEE
FMP48_Scer/1-285	YTKLRSI	QSGTF	STVYK	AWSTTHN	-----	RYVALKITPKYK TSE
KSP1_Scer/1-260	YQKIEDI	SEGSY	GVYSL	AKDVREK	-----	RLVAVKYIFKLE DLE
CAK1_Scer/1-304	-MKLDS	IDITHC	QLVK	STRTARIYR	-----	SDTYAIKCLALDFDIP

Table S4, continued

	61	70	80	90	100	110
TOS3_Scer/1-295	-----	-----	-----	-----	FEKRSG----	YSLQLKV-ENPRVNQEIIE
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	-----	-----	-----	-----	-----	-----QKKVKYVSIKKT
KCC4_Scer/1-265	NGN-----	-----	-----	-----	-----	-----HSNDDSVLPYNIEREIV
GIN4_Scer/1-271	GNVSGTSTI-----	-----	-----	-----	-----	-----VGSTTPDALPYGIEREII
HSL1_Scer/1-289	HCSNNGTVPNSYSSSMVTSNVSSPSIA-----	-----	-----	-----	-----	-----SREHSNHSQTNPYGIEREIV
SNF1_Scer/1-252	SDMQG-----	-----	-----	-----	-----	-----RIEREIS
KIN1_Scer/1-279	LHKEQMLPPPKNQDVLER-----	-----	-----	-----	-----	-----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-----	-----	-----	-----	-----	-----MDGVTRERE
RCK2_Scer/1-316	INGDHRKKDK-----	-----	-----	-----	-----	-----GKDSTKTSSRDQVLKEVA
APG1_Scer/1-302	KKLL-----	-----	-----	-----	-----	-----ENLEIEIA
MEK1_Scer/1-284	-----	-----	-----	-----	-----	-----PNKFDKEAR
YKL171W_Scer/1-283	-----	-----	-----	-----	-----	-----IKKHVLEVA
CDC5_Scer/1-256	EKTR-----	-----	-----	-----	-----	-----KKLLSEIQ
PSK2_Scer/1-259	DT-----	-----	-----	-----	-----	-----WVRDRKLGITIPSEIQ
YDL025C_Scer/1-274	EGTN-----	-----	-----	-----	-----	-----QSQLANYSKKVTTEFC
SKY1_Scer/1-312	-----	-----	-----	-----	-----	-----EAAEDEIK
YAK1_Scer/1-257	-----	-----	-----	-----	-----	-----TQSITTEAK
PTK2_Scer/1-305	E-----	-----	-----	-----	-----	-----SPEKFKRCSKEFI
PHO85_Scer/1-291	-----	-----	-----	-----	-----	-----EGTPSTAIRESIS
CDC28_Scer/1-288	-----	-----	-----	-----	-----	-----EGVPSTAIRESIS
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-----	-----	-----	-----	-----	-----TSLRYKETLSRLENLSTRELO
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	KEADMGNDKIHKNSVKLQKKLAKLKFESKNVVRVPSIDLESINMSEEDFKKLPHYKEIS	-----	-----	-----	-----	-----KQLIMNELL
SKM1_Scer/1-280	-----	-----	-----	-----	-----	-----KELIVNEIL
CLA4_Scer/1-280	-----	-----	-----	-----	-----	-----KELINEIL
STE20_Scer/1-252	-----	-----	-----	-----	-----	-----LNDIMAEIS
CDC15_Scer/1-248	-----	-----	-----	-----	-----	-----ANMKNEYD
FMP48_Scer/1-285	-----	-----	-----	-----	-----	-----AMYEVD
KSP1_Scer/1-260	-----	-----	-----	-----	-----	-----PHNAKFEVS
CAK1_Scer/1-304	-----	-----	-----	-----	-----	-----

