

Supplemental Information

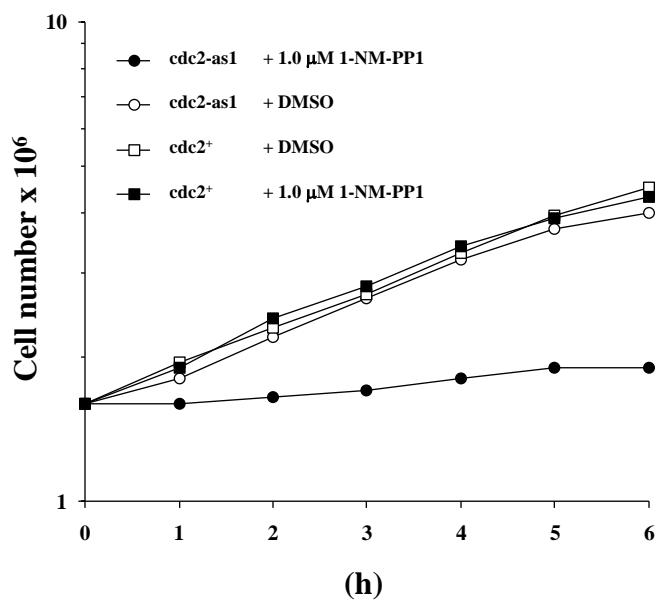
Generation of a set of conditional analog-sensitive alleles of essential protein kinases in the fission yeast *Schizosaccharomyces pombe*

Lubos Cipak, Chao Zhang, Ines Kovacicova, Cornelia Rumpf, Eva Miadokova, Kevan M. Shokat and Juraj Gregan

Supplemental Figure Legends

Figure S1. Growth, cellular morphology and FACS analysis of analog-sensitive protein kinases.

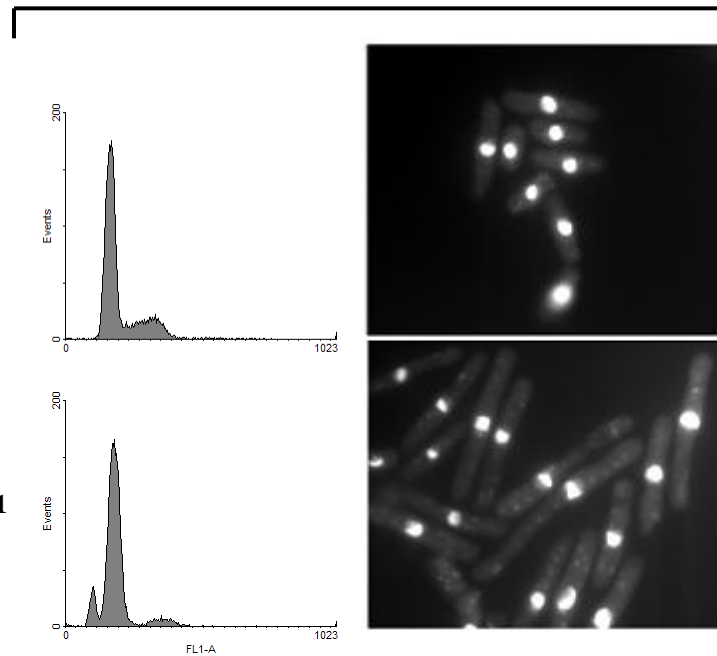
Cells were grown in liquid YES medium supplemented with ATP analog or DMSO at 32 °C, with the exception of the temperature-sensitive mutants, *cdc2-as1* and *pat1-as2* which were grown at 25 °C. At the indicated time point the number of cells was determined. Four hours after adding the inhibitor/DMSO, cells were harvested and the morphology of the cells was analyzed by microscopy and the cellular DNA content by flow cytometry.

