

**Table S1. *Saccharomyces cerevisiae* Strains Used in These Studies, Related to Figures 1 and 4**

Name	Relevant genotype
<b>Microarray experiments</b>	
BY4741	MAT $\alpha$ <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>
RDKY7796, RDKY7797	BY4741 <i>sml1::HPH<sup>a</sup></i>
RDKY8051, RDKY8052	BY4741 <i>ste11::KanMX4<sup>b</sup></i>
RDKY7798, RDKY7799	BY4741 <i>ste11::KanMX4 sml1::HPH<sup>a</sup></i>
RDKY7804, RDKY7805	BY4741 <i>rfx1::KanMX4 sml1::HPH<sup>a</sup></i>
RDKY7802, RDKY7803	BY4741 <i>chk1::KanMX4 sml1::HPH<sup>a</sup></i>
RDKY7800, RDKY7801	BY4741 <i>dun1::KanMX4 sml1::HPH<sup>a</sup></i>
RDKY7813 <sup>c</sup>	BY4741 <i>rad53::KanMX4 sml1::HPH</i>
RDKY7814 <sup>c</sup>	BY4741 <i>rad53::KanMX4 sml1::HPH</i>
RDKY7807	RKDY7803 <i>chk1::KanMX4 dun1::natNT2 sml1::HPH</i>
RDKY7808	RKDY7802 <i>chk1::KanMX4 dun1::natNT2 sml1::HPH</i>
RDKY7809	RKDY7802 <i>chk1::KanMX4 rad53::natNT2 sml1::HPH</i>
RDKY7810	RKDY7803 <i>chk1::KanMX4 rad53::natNT2 sml1::HPH</i>
RDKY7811	RKDY7800 <i>dun1::KanMX4 rad53::natNT2 sml1::HPH</i>
RDKY7812	RKDY7801 <i>dun1::KanMX4 rad53::natNT2 sml1::HPH</i>
RDKY7806, RDKY7835	BY4741 <i>tel1::KanMX4 sml1::HPH<sup>a</sup></i>
RDKY7815 <sup>c</sup>	BY4741 <i>mec1::KanMX4 sml1::HPH</i>
RDKY7816 <sup>c</sup>	BY4741 <i>mec1::KanMX4 sml1::HPH</i>
NA <sup>d</sup>	RDKY7815 <i>mec1::KanMX4 tel1::natNT2 sml1::HPH</i>
NA <sup>d</sup>	RDKY7816 <i>mec1::KanMX4 tel1::natNT2 sml1::HPH</i>
RDKY7978, RDKY7979	BY4741 <i>hap1::KanMX4</i>
RDKY7976, RDKY7977	BY4741 <i>hap4::KanMX4<sup>b</sup></i>
RDKY7970, RDKY7971	BY4741 <i>rcs1::KanMX4<sup>b</sup></i>
RDKY7972, RDKY7973	BY4741 <i>sut1::KanMX4<sup>b</sup></i>
RDKY7974, RDKY7975	BY4741 <i>yap7::KanMX4<sup>d</sup></i>
<b>Mass Spec. experiments</b>	
SCY251 <sup>e</sup>	MAT $\alpha$ <i>ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2ΔBgl ade2Δ1 hom3-10 ade8 arg4Δ</i>
RDKY7855	SCY251 <i>sml1::HPH</i>
RDKY7856	RDKY7855 <i>rad53::natNT2</i>
RDKY7857	SCY251 <i>SWI6-TAP:kanMX4</i>
RDKY7858, RDKY7859	RDKY7857 <i>SWI6-TAP:kanMX4 sml1::HPH2</i>
RDKY7860	RDKY7858 <i>SWI6-TAP:kanMX4 sml1::HPH2 rad53::natNT2</i>
RDKY7879	RDKY7859 <i>SWI6-TAP:kanMX4 sml1::HPH2 rad53::natNT2</i>
RDKY7861, RDKY7862	RDKY7855 <i>GCN4-TAP:KanMX4</i>
RDKY7863, RDKY7864	RDKY7856 <i>rad53::natNT2 GCN4-TAP:KanMX4</i>
RDKY7865	RDKY7855 <i>NDD1-TAP:KanMX4</i>