Table S4, continued

	121	130	140	150	160	170
TOS3_Scer/1-295	VM-----					KRCHHENVVELYEILNDPE
TPK3_Scer/1-255	MLSIVS-----					HPFIIRMWGTFO---
TPK1_Scer/1-255	MLSIVT-----					HPFIIRMWGTFO---
TPK2_Scer/1-255	MLKLVE-----					HPFLIRMWGTFO---
YPK1_Scer/1-256	VLARVD-----					CPFIVPLKFSFO---
YPK2_Scer/1-256	VLARVD-----					CPFIVPLKFSFO---
SCH9_Scer/1-260	ILVTTASKS-----					SPFIVGLKFSFO---
YNR047W_Scer/1-282	ILATSN-----					HPFIVTLYHSFO---
RIM15_Scer/1-269	IMMVQSD-----					KPYVARLFASFQ---
IPL1_Scer/1-252	IQTSLN-----					HPNLTKSYGYFH---
PKH2_Scer/1-265	ALQKLNNS-----					PSVVRLFSTFO---
KCC4_Scer/1-265	IMKLLS-----					HPNVLSLYDVWE---
GIN4_Scer/1-271	IMKLLN-----					HPNVLRLYDVWE---
HSL1_Scer/1-289	IMKLIS-----					HTNVMALFEVWE---
SNF1_Scer/1-252	YLRLLR-----					HPHIKLYDVIK---
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-----					HNRILPLLDWKLLDN
CDC5_Scer/1-256	IHKSMS-----					HPNIVQFIDCFE---
PSK2_Scer/1-259	IMATLNKN-----					SQENILKLLDFEDDD
YDL025C_Scer/1-274	IGSTLH-----					HENIVETLDMLTEG-
SKY1_Scer/1-312	LLQRVNDADN-----					TKEDSMGANHILKLLDHFNHKG
YAK1_Scer/1-257	ILELLNOK-----					IDPTNKHFFLRMYDSFV
PTK2_Scer/1-305	IAKHLSHN-----					VHITNTFYLLKVPPTTY
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-----					HPNTVGLQYFFY---
MCK1_Scer/1-293	ILRIAD-----					HPNIVKLQYFFT--HL
CKA1_Scer/1-324	ILTDLSEKVPPTTLPFQKDQYYTNQKEDVLFIRPYIFDQPHNGHANIHLFDI IKDPI					
CDC7_Scer/1-272	LLYIMT-----					GSSRVAPLCAKRRVR-
YCK1_Scer/1-261	TYKIL-----					NGTPNIPYAYYFGQEG
YCK2_Scer/1-261	TYKIL-----					AGTPGIPQEYFGQEG
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-----					HQYVVRYAAWLEED
KIN3_Scer/1-319	ILSQL-----					KHENIVEFYNWDFDEQ
PRK1_Scer/1-277	AMKLLRNN-----					KHVVSYIDSHAARS
ARK1_Scer/1-277	AMRLKNN-----					RYVVSYIDSHAACA
AKL1_Scer/1-285	VMKCLKGA-----					PNIVQYFDSNASRR
VHS1_Scer/1-321	LHLRVHH-----					HKNIVTIEVLQS--
SKM1_Scer/1-280	VMNDS-----					ROENIVNFLEAYIDD
CLA4_Scer/1-280	VMKDS-----					RHKNIVNFLEAYLRTD
STE20_Scer/1-252	VMKGS-----					KHPNIVNFIDSYVLKG
CDC15_Scer/1-248	LLKNL-----					NHN-NIVKYHGFIKRS
FMP48_Scer/1-285	VMKILSSCN-----					PHPNICSMDFYTDSD
KSP1_Scer/1-260	IQTKIG-----					RHQNIAALLDFFDSD--
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	-----		DSQOVFMVMDYIEGGELFSLLRK-----			
TPK1_Scer/1-255	-----		DAQQIFMIMDYIEGGELFSLLRK-----			
TPK2_Scer/1-255	-----		DARNIFMVM DYIEGGELFSLLRK-----			
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	-----		NKDNLFVMEYLPGGDLATLIK-----			
IPL1_Scer/1-252	-----		DEKRVYLLMEYLVNGEMYKLLRL-----			
PKH2_Scer/1-265	-----		DESSLYFLLEYAPNGDFLSLMKK-----			
KCC4_Scer/1-265	-----		TNNNLYLILEYAEKGELFNLLVD-----			
GIN4_Scer/1-271	-----		TNTDLYLVLEYAEKGELFNLLVE-----			
HSL1_Scer/1-289	-----		NKSELYLVLEYVDGGELFDYLV-----			
SNF1_Scer/1-252	-----		SKDEIIMVIEYA-GNELFDYIVQ-----			
KIN1_Scer/1-279	-----		LSNHFYMLFEYVSGGQLLDYIIQ-----			
KIN4_Scer/1-268	-----		NSKYIGIVLEFVSGGEFYKYIQ-----			
YPL141C_Scer/1-273	-----		NSRYIGIVLEYACGGEFYKYIQ-----			
CMK1_Scer/1-263	-----		SKDKFYIITQLAKGGELFDRILK-----			
CMK2_Scer/1-263	-----		SKDKFYIVTQLATGGELFDRILS-----			
RAD53_Scer/1-269	-----		DTESYVMMEFVSGGDLMDVAA-----			
RCK2_Scer/1-316	-----		TDSYYYIIQELLTGGEIFGEIVR-----			
APG1_Scer/1-302	-----		TSTDFYLIMEYCALGDLTFLK-----			
MEK1_Scer/1-284	-----		RNNHLYIFQDLIPGGDLFSYLAG-----			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-LDLDLKRMEG-----			
SLT2_Scer/1-296	PDG-----		SINGLYLYEEL-MECDMHQI IKSGQ-----			
KSS1_Scer/1-301	ID-----		KLNAVYLVEEL-METDLQKVINNQNS-----			
FUS3_Scer/1-297	FE-----		NFNEVYIIQEL-MQTDLHRVISTQ-----			
HOG1_Scer/1-280	-----		PLEDIYFVTEL-QGTDLHRLLOTR-----			
RIM11_Scer/1-284	ERDSQ-----		DEIYLNILEY-MPQSLYQRLRHF-VHQRT-----			
MRK1_Scer/1-284	EKDEE-----		DEVYLNVLVDY-MPQSLYQRLRHF-VNLKM-----			
MCK1_Scer/1-293	SPQDN-----		KVYQHAMEC-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-----		HNILIDLLGP-SLEDLFEW-----			
HRR25_Scer/1-259	E-----		YNAMVIDLLGP-SLEDLFNY-----			
PRR1_Scer/1-317	VTSKKPLCDLI IKTPRALPPCDMIMSYCPAGDLLAAVMAR-----					
MPS1_Scer/1-277	-----		LLYLIMECGDHLSQILNQ-----			
GCN2_Scer/1-268	S-----		MLFIQMEYCENRTLYDLIHS-----			
KIN3_Scer/1-319	K-----		EVLYLYMEYCSRGDLSQMIKH-----			YKQEHKY
PRK1_Scer/1-277	VNG-----		IAYEVFVLMFCERGGLIDFMNT-----			RLQNR
ARK1_Scer/1-277	MLHN-----		GSYEVFVLMFCERGGLIDFMNT-----			RLQNR
AKL1_Scer/1-285	RDGV-----		QGFEVLLLMELCPNKSLDYMNQ-----			RLSTK
VHS1_Scer/1-321	-----		AVCTFIVMDYYPDTLFTSIVDN-----			
SKM1_Scer/1-280	E-----		ELWVIMEYMEGGCLTDILDA-----			VARSNTG
CLA4_Scer/1-280	D-----		DLWVMEFMEGGSLTDIEN-----			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-----		DLLLLFPFEEMLNLYEFMQM-----			HYKRDRRKKNPYYD