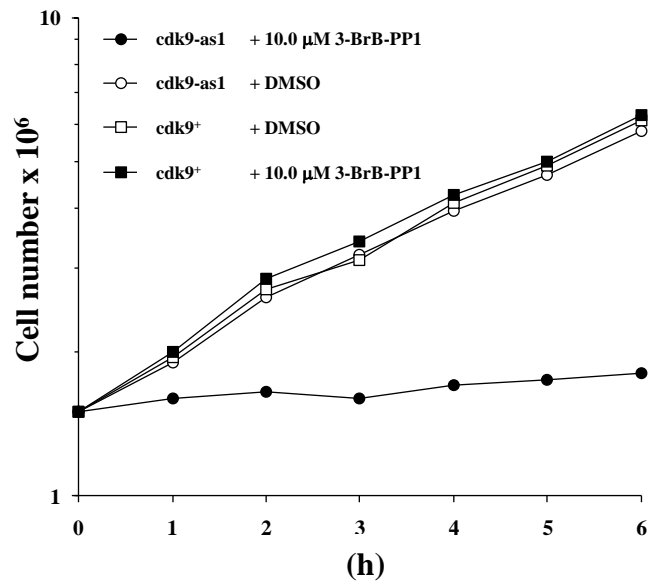


+ DMSO

+ 1.0 μM 1-NM-PP1
4 h incubation

cdc2-as1

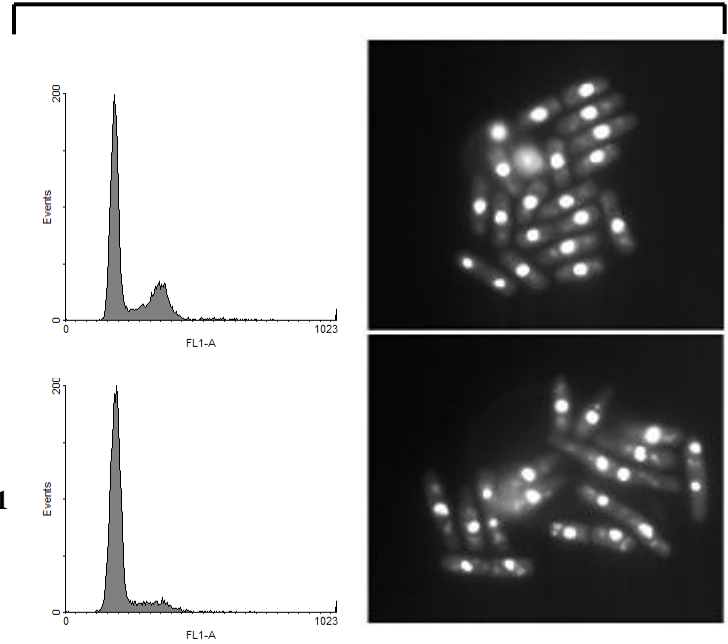


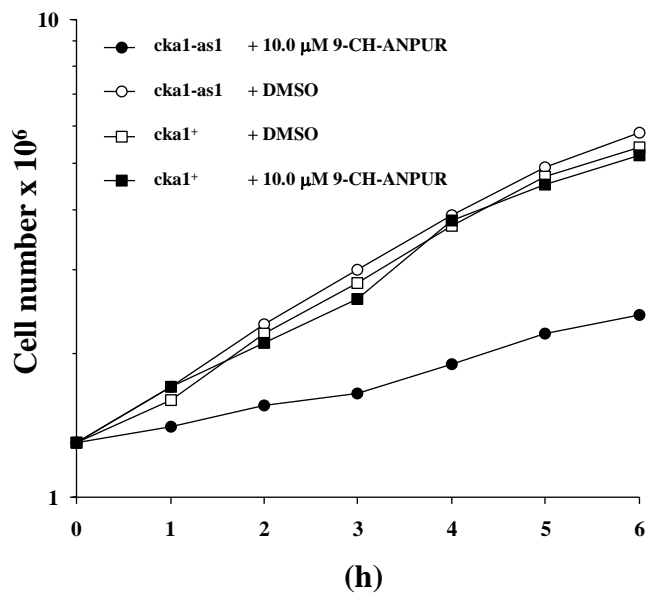


+ 10.0 μ M 3-BrB-PP1
4 h incubation

+ DMSO

cdk9-as1

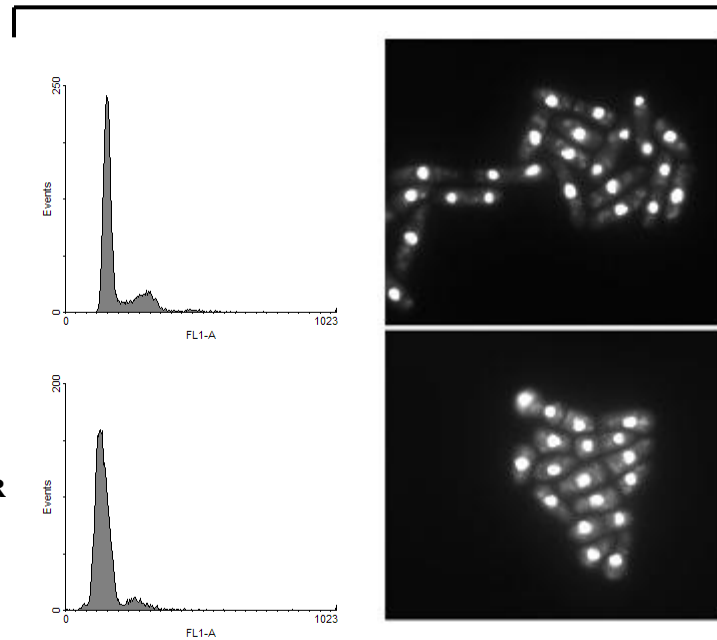


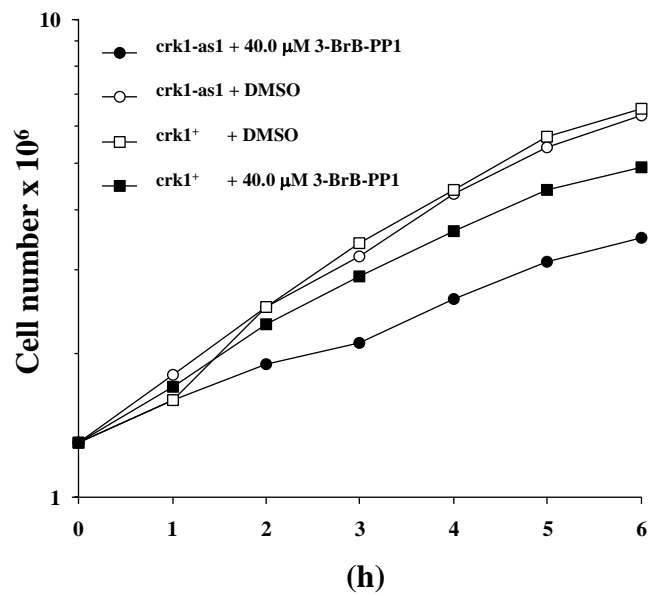


+ 10.0 μ M 9-CH-ANPUR
4 h incubation

+ DMSO

cka1-as1

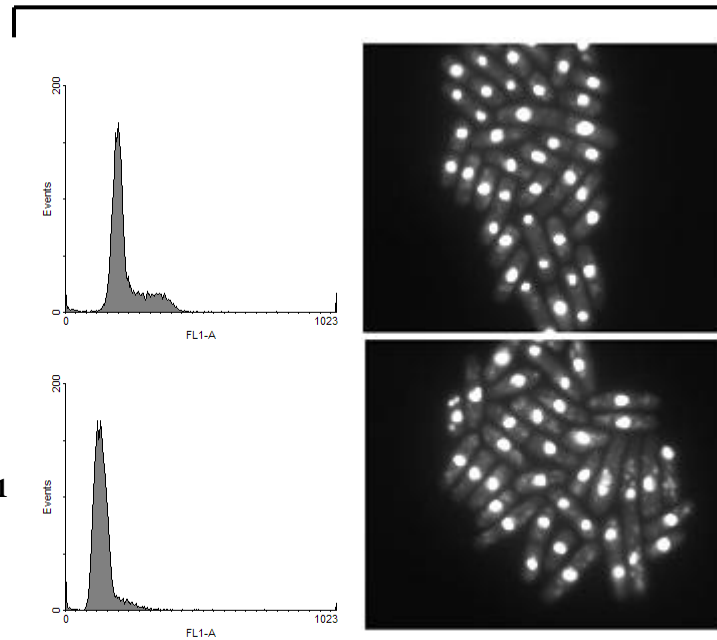