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RDKY7866	RDKY7856 <i>rad53::natNT2 NDD1-TAP:KanMX4</i>
RDKY7867, RDKY7868	RDKY7855 <i>MCM1-TAP:KanMX4</i>
RDKY7869, RDKY7870	RDKY7856 <i>rad53::natNT2 MCM1-TAP:KanMX4</i>
RDKY7871, RDKY7872	RDKY7855 <i>MSN4-TAP:KanMX4</i>
RDKY7873, RDKY7874	RDKY7856 <i>rad53::natNT2 MSN4-TAP:KanMX4</i>
RDKY7875, RDKY7876	RDKY7855 <i>FKH2-TAP:KanMX4</i>
RDKY7877, RDKY7878	RDKY7856 <i>rad53::natNT2 FKH2-TAP:KanMX4</i>

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<sup>a</sup>These strains were constructed from BY4741 or the corresponding *STE1*, *RFX1*, *CHK1*, *DUN1* or *TEL1* gene deletion mutants derived from BY4741 from the Yeast Knockout Collection (Open Biosystems) by deleting *SML1* as indicated.

<sup>b</sup>*STE11*, *HAP4*, *RCS1*, *SUT1*, and *YAP7* gene deletion strains from the Yeast Knockout Collection (Open Biosystems) were streaked out, and two isolated colonies were selected for the studies here.

<sup>c</sup>These strains are *rad53Δ* and *mec1Δ* mutants from our laboratory collection, constructed by Dr. Christopher Putnam (Ludwig Institute San Diego Branch).

<sup>d</sup>NA, not available because strains were made freshly for each experiment and were not saved. Since deleting *TEL1* in the indicated *mec1Δ* strain results in extreme genome instability, the double mutant strain was used with minimal passaging.

<sup>e</sup>SCY251 was provided by Drs. Sheng-hong Chen and Huilin Zhou (Ludwig Institute San Diego Branch) and was constructed by deleting *ARG4* in the S288c strain RDKY3023 (also known as RDKY2672), which is *MATa ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2ΔBgl ade2Δ1 ade8* (Tishkoff et al., 1997).

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**Table S3. Summary of Genes Showing Significant Differences for Each Comparison in Table S2, Related to Figure 2**

Comparison (Kinases)	UP	DOWN	TOTAL	Comparison (TFs)	UP	DOWN	TOTAL
<b>Differential Expression</b>				<b>Differential Expression</b>			
BY4741.MMSVsNT <sup>a,b</sup>	897	759	1656	WT.MMSVsNT <sup>a,b</sup>	905	764	1669
WT.MMSVsNT <sup>a,b</sup>	950	722	1672	<i>sml1Δ</i> .MMSVsNT <sup>a,b</sup>	954	719	1673
<i>ste11Δ</i> .MMSVsNT <sup>a</sup>	937	690	1627	<i>dun1Δ sml1Δ</i> .MMSVsNT <sup>a</sup>	687	486	1173
<i>dun1Δ</i> .MMSVsNT <sup>a</sup>	677	476	1153	<i>rad53Δ sml1Δ</i> .MMSVsNT <sup>a</sup>	539	434	973
<i>rad53Δ</i> .MMSVsNT <sup>a</sup>	536	436	972	<i>ste11Δ</i> .MMSVsNT <sup>a</sup>	940	694	1634
<i>rfx1Δ</i> .MMSVsNT <sup>a</sup>	902	732	1634	<i>rfx1Δ sml1Δ</i> .MMSVsNT <sup>a</sup>	905	749	1654
<i>tel1Δ</i> .MMSVsNT	876	651	1527	<i>sut1Δ</i> .MMSVsNT	635	462	1097
<i>chk1Δ</i> .MMSVsNT	935	649	1584	<i>rcs1Δ</i> .MMSVsNT	880	671	1551
<i>chk1Δ rad53Δ</i> .MMSVsNT	309	235	544	<i>yap7Δ</i> .MMSVsNT	802	591	1393
<i>chk1Δ dun1Δ</i> .MMSVsNT	649	482	1131	<i>hap1Δ</i> .MMSVsNT	848	614	1462
<i>dun1Δ rad53Δ</i> .MMSVsNT	494	410	904	<i>hap4Δ</i> .MMSVsNT	791	640	1431
<i>tel1Δ mec1Δ</i> .MMSVsNT	453	332	785				
<i>mec1Δ</i> .MMSVsNT	716	566	1282				
<b>effect of <i>SML1</i> deletion</b>				<b>effect of <i>SML1</i> deletion</b>			
WTvsBY4741	1	1	2	<i>sml1Δ</i> vsWT	1	0	1
<b>mutant vs. WT</b>				<b>mutant vs. WT</b>			
<i>ste11Δ</i> vsWT	8	10	18	<i>ste11Δ</i> vsWT	6	8	14
<i>dun1Δ</i> vsWT	6	109	115	<i>rfx1Δ sml1Δ</i> vsWT	16	45	61
<i>rad53Δ</i> vsWT	121	604	725	<i>sut1Δ</i> vsWT	22	215	237
<i>rfx1Δ</i> vsWT	31	41	72	<i>rcs1Δ</i> vsWT	29	36	65
<i>tel1Δ</i> vsWT	8	27	35	<i>yap7Δ</i> vsWT	13	36	49
<i>chk1Δ</i> vsWT	2	2	4	<i>hap1Δ</i> vsWT	25	60	85
<i>chk1Δ rad53Δ</i> vsWT	24	479	503	<i>hap4Δ</i> vsWT	28	114	142
<i>dun1Δ rad53Δ</i> vsWT	77	463	540				
<i>tel1Δ mec1Δ</i> vsWT	47	459	506				
<i>mec1Δ</i> vsWT	33	146	179				
<i>chk1Δ dun1Δ</i> vsWT	10	98	108				
<b>other comparisons</b>							
<i>tel1Δ mec1Δ</i> vs <i>rad53Δ</i>	39	19	58				
<i>tel1Δ mec1Δ</i> vs <i>mec1Δ</i>	50	143	193				
<i>dun1Δ rad53Δ</i> vs <i>rad53Δ</i>	9	1	10				
<i>chk1Δ rad53Δ</i> vs <i>rad53Δ</i>	15	6	21				
<i>chk1Δ dun1Δ</i> vs <i>dun1Δ</i>	16	11	27				