Table S4, continued

	241	250	260	270	280	290
TOS3_Scer/1-295	KA--VGP	-----	-----	SILTFQOSRKVVLDVVS	GLLEYLHSQ	-----
TPK3_Scer/1-255	SQ--RFP	-----	-----	NPVAKFYAAEVCLALEY	LHSHK	-----
TPK1_Scer/1-255	SQ--RFP	-----	-----	NPVAKFYAAEVCLALEY	LHSHK	-----
TPK2_Scer/1-255	SQ--RFP	-----	-----	NPVAKFYAAEVILALEY	LHSHN	-----
YPK1_Scer/1-256	EG--RFD	-----	-----	LSRARFYTAELLCALDN	LHKLD	-----
YPK2_Scer/1-256	EG--RFS	-----	-----	LARSRFYIAELLCALDSL	LHKLD	-----
SCH9_Scer/1-260	EG--RFS	-----	-----	EDRAKFYIAELVLALEH	LHDND	-----
YNR047W_Scer/1-282	TK--CIC	-----	-----	EDDARFYASEVTAALEY	LHLLG	-----
RIM15_Scer/1-269	MG--YLP	-----	-----	DQWAKQYLTEIVGVNDM	HQNG	-----
IPL1_Scer/1-252	HG--PFN	-----	-----	DILASDYIYQIANALDY	MHKKN	-----
PKH2_Scer/1-265	YG--SLD	-----	-----	ETCARYYAAQIIDAIDY	LHNSG	-----
KCC4_Scer/1-265	HG--PLP	-----	-----	EREAINCFRQIIIGISY	CHALG	-----
GIN4_Scer/1-271	RG--PLP	-----	-----	EHEAIRFFRQIIIGVSY	CHALG	-----
HSL1_Scer/1-289	KG--KLP	-----	-----	EREAIHYFKQIVEGVSY	CHSFN	-----
SNF1_Scer/1-252	RD--KMS	-----	-----	EQEARKFFQQIISAVEY	CHRHK	-----
KIN1_Scer/1-279	HG--SIR	-----	-----	EHQARKFARGIASALIY	LHANN	-----
KIN4_Scer/1-268	KR--RLK	-----	-----	ESSACRLFAQLISGVNY	MHYKG	-----
YPL141C_Scer/1-273	KR--RLK	-----	-----	EMNACRLFSQLISGVHY	IHSGK	-----
CMK1_Scer/1-263	KG--KFT	-----	-----	EEDAVRILVEILSAVKY	MHSQN	-----
CMK2_Scer/1-263	RG--KFT	-----	-----	EVDAVEIVQILGAVEYM	HSKN	-----
RAD53_Scer/1-269	HG--AVG	-----	-----	EDAGREISRQILTAIKY	IHSMG	-----
RCK2_Scer/1-316	LT--YFS	-----	-----	EDLSRHVIKQLALAVKH	MHSLG	-----
APG1_Scer/1-302	RK--ELMENHPLLRTVFE	KYPPPS	ENHNLH	RAFVLSYLOQLASALK	FRLSKN	-----
MEK1_Scer/1-284	LT--SMS	-----	-----	ETESLLIVFQILQALNY	LHDQD	-----
YKL171W_Scer/1-283	DEFKRSKIPFAER	-----	-----	CRLTIFLSLQLLSALKY	MHSKT	-----
CDC5_Scer/1-256	RK--VLT	-----	-----	EPEVRFFTTQICGAIKY	MHSRR	-----
PSK2_Scer/1-259	KK--DMV	-----	-----	EHEAKLVFKQVVASIKH	LHDQ	-----
YDL025C_Scer/1-274	-N--LMT	-----	-----	QDEVNCYFKQLCHGVNY	LHSMG	-----
SKY1_Scer/1-312	EH--RG	-----	-----	IPLIYVKQISKQLLLGL	DYMHRRCG	-----
YAK1_Scer/1-257	NKF--HG	-----	-----	LSIQILIRFTTQILD	SLCVLKEK	-----
PTK2_Scer/1-305	TG--WKN	-----	-----	VPFNEKYCLFKQVAQGI	KFCHDNG	-----
PHO85_Scer/1-291	-----	RTVGN	-----	TPRGLELNLVKYFQWQL	LQGLAFCHENK	-----
CDC28_Scer/1-288	-----	IPKDQ	-----	PLGADIVKFKMMQLCKG	IAYCHSHR	-----
SLT2_Scer/1-296	-----	-----	-----	PLTDAHYQSFTYQILC	GLKYIHSAD	-----
KSS1_Scer/1-301	-----	-----	-----	GFSTLSDDHVQYFTYQ	ILRALKSIHSAQ	-----
FUS3_Scer/1-297	-----	-----	-----	MLSDDHIQYFIYQTL	RAVKVLHGSN	-----
HOG1_Scer/1-280	-----	-----	-----	PLEKQFVQYFLYQILR	GLKYVHSAG	-----
RIM11_Scer/1-284	-----	-----	-----	PMSRLEIKYMFQLFKSL	NYLHFFAN	-----
MRK1_Scer/1-284	-----	-----	-----	QMPRVEIKFYAYQLFK	KALNYLHNVPR	-----
MCK1_Scer/1-293	-----	-----	-----	EMPLKHIRLYTYQIAR	GMLYLHGLG	-----
CKA1_Scer/1-324	-----	-----	-----	KLTDLEIRFYMFELLK	ALDYCHSMG	-----
CDC7_Scer/1-272	-----	-----	-----	DLPIKGIKKYIWELLR	ALKFVHSHK	-----
YCK1_Scer/1-261	CG--R	-----	-----	KFSVKTVVQVAVQMIT	LIEDLHAHD	-----
YCK2_Scer/1-261	CG--R	-----	-----	RFSVKTVVQVAVQMIT	LIEDLHAHD	-----
YCK3_Scer/1-296	CG--R	-----	-----	KFSVKTTCMVAQMIDR	VRAIHDDH	-----
HRR25_Scer/1-259	CH--R	-----	-----	RFSFKTVIMLALQMFC	RIQYIHGRS	-----
PRR1_Scer/1-317	NG--RLE	-----	-----	AWLIQRIFTEVVLA	VKYLHENS	-----
MPS1_Scer/1-277	RSGMPLD	-----	-----	FNFVRFYTKEMLLC	IKVVHDAG	-----
GCN2_Scer/1-268	ENLNQ	-----	-----	RDEYWRLFROILEALSY	IHSQ	-----
KIN3_Scer/1-319	-----	IP	-----	EKIVWGILAQLLTALY	KCHYGVELPTLTT	-----
PRK1_Scer/1-277	-----	LQ	-----	ESEILEIMSQTVQGIT	TAMHALQP	-----
ARK1_Scer/1-277	-----	LH	-----	EFEILQIMSQVTQGV	AAMHALQP	-----
AKL1_Scer/1-285	-----	LT	-----	EAEIVKIMYDVALS	ISQMHYLPV	-----
VHS1_Scer/1-321	-R--HFVTN	-----	-----	GLLVKKVFLQIC	SALNYCHEHG	-----
SKM1_Scer/1-280	EHSSPLN	-----	-----	ENQMAYIVKETCQGL	KFLHKN	-----
CLA4_Scer/1-280	SH--SPLT	-----	-----	EPQIAYIVRET	CQGLKFLHDK	-----
STE20_Scer/1-252	CI---LT	-----	-----	EGQIGAVCRETLSGL	EFLHSHK	-----
CDC15_Scer/1-248	--SSTGLS	-----	-----	ENESKTYVTQTL	LGLKYLHGE	-----
FMP48_Scer/1-285	PSSPSLI	-----	-----	QIDMQKIIKQLCSA	ISFAHSLG	-----
KSP1_Scer/1-260	----AVPKK	-----	-----	TKSITHIITQIMDA	IEYVHNKG	-----
CAK1_Scer/1-304	LL--NPS-IPIV	-----	ADPPVQKYTNQLD	VNRYSLSFFRQMV	EGIAFLHENK	-----