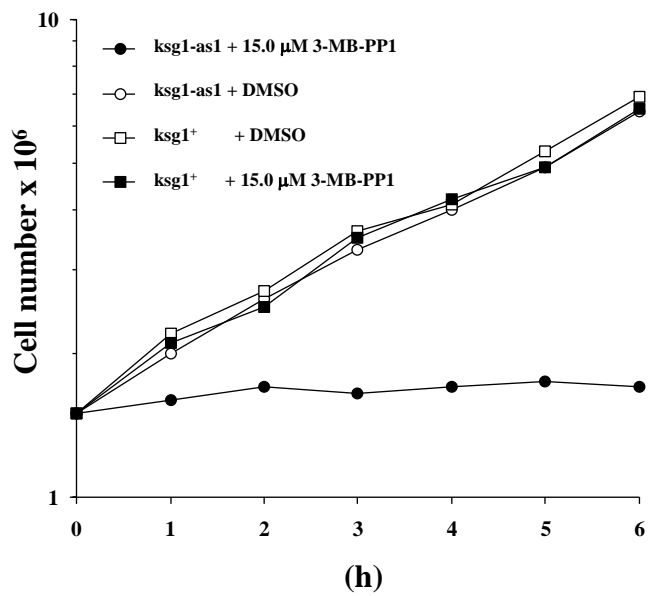


+ DMSO

+ 40.0 μM 3-BrB-PP1
4 h incubation

crk1-as1

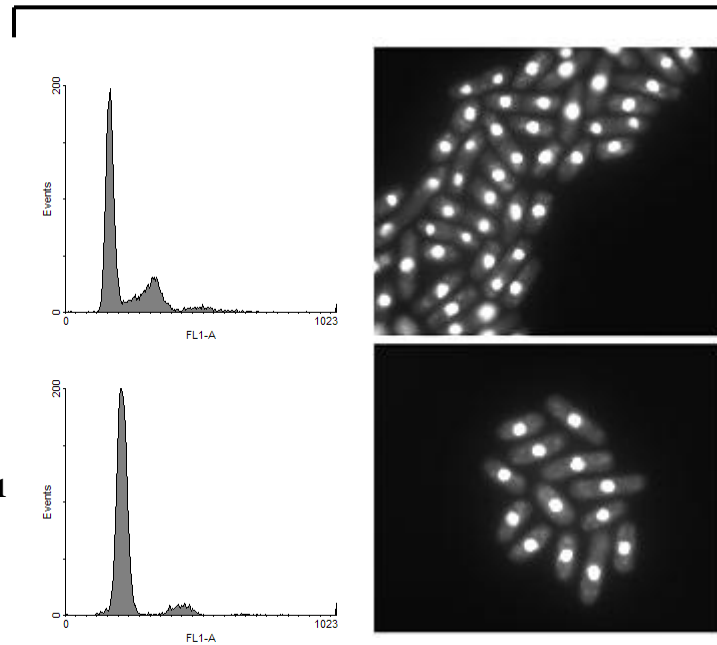


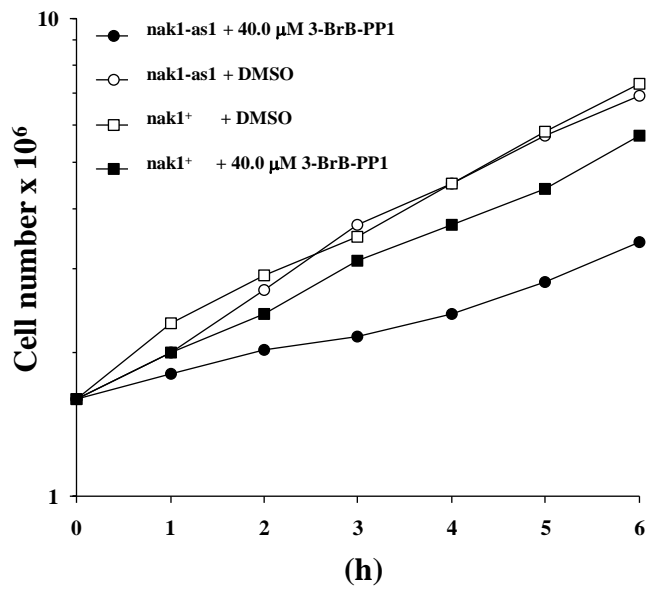


+ DMSO

+ 15.0 μM 3-MB-PP1
4 h incubation

$ksg1-as1$

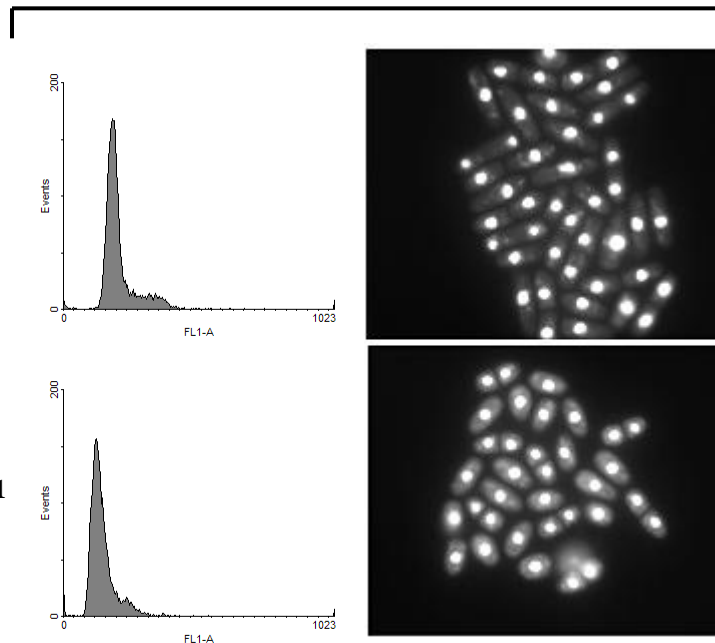


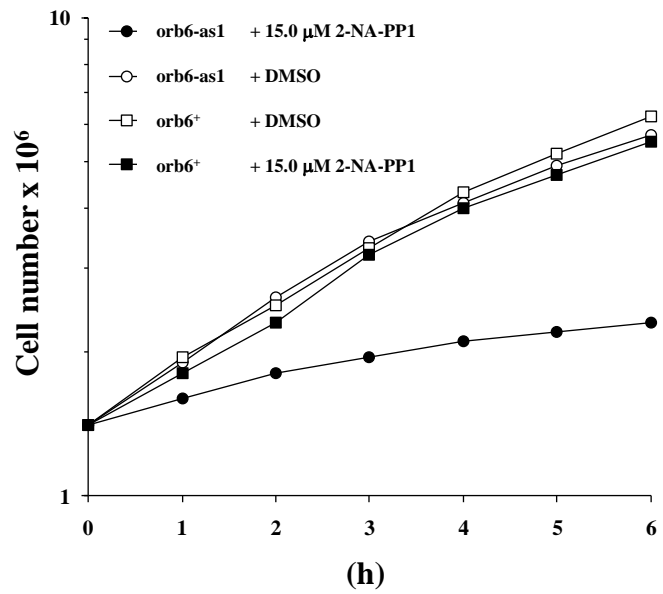


+ DMSO

+ 40.0 μM 3-BrB-PP1
4 h incubation

nak1-as1

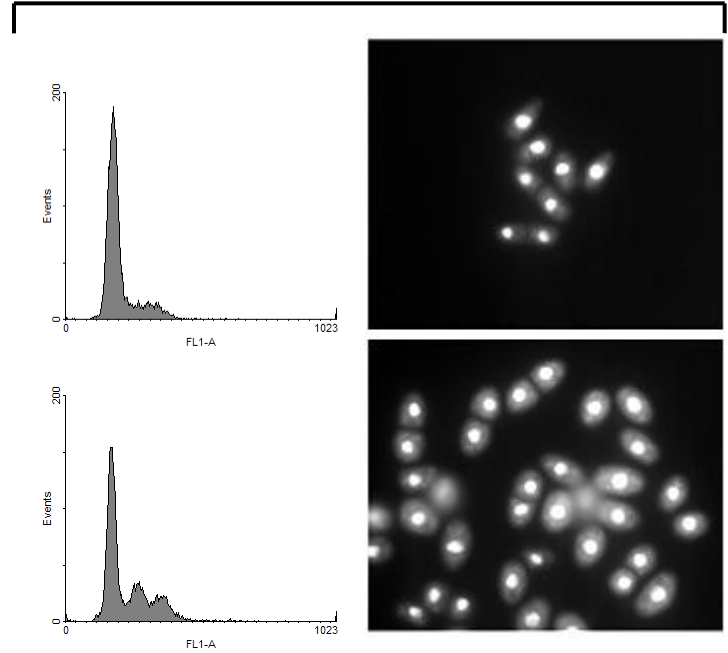


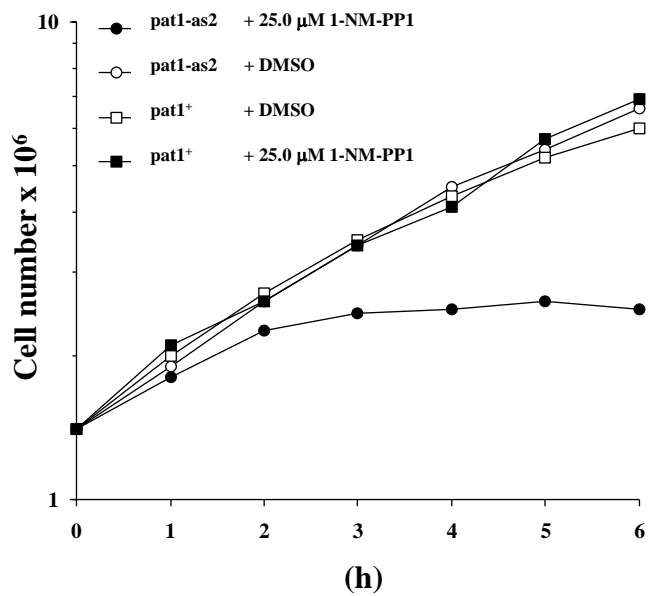