Shown are the total numbers of genes showing significant differences for each comparison. Yellow cells indicate upregulated genes, and blue cells indicate downregulated genes. Red cells indicate genes that show a significant decrease in differential expression in the mutant relative to wild-type and are, thus, checkpoint kinase-dependent genes, while green cells indicate genes that show an increase in differential expression in the mutant (shown in Figure 2B). The

checkpoint kinase mutant analysis is detailed in the left half of the table, while the transcription factor mutant analysis is presented on the right.

<sup>a</sup>The same microarray data was used for both sets of analyses for BY4741, *sml1Δ*, *ste11Δ*, *dun1Δ* (*sml1Δ*), *rad53Δ* (*sml1Δ*), and *rfx1Δ* (*sml1Δ*).

<sup>b</sup>The wild-type (WT) sample for the kinase mutant analyses was *sml1Δ*, while BY4741 served as WT for the TF mutant analyses.

**Table S5. Overlap of Genes with Kinase-Dependent Differential Expression, Related to Figure 2**

	<i>chk1Δ</i>	<i>tel1Δ</i>	<i>rfx1Δ</i>	<i>dun1Δ</i>	<i>chk1Δ dun1Δ</i>	<i>mec1Δ</i>	<i>rad53Δ</i>	<i>chk1Δ rad53Δ</i>	<i>dun1Δ rad53Δ</i>	<i>tel1Δ mec1Δ</i>
<i>chk1Δ</i>	2/2	0/27	0/41	1/109	1/98	1/146	1/604	1/479	1/463	1/459
<i>tel1Δ</i>	0/2	27/27	8/41	13/109	9/98	13/146	21/604	20/479	20/463	22/459
<i>rfx1Δ</i>	0/2	8/27	41/41	16/109	17/98	27/146	39/604	39/479	40/463	40/459
<i>dun1Δ</i>	1/2	13/27	16/41	109/109	56/98	58/146	96/604	100/479	99/463	97/459
<i>chk1Δ dun1Δ</i>	1/2	9/27	17/41	56/109	98/98	45/146	88/604	90/479	88/463	87/459
<i>mec1Δ</i>	1/2	13/27	27/41	58/109	45/98	146/146	133/604	134/479	131/463	138/459
<i>rad53Δ</i>	1/2	21/27	39/41	96/109	88/98	133/146	604/604	402/479	413/463	368/459
<i>chk1Δ rad53Δ</i>	1/2	20/27	39/41	100/109	90/98	134/146	402/604	479/479	377/463	363/459
<i>dun1Δ rad53Δ</i>	1/2	20/27	40/41	99/109	88/98	131/146	413/604	377/479	463/463	348/459
<i>tel1Δ mec1Δ</i>	1/2	22/27	40/41	97/109	87/98	138/146	368/604	363/479	348/463	459/459

The numerator contains the total number of genes with reduced differential expression in both mutant strains, while the denominator contains the number of genes with reduced differential expression in the mutant in the top row.