Table S4, continued

	301	310	320	330	340	350
TOS3_Scer/1-295	-----	ITHRDLKPSNLLI	-----	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	-----	IIHHDLPENILL	-----	DNAG	-----	-----
IPL1_Scer/1-252	-----	IIHRDLKPENILL	-----	GFNN	-----	-----
PKH2_Scer/1-265	-----	IIHRDLKPENILL	-----	DGEM	-----	-----
KCC4_Scer/1-265	-----	IVHRDLKPENILL	-----	DSFY	-----	-----
GIN4_Scer/1-271	-----	IVHRDLKPENILL	-----	DKHY	-----	-----
HSL1_Scer/1-289	-----	ICHRDLKPENILL	-----	DKKN	-----	-----
SNF1_Scer/1-252	-----	IVHRDLKPENILL	-----	DEHL	-----	-----
KIN1_Scer/1-279	-----	IVHRDLKIENIMI	-----	SDSS	-----	-----
KIN4_Scer/1-268	-----	LVHRDLKLENLLI	-----	DKHE	-----	-----
YPL141C_Scer/1-273	-----	LVHRDLKLENLLI	-----	DKNE	-----	-----
CMK1_Scer/1-263	-----	IVHRDLKPENLLY	-----	IDKSDES	-----	-----
CMK2_Scer/1-263	-----	VVHRDLKPENVLY	-----	VDKSENS	-----	-----
RAD53_Scer/1-269	-----	ISHRDLKPDNILL	-----	EQDDP	-----	-----
RCK2_Scer/1-316	-----	VVHRDLKPENLLFEP	IEFTRSIKPKLRKSD	-----	-----	-----
APG1_Scer/1-302	-----	LVHRDLKPQNLLI	-----	STPLIGY	-----	-----
MEK1_Scer/1-284	-----	IVHRDLKLDNILL	-----	CTPEP	-----	-----
YKL171W_Scer/1-283	-----	IVHGDIKLENCLL	-----	QKEGKKS	-----	-----
CDC5_Scer/1-256	-----	VIHRDLKLGNIFF	-----	DSNY	-----	-----
PSK2_Scer/1-259	-----	IVHRDLKDNVIV	-----	DSHG	-----	-----
YDL025C_Scer/1-274	-----	LAHRDLKLDNCVV	-----	TKDG	-----	-----
SKY1_Scer/1-312	-----	IIHTDLKPENVLM	-----	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	-----	VIHRDLKPSNLLI	-----	NSN	-----	-----
FUS3_Scer/1-297	-----	VIHRDLKPSNLLI	-----	NSN	-----	-----
HOG1_Scer/1-280	-----	VIHRDLKPSNILL	-----	NEN	-----	-----
RIM11_Scer/1-284	-----	VCHRDIKPQNLLV	-----	DPET	-----	-----
MRK1_Scer/1-284	-----	ICHRDIKPQNLLV	-----	DPTT	-----	-----
MCK1_Scer/1-293	-----	VCHRDIKPSNVLV	-----	DPET	-----	-----
CKA1_Scer/1-324	-----	IMHRDVKPHNVM	-----	DHKN	-----	-----
CDC7_Scer/1-272	-----	IIHRDLKPTNPLF	-----	NLEL	-----	-----
YCK1_Scer/1-261	-----	LIYRDLKPDNFLI	-----	GRPG	-----	-----
YCK2_Scer/1-261	-----	LIYRDLKPDNFLI	-----	GRPG	-----	-----
YCK3_Scer/1-296	-----	LIYRDLKPDNFLI	-----	SQYQRISPEGKVIKSCA	-----	-----
HRR25_Scer/1-259	-----	FIHRDLKPDNFLM	-----	GVGR	-----	-----
PRR1_Scer/1-317	-----	IIHRDLKLENILL	-----	KYSFDDIN	-----	-----
MPS1_Scer/1-277	-----	IVHSDLKPANFVL	-----	VKG	-----	-----
GCN2_Scer/1-268	-----	IIHRDLKPMNIFI	-----	DESR	-----	-----
KIN3_Scer/1-319	IYDRMKPPVKGNIVHRDLKPGNIFLSYDDS	-D	-----	YNINEQVDGHEE	-----	VNSNYY
PRK1_Scer/1-277	-----	PLIHRDLKIENVLI	-----	SHDG	-----	-----
ARK1_Scer/1-277	-----	PLIHRDLKIENVLI	-----	SANN	-----	-----
AKL1_Scer/1-285	-----	SLIHRDLKIENVLI	-----	DAKN	-----	-----
VHS1_Scer/1-321	-----	IYHCDIKPENLLL	-----	DTED	-----	-----
SKM1_Scer/1-280	-----	KIIHRDLKSDNILL	-----	NSQG	-----	-----
CLA4_Scer/1-280	-----	HIHRDLKSDNVLL	-----	DTRA	-----	-----
STE20_Scer/1-252	-----	GVLHRDLKSDNILL	-----	SMEG	-----	-----
CDC15_Scer/1-248	-----	GVIHRDLKAAANILL	-----	SADN	-----	-----
FMP48_Scer/1-285	-----	IAHRDLKPENILL	-----	TING	-----	-----
KSP1_Scer/1-260	-----	IYHRDLKPENILL	-----	SGIDW	-----	-----
CAK1_Scer/1-304	-----	IIHRDLKPQNIML	-----	TNNTSTVS	-----	-----