+ 15.0 μM 2-NA-PP1
4 h incubation

+ DMSO

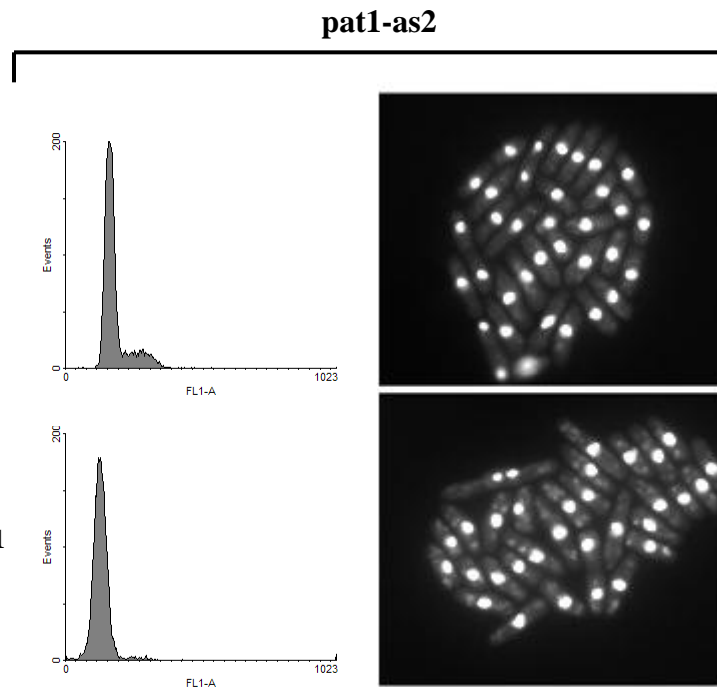
orb6-as1

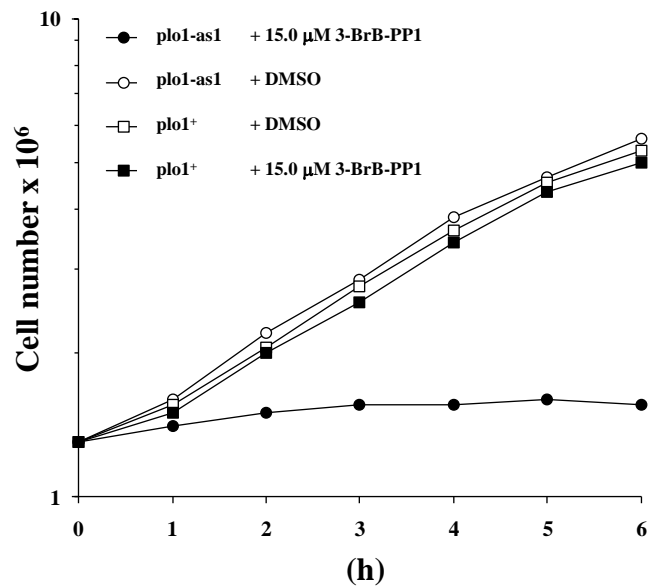




+ DMSO

+ 25.0 μM 1-NM-PP1
4 h incubation

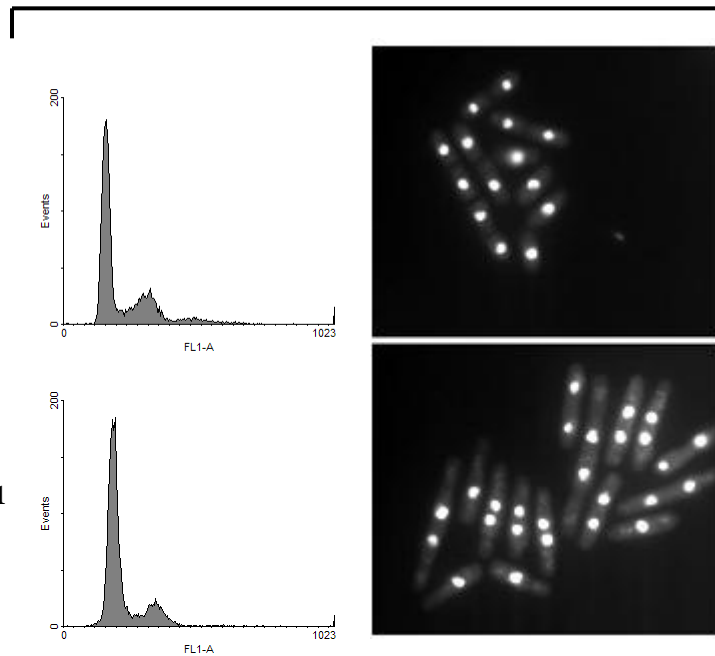


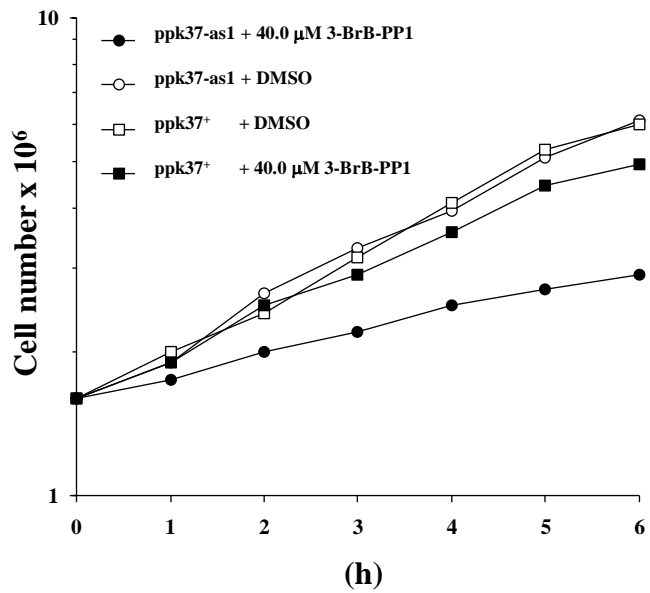


+ DMSO

+ 15.0 μM 3-BrB-PP1
4 h incubation

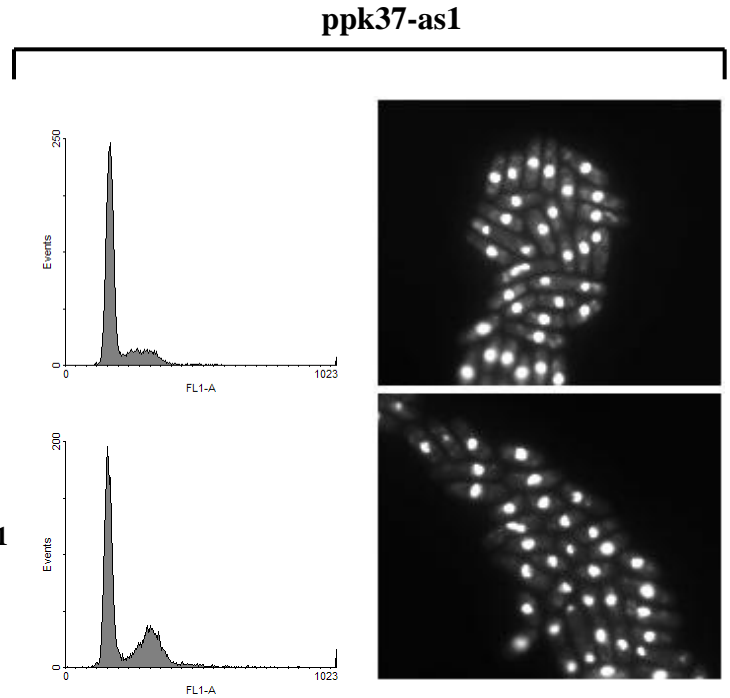
plo1-as1

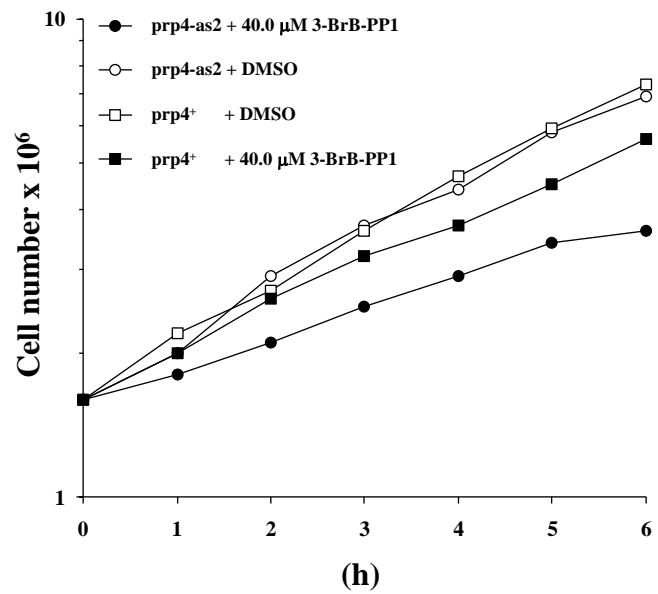