Table S4, continued

	421	430	440	450	460	470
TOS3_Scer/1-295	QLLKSRLALGTPAFFAPELCS	TEKEYS	-----	-----	-----	-----
TPK3_Scer/1-255	-DVTYTLCGTPDYIAPEVVST	-----	-----	-----	-----	-----
TPK1_Scer/1-255	-DVTYTLCGTPDYIAPEVVST	-----	-----	-----	-----	-----
TPK2_Scer/1-255	-TVTWTLCGTPDYIAPEVITT	-----	-----	-----	-----	-----
YPK1_Scer/1-256	DDKTDTFCGTPEYLAPPELLG	-----	-----	-----	-----	-----
YPK2_Scer/1-256	NDKTDTFCGTPEYLAPPELLG	-----	-----	-----	-----	-----
SCH9_Scer/1-260	--RTNTFCGTTEYLAPELLDE	-----	-----	-----	-----	-----
YNR047W_Scer/1-282	GFRTNFSVFGTEEYIAPEVIRG	-----	-----	-----	-----	-----
RIM15_Scer/1-269	KKQNKKFFGTPDYLAPEITGK	-----	-----	-----	-----	-----
IPL1_Scer/1-252	ENRRRTVCGTIDYLSPEMVES	-----	-----	-----	-----	-----
PKH2_Scer/1-265	STRSKSFVGTAEYVSPPELLND	-----	-----	-----	-----	-----
KCC4_Scer/1-265	ADLLETSCGSPHYAAPEIVSG	-----	-----	-----	-----	-----
GIN4_Scer/1-271	GKLETSCGSPHYAAPEIVSG	-----	-----	-----	-----	-----
HSL1_Scer/1-289	NKLLKTSCGSPHYASPEIVMG	-----	-----	-----	-----	-----
SNF1_Scer/1-252	GNFLKTSCGSPNYAAPEIVSG	-----	-----	-----	-----	-----
KIN1_Scer/1-279	RKQLHTFCGSLYFAAPELLKA	-----	-----	-----	-----	-----
KIN4_Scer/1-268	NELMKTSCGSPCYAAPELVVST	-----	-----	-----	-----	-----
YPL141C_Scer/1-273	NELMKTSCGSPCYAAPELVISA	-----	-----	-----	-----	-----
CMK1_Scer/1-263	EELLYKPAGSLGYVAPEVLT	-----	-----	-----	-----	-----
CMK2_Scer/1-263	EDLIYKAAGSLGYVAPEVLT	-----	-----	-----	-----	-----
RAD53_Scer/1-269	GSFMKTFCGTLAYVAPEVIRGK	DTSVSPDEYEE	----	----	----	----
RCK2_Scer/1-316	--KNTKTPCGTVGYTAPEVVKD	-----	-----	-----	-----	-----
APG1_Scer/1-302	TSLAETLCGSPLYMAPEILN	-----	-----	-----	-----	-----
MEK1_Scer/1-284	KERMHTVVGTPPEYCAPEVGF	FRANRKA	YQSF	SRAATLE	QRGYDSK	CDLWSLGVITHIMLT
YKL171W_Scer/1-283	EPEPSKYIGSLPYASPELLDFP	-----	-----	-----	-----	-----
CDC5_Scer/1-256	SERKYTCGTPNYIAPEVLMGK	-----	-----	-----	-----	-----
PSK2_Scer/1-259	--PFDVFGTMDYAAPEVLGSS	-----	-----	-----	-----	-----
YDL025C_Scer/1-274	IVKSHGIVGSDPYLAPELLKQT	-----	-----	-----	-----	-----
SKY1_Scer/1-312	--EHYTNSIQTREYRSPEVLLG	-----	-----	-----	-----	-----
YAK1_Scer/1-257	--RTVYTYIQSRFYRAPEIILG	-----	-----	-----	-----	-----
PTK2_Scer/1-305	VKTCQGMIGSPPYTPPEVMY	FD	AKKH	HYPEK	----	----
PHO85_Scer/1-291	VNTFSSEVVTLWYRAPDVLMSG	-----	-----	-----	-----	-----
CDC28_Scer/1-288	LRAYTHEIVTLWYRAPEVLLGG	-----	-----	-----	-----	-----
SLT2_Scer/1-296	--FLTEYVATRWRYPEIMLSY	-----	-----	-----	-----	-----
KSS1_Scer/1-301	VGFMTEYVATRWRYPEIMLTF	-----	-----	-----	-----	-----
FUS3_Scer/1-297	QSGMTEYVATRWRYPEVMLTS	-----	-----	-----	-----	-----
HOG1_Scer/1-280	--PQMTGYVSTRYYRAPEIMLTW	-----	-----	-----	-----	-----
RIM11_Scer/1-284	TEPNVSYICSRYYRAPELIFGA	-----	-----	-----	-----	-----
MRK1_Scer/1-284	DQPNVSYICSRYYRAPELIFGA	-----	-----	-----	-----	-----
MCK1_Scer/1-293	NQPSISYICSRFYRAPELIIGC	-----	-----	-----	-----	-----
CKA1_Scer/1-324	NMEYNVRVASRFFKPELLVDY	-----	-----	-----	-----	-----
CDC7_Scer/1-272	RIKRANRAGTRGFRAPEVLMKC	-----	-----	-----	-----	-----
YCK1_Scer/1-261	YREKKSLSGTARYMSINTHLG	-----	-----	-----	-----	-----
YCK2_Scer/1-261	YREKKSLSGTARYMSINTHLG	-----	-----	-----	-----	-----
YCK3_Scer/1-296	YREKKSLSGTARYMSINTHFG	-----	-----	-----	-----	-----
HRR25_Scer/1-259	YRENKSLTGTARYASVNTHLG	-----	-----	-----	-----	-----
PRR1_Scer/1-317	NEMCTARCGSEDYVSPPEILMGVP	-----	-----	-----	-----	-----
MPS1_Scer/1-277	NIYRETQIGTPNYMAPEALVAMNY	TQNS	ENQHEG	----	----	----
GCN2_Scer/1-268	SDNLTSAIGTAMYVATEVLDGTG	-----	-----	-----	-----	-----
KIN3_Scer/1-319	IQFATTYVGTPTYMSPEVLMQD	-----	-----	-----	-----	-----
PRK1_Scer/1-277	VQHDILTNTTAQYRSPPEMIDLYR	-----	-----	-----	-----	-----
ARK1_Scer/1-277	VQODILKNTTAQYRSPPEMIDTFR	-----	-----	-----	-----	-----
AKL1_Scer/1-285	LTQNIYVHTTPQYRSPPEMIDLYR	-----	-----	-----	-----	-----
VHS1_Scer/1-321	YIKPNVCISSYMPPERISFDGRV	SSSKSGG	----	----	----	----
SKM1_Scer/1-280	RSKRATMVGTPYWMapeivN	-----	-----	-----	-----	-----
CLA4_Scer/1-280	RSKRATMVGTPYWMapeivN	-----	-----	-----	-----	-----
STE20_Scer/1-252	NLKRRTMVGTPYWMapeivN	-----	-----	-----	-----	-----
CDC15_Scer/1-248	--SSALTLAGTLNWMapeiLG	-----	-----	-----	-----	-----
FMP48_Scer/1-285	--KSNDFQIGTDNYRAPETFSGRVS	NSCF	KKNF	DRSSAP	LYNTYQADY	WSLGATIFYLMF
KSP1_Scer/1-260	--TSMDRNVGSEYMSPELFD	SNLDIKER	-----	-----	-----	-----
CAK1_Scer/1-304	MDSKVTDISTGIYKAPEVLF	-----	-----	-----	-----	-----
						VKCYDGGVDVWSLLIIISQWFR

Table S4, continued

	481	490	500	510	520	530
TOS3_Scer/1-295	-----GKLPFNANS-----	-----GLELFDSIINKPLEFFPSY-----				
TPK3_Scer/1-255	-----GYTPFYNSN-----	-----TMKTYENILNAELKFP-----				
TPK1_Scer/1-255	-----GYTPFYDSN-----	-----TMKTYEKILNAELRFP-----				
TPK2_Scer/1-255	-----GYTPFYDFT-----	-----PMKTYEKILQKVVYP-----				
YPK1_Scer/1-256	-----GLPPYYDED-----	-----VPKMYKKILQEPLVFP-----				
YPK2_Scer/1-256	-----GLPPYYDEN-----	-----VPVMYKKILQQPLLFP-----				
SCH9_Scer/1-260	-----GWSPFFAEN-----	-----NQKMYQKIAFGKVKFPR-----				
YNR047W_Scer/1-282	-----GTFPFKGDN-----	-----TNETFTNILKNEVSFPNN-----				
RIM15_Scer/1-269	-----GYPPFHAET-----	-----PDAVFKKILSGVIQWPEFK-----				
IPL1_Scer/1-252	-----GAPPFEEEM-----	-----KDTTYKRIAALDIKMP-----				
PKH2_Scer/1-265	-----GKPPFKATN-----	-----EYLTFQKVMKVQYAFTPG-----				
KCC4_Scer/1-265	-----GRLPFDEEN-----	-----GNVRDLLLKVQKQF-----				
GIN4_Scer/1-271	-----GRLPFDEED-----	-----GNIRTLILLKVQKGEF-----				
HSL1_Scer/1-289	-----GHLPFNDN-----	-----NIKLLLLKVQSGKY-----				
SNF1_Scer/1-252	-----RRLPFDEE-----	-----SIPVLFKNISNGVY-----				
KIN1_Scer/1-279	-----GKVPFDDE-----	-----NSSVLHEKIKQKQV-----				
KIN4_Scer/1-268	-----GYLPWDDDHEN-----	-----PTGDDIARLYKYITQTPL-----				
YPL141C_Scer/1-273	-----GYLPWDDDPNN-----	-----PEGSDIGRLYNYINSTPL-----				
CMK1_Scer/1-263	-----GYSAFRAER-----	-----VQDFLDECTTGEYYPV-----				
CMK2_Scer/1-263	-----GYSFFIAES-----	-----VEGFMEECTASRYPV-----				
RAD53_Scer/1-269	-----GHLPFSGST-----	-----QDQLYKQIGRGSY-----				
RCK2_Scer/1-316	-----GFPFFYDEK-----	-----IDTLTEKISRGEY-----				
APG1_Scer/1-302	-----GTPPFRASN-----	-----HLELFKKIKRAN-----				
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-----	-----KKSFSNFRFLT-----				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-----	-----DEEQKLIIFDIMGTP-----				NESLWPSVTK
CDC28_Scer/1-288	-----RKPIFSGDS-----	-----EIDQIFKIFRVLGTP-----				NEAIWPDIVY
SLT2_Scer/1-296	-----GKPIFKGKD-----	-----YVNQLNQILOVLGTP-----				PDETLLRIGSK
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-----	-----GIDQLVEI IKILGTP-----				SKQEICSMNPN
MRK1_Scer/1-284	-----GKPLFSGES-----	-----GIDQLVEI IKIMGIP-----				TKDEISGMNPN
MCK1_Scer/1-293	-----GKAIFQOQE-----	-----PLLQREIAKLLGPP-----				DKRFIFFSNPA
CKA1_Scer/1-324	-----KREPFHGT-----	-----NTDQLVKIVKVLGTSDFEKYLLKYEIT-----				
CDC7_Scer/1-272	-----RRFPMFQSLD-----	-----DADSLLELCTIFGWKELRKAALHGLGFEASGLID-----				
YCK1_Scer/1-261	-----GHLPWQGLKAP-----	-----NNKQKYEKIG-----				
YCK2_Scer/1-261	-----GQLPWQGLKAP-----	-----NNKQKYEKIG-----				
YCK3_Scer/1-296	-----GSLPWQGLKAP-----	-----NNKLYEKIG-----				
HRR25_Scer/1-259	-----GSLPWQGLKAT-----	-----TKKQYDRIM-----				
PRR1_Scer/1-317	-----DRLPFDPPPNAS-----	-----ARQSRATSHRIARFDWR-----				
MPS1_Scer/1-277	-----GKPPYGSFQ-----	-----GQNRLLAIMNPDVKIPFPEHTS-----				
GCN2_Scer/1-268	-----PFST-----	-----GMERNILKRLRSVSI-----				
KIN3_Scer/1-319	-----LHPPFOAKN-----	-----YLELQTKIKNG-----				
PRK1_Scer/1-277	-----YTPPFEKS-----	-----GEAGILHARYQYPSFP-----				
ARK1_Scer/1-277	-----YTPPFEKG-----	-----GDLAILSGKFEPFLYP-----				
AKL1_Scer/1-285	-----FTTPFEMT-----	-----GQFAILHSHKYEFVFN-----				
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-----				