+ 40.0 μM 3-BrB-PP1
4 h incubation

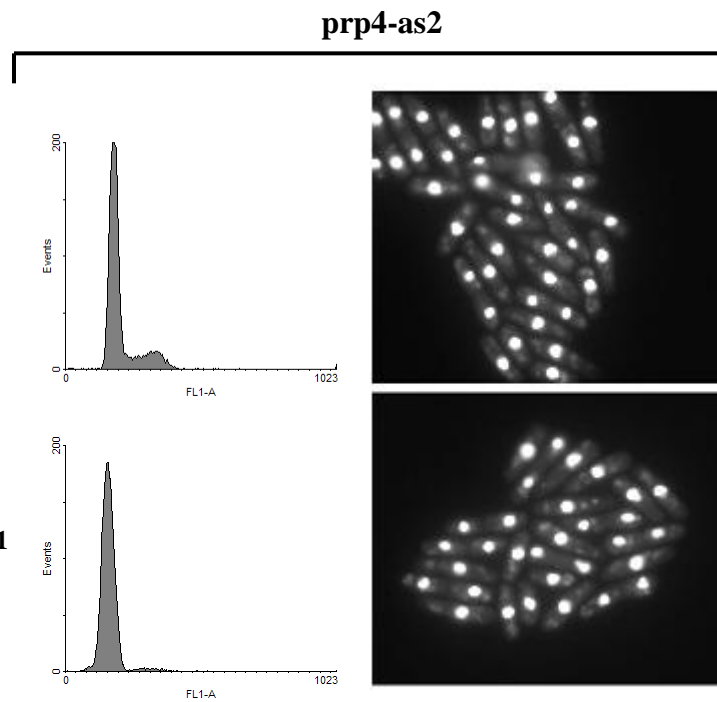
+ DMSO

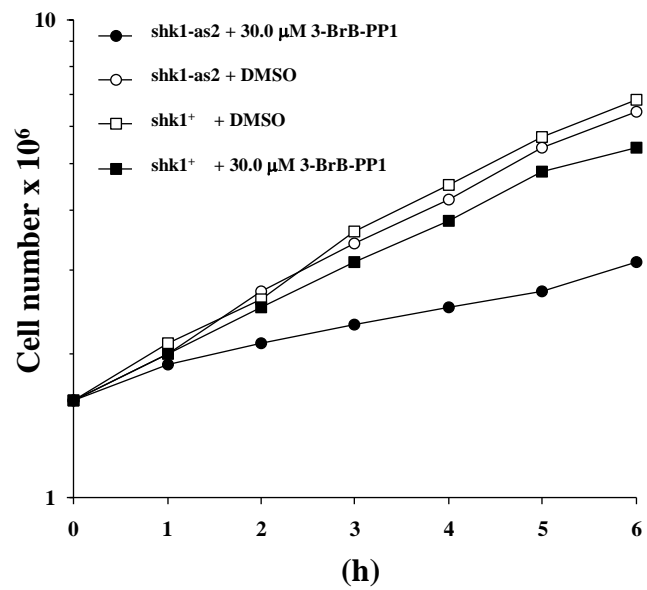




+ 40.0 μM 3-BrB-PP1
4 h incubation

+ DMSO

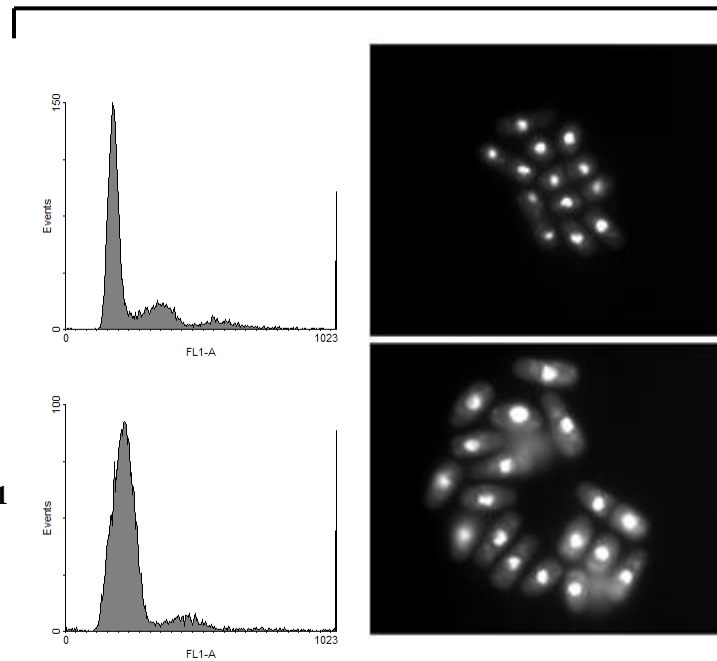


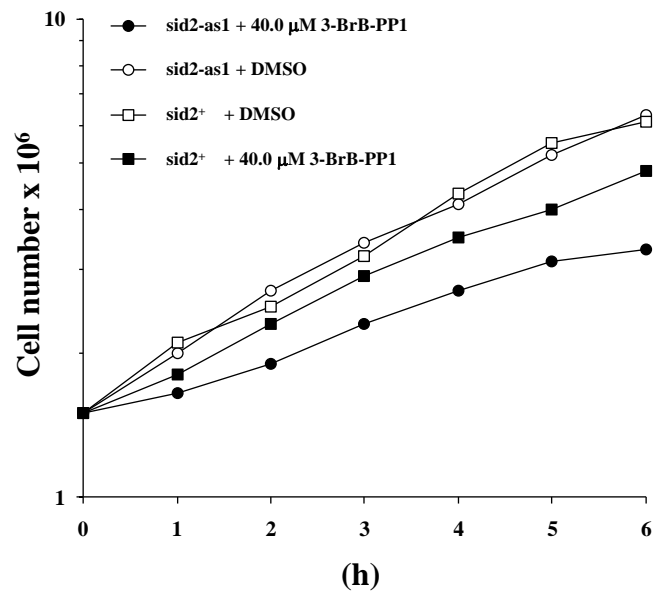


+ DMSO

+ 30.0 μM 3-BrB-PP1
4 h incubation

shk1-as2





+ DMSO

+ 40.0 μM 3-BrB-PP1
4 h incubation

sid2-as1

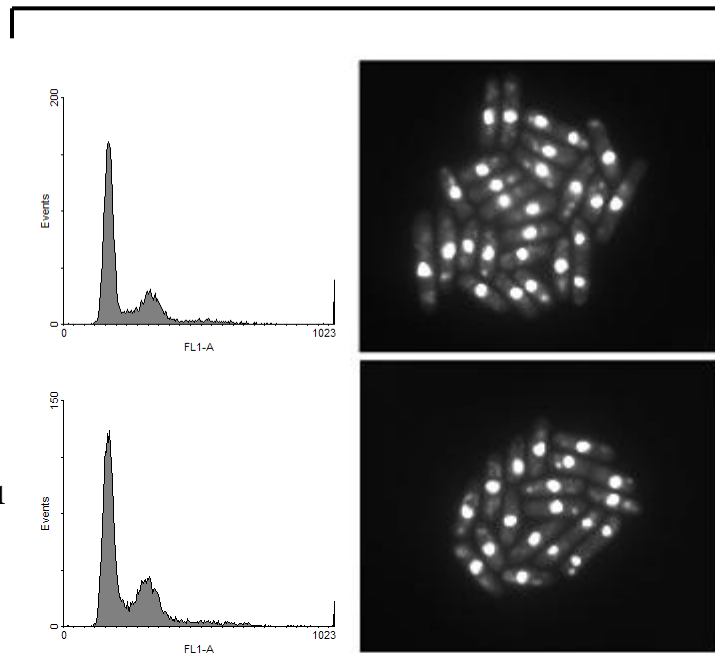


Table S1. Primers used for deletion of essential kinases.

<i>cdc2</i>	up-out	5'-aaaatctagacacctgtgtgtacacgggatagttc-3'
	up-in	5'-aaaactcgagtgcttatgttgtgtgtgctgtggg-3'
	down-out	5'-aaaatctagagaacggcgataattcccttgacag-3'
	down-in	5'-aaaaagatctcaacaatgggcaatttacagggc-3'
	up-check	5'-ggaggtaatgactttgcaatga-3'
	down-check	5'-tggttctcctgcgctgttt-3'

<i>cdc7</i>	up-out	5'-aaaatctagatgggtacggaaactggattgttc-3'
	up-in	5'-aaaactcgagcgatgtagcatttgctgcgtttg-3'
	down-out	5'-aaaatctagaatcgcgtcctccatatacaaaagcc-3'
	down-in	5'-aaaaagatctcgcattcccagtgacagac-3'
	up-check	5'-atcgccgctgttgatag-3'
	down-check	5'-ttaaagcatcgcgtcctcc-3'

<i>cdk9</i>	up-out	5'-aaaatctagacaccagggtcggttgagttc-3'
	up-in	5'-aaaactcgagccgatcgatgcgcgaaatg-3'
	down-out	5'-aaaatctagaggtgggagagggtggtcaag-3'
	down-in	5'-aaaaagatctaataggcctttccgcgtgatg-3'
	up-check	5'-cccgatttggtggtcattca-3'
	down-check	5'-ccatgtgcttcgacattccc-3'