Table S4, continued

	541	550	560	570	580	590
TOS3_Scer/1-295	-----		EEMLNGATSGIT	-----	MEEYTD	AKDLLKLLQKDPDKR
TPK3_Scer/1-255	-----			-----	PPFHPDAQ	DLLKLLITRDLSER
TPK1_Scer/1-255	-----			-----	PPFNEDVK	DLLSRLITRDLSQL
TPK2_Scer/1-255	-----			-----	PYFHPDVV	DLLSKLITADLTRR
YPK1_Scer/1-256	-----			-----	DGFDRDAK	DLLIGLLSRDPTRR
YPK2_Scer/1-256	-----			-----	DGFDPAAK	DLLIGLLSRDPSRR
SCH9_Scer/1-260	-----			-----	DVLSQEGRS	FVKGLLRNPKHR
YNR047W_Scer/1-282	-----			-----	NEISRTCK	DLIKLLTKNESKR
RIM15_Scer/1-269	-----			-----	NEEEEREFL	TPEAKDLIEKLLVDPKR
IPL1_Scer/1-252	-----			-----	SNISQDAQ	DILKLLKYDPKDR
PKH2_Scer/1-265	-----			-----	FPLIIRDV	KKILVKNLDRR
KCC4_Scer/1-265	-----			-----	EMPNDTEI	SRDAQDLIGKILVDPQR
GIN4_Scer/1-271	-----			-----	EMPSDDEI	SREAQDLIRKILTVDPERR
HSL1_Scer/1-289	-----			-----	QMPSN--	LSSEARDLISKILVIDPEKR
SNF1_Scer/1-252	-----			-----	TLPKF--	LSPGAAGLIKRMILVPLNR
KIN1_Scer/1-279	-----			-----	EYPQH--	LSIEVISLLSKMLVDPKRR
KIN4_Scer/1-268	-----			-----	KFPEY--	ITPIPRDLLRRILVNPRRR
YPL141C_Scer/1-273	-----			-----	KFPDY--	ILPIPRDLLRRMLVSDPKKR
CMK1_Scer/1-263	-----			-----	KFHRPYW	SDSVSNKAKQFILKALNLDPSKR
CMK2_Scer/1-263	-----			-----	TFHMPYW	DNISIDVKRFILKALRLNPADR
RAD53_Scer/1-269	-----			-----	HEGPLKDF	RISEEARDFIDSLQLQVDPNRR
RCK2_Scer/1-316	-----			-----	TFLKPWW	DEISAGAKNAVAKLLELEPSKR
APG1_Scer/1-302	-----			-----	VITFPSYC	NIEPELKELICSLTTFDPAQR
MEK1_Scer/1-284	-----			-----	FKLQWDI	VSDNAKSFVKDLLQTDVVKR
YKL171W_Scer/1-283	-----			-----	LAQVCKF	DRKKNEGTTIGQLYDVTVIGCLTIDLK
CDC5_Scer/1-256	-----			-----	DFSFP	PRDKPISDEGKILIRDILSLDPIER
PSK2_Scer/1-259	-----			-----	EHVSEEC	ISLIKRILTREVDKR
YDL025C_Scer/1-274	-----	DIVRG	-----	-----	PNKILR	LRLPRHSRTIIGRMLALEPKQR
SKY1_Scer/1-312	-----	TFFNSRGL	LRNISK	LKFWPLEDVLTE	-----	KYKFSKDEAKEISDFLSPMLQLDPRKR
YAK1_Scer/1-257	-----			-----	PSWMIR	-----
PTK2_Scer/1-305	PGPGSE	-----		-----	YSLARN	FKNATDTRIARWLADPNPATR
PHO85_Scer/1-291	-----	LPKYNPNI	---QOR---	PPRDLRQV	LQPHT	---KEPLDGNLMDFLHGLLQLNPDMR
CDC28_Scer/1-288	-----	LPDFKPSF	---PQW---	RRKDL	SQVVP	-----SLDPRGIDLLDKLLAYDPINR
SLT2_Scer/1-296	-----	NVQDY	---IHQLGF	IPKVPFVNLYPN	-----	ANSQALDLEQLAFDPQKR
KSS1_Scer/1-301	-----	RAKEY	---IANLPMR	PPLPWETVWSKT	-----	DLNPDMLDLLDKMLQFNPDKR
FUS3_Scer/1-297	-----	RAREY	---IKSLPMY	PAAPLEKMFPR	-----	VNPKGIDLLQRMVLPDAKR
HOG1_Scer/1-280	-----	NTLKF	---VTSLPHR	DIPIPFSERFKT	-----	VEPDAVDLLEKMLVDPKRR
RIM11_Scer/1-284	-----	YMEH	---KFPQIK	PIPLSRVFKK	-----	EDDQTVFELADVLLKYDPLER
MRK1_Scer/1-284	-----	YEDH	---VFPNIK	ITLAEIFKA	-----	EDPDTLDDLTKTKLHPCER
MCK1_Scer/1-293	-----	YDGPLF	SKPLFSGSSQ	RFEKYFGH	-----	SGPDGIDLLMKILVYEPQQR
CKA1_Scer/1-324	-----	LPREFYD	---MDQYIR	KPWHRFIN	---DGNKHL	SGNDEIIDLDNLLRYDHQER
CDC7_Scer/1-272	-----	KPNGYSNG	---LKEFVY	DLLNKECTIGTFPE	---	HYWCQVLEQCFEMDPQKR
YCK1_Scer/1-261	-----			-----	EKKRSTNVY	-----DLAQG
YCK2_Scer/1-261	-----			-----	EKKRLTNVY	-----DLAQG
YCK3_Scer/1-296	-----			-----	MTKQKLNPD	-----DLLLLNNAIPYQFATYLKYA
HRR25_Scer/1-259	-----			-----	EKKLNV	SVE-----TLC
PRR1_Scer/1-317	-----			-----	WYRLSDY	KTNVGVKQIVENTLTRK
MPS1_Scer/1-277	-----			-----	NNEKIPK	SAIELMKACLYRNPDKR
GCN2_Scer/1-268	-----			-----	FPPDFDDN	-----KMKVEKKIIRLLIDHDPNKR
KIN3_Scer/1-319	-----			-----	KCDTVPEY	-----YSRGLNAIHSMDIVNLRTR
PRK1_Scer/1-277	-----			-----	QYSDRL	KNLIRLMLMEAPSQR
ARK1_Scer/1-277	-----			-----	NYSEQL	KGLIRDILVQDPRHR
AKL1_Scer/1-285	-----			-----	KYSSKL	INLIIIMLAENPNLR
VHS1_Scer/1-321	-----			-----	ILKQIL	PLSDDFYSLLSKILQVNPKNR
SKM1_Scer/1-280	-----			-----	GSPKLRHPE	-----SVSKQTKQFLDACLVNVESR
CLA4_Scer/1-280	-----			-----	GTPKLRHPE	-----SLSLEIKRFLSVCLQVDVRYR
STE20_Scer/1-252	-----			-----	GTPKLRHPE	-----NLSSSLKKFLDWCLCPEPEDR
CDC15_Scer/1-248	-----			-----	DTYPPS	-----SFSEPLKDFLSKCFVKNMYKR
FMP48_Scer/1-285	-----			-----	PFAFIYR	KYVVPRLSCGYNDEEDLHVSILQHTR
KSP1_Scer/1-260	-----			-----	VFSTMAY	DFQVLRYSLTIDPANRD
CAK1_Scer/1-304	-----			-----	IFEKVKQ	KFINCILGMVSPSPNER

Table S4, continued

	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-----	LGDKMHPWI-----	
PKH2_Scer/1-265	LT-----	ISQIKEHHFF-----	
KCC4_Scer/1-265	IK-----	IRDILSHPLL-----	
GIN4_Scer/1-271	IK-----	TRDILKHPLL-----	
HSL1_Scer/1-289	IT-----	TOEILKHPLI-----	
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-----	MDDLFDNPPFF-----	
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-----	LSFEPCPDYEGYRLLLL	
YCK2_Scer/1-261	RN-----	LSFEETPDYEGYRMLLL	
YCK3_Scer/1-296	RS-----	LKFDEDPDYD----YLI	
HRR25_Scer/1-259	KN-----	LKFDEKPDYLFARLFK	
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-----	ICQVLEEVSR-----	
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-----	CQILQELE-----	

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