<i>cka1</i>	up-out	5'-aaaatctagagcgacaattttaacgcctccagg-3'
	up-in	5'-aaaactcgagtgtccctctatgttaataatgcaaatcc-3'
	down-out	5'-aaaatctagattcattccatcgcttcttagattgc-3'
	down-in	5'-aaaaagatctcggacagcctttcattactagtccc-3'

up-check 5'-tttcgactgggaaagatgtgtct-3'

down-check 5'-cagctcgcaagattaacgc-3'

crk1 up-out 5'-aaaatctagagggtccttcgaccattcctgc-3'

up-in 5'-aaaactcgagcaaaaacacacaaacaattccagcaag-3'

down-out 5'-aaaatctagagaggggcagcaacacgcttc-3'

down-in 5'-aaaaagatctttggaatgatgtgactgtatgcgg-3'

up-check 5'-cgaaagtgaacgtgctgcaa-3'

down-check 5'-tcaaaggaaggcactcacgg-3'

hsk1 up-out 5'-aaaatctagatggcgcatgtatcatttcaagg-3'

up-in 5'-aaaactcgagtgctgccaaggaaggaacc-3'

down-out 5'-aaaatctagaatcaagcaagctggtggtgctc-3'

down-in 5'-aaaaagatctgctggcaatttctcatcaagcaatc-3'

up-check 5'-tgagcgtgccacgtaaaagc-3'

down-check 5'-ggcgaagaaacgagatgcct-3'

ksg1 up-out 5'-aaaatctagagaattccacaccegaaggaatgc-3'

up-in 5'-aaaaaagcttgcaatgtcatcaaaagaaaattgcac-3'

down-out 5'-aaaatctagagctgttgaagaatgggttccg-3'

down-in 5'-aaaaggatcctgaacacttccatccatccctc-3'

up-check 5'-ttcccgatattcagctacga-3'

down-check 5'-tctgctcgaggcaggaaag-3'

nak1 up-out 5'-aaaatctagacaccagacaacctgcatactcgg-3'

up-in 5'-aaaactcgagtgctggtgtagcgggtacg-3'

down-out 5'-aaaatctagatcgacgggttgatgctttgg-3'

down-in 5'-aaaagatcttgcccacggttaaggaatagagtcg-3'

up-check 5'-ccatgcaagatgagcagcaa-3'

down-check 5'-cccaacattttccctccgaa-3'

orb6 up-out 5'-aaaatctagaggcaaacgcgcatttaaaca-3'

up-in 5'-aaaactcgagtgaaagaaatggtgcaataaatgga-3'

down-out 5'-aaaatctagaagccaatgcaccatctgtcg-3'

down-in 5'-aaaagatcttttgttcattgtcctttgagcatc-3'

up-check 5'-tcaaccagatttgccgatgg-3'

down-check 5'-cgctctcggttgagcaaga-3'

pat1 up-out 5'-aaaatctagacgcaagcgttgattgtcgat-3'

up-in 5'-aaaactcgaggtccaattgatggcgaaaa-3'

down-out 5'-aaaatctagattcgtattccaaaagcttagttgc-3'

down-in 5'-aaaagatcttcgtaccgcacgttgttt-3'

up-check 5'-tccccagtccaactttcca-3'

down-check 5'-cgacctagtccaactttctatgcc-3'

plol up-out 5'-aaaatctagacgcggttgagaaatcgatggg-3'

up-in 5'-aaaactcgagaacgcaggtgtaactttgaggg-3'

down-out 5'-aaaatctagatgttctgctatttgattcaactg-3'

down-in 5'-aaaagatctaacgcaggagctcttgacgaataag-3'

up-check 5'-gtccaccacatcgtttcca-3'

down-check 5'-cgattccgaagcactactggg-3'

ppk37 up-out 5'-aaaatctagaccgtcctcccctacttg-3'

up-in 5'-aaaactcgagtggatgtagatggacggcg-3'

down-out 5'-aaaatctagacggggcggattcatcgttaag-3'
down-in 5'-aaaaagatcttgatgtgtttgtgccctttgc-3'
up-check 5'-gcgttaacgaggagcaatcg-3'
down-check 5'-ttttgcgggttagctcgg-3'

prp4 up-out 5'-aaaatctagaatcagcattgcgaatctatcattgcc-3'
up-in 5'-aaaactcgagtgttcaatgcctgatgaaaag-3'
down-out 5'-aaaatctagatggtgccgctatgtgtacagg-3'
down-in 5'-aaaaagatctacgcccaactggtttgtttgac-3'
up-check 5'-ctatggtggcaggtcgcac-3'
down-check 5'-tgcacaggtgccgagttt-3'

shk1 up-out 5'-aaaatctagaagggcaattccaaaagaactgc-3'
up-in 5'-aaaaaagcttaagaggcgcaaagggcacag-3'
down-out 5'-aaaatctagagcaggcgttctacttgagattcc-3'
down-in 5'-aaaaggatcctccatatccgcaggtgggc-3'
up-check 5'-tgcgatacgatgtatttgacca-3'
down-check 5'-tgtgaaatattccagcaggcg-3'

sid1 up-out 5'-aaaagctagcagctccttgaccaaaccga-3'
up-in 5'-aaaactcgaggcacaagctagatgcaccacg-3'
down-out 5'-aaaagctagcgcctgcctggtgtgatcgta-3'
down-in 5'-aaaaagatctcaacctgtttacagcattcaca-3'
up-check 5'-catgaaatggatgattgggca-3'
down-check 5'-agcgaaaaagaaatgccgga-3'

sid2 up-out 5'-aaaatctagaatcgtaagtttggcgacacc-3'

up-in 5'-aaaactcgagaacatccaacgttttggcccc-3'
down-out 5'-aaaatctagagaatgttacagctcttctgctgttgcc-3'
down-in 5'-aaaagatctcgaagatactgagcttcgctttgatg-3'
up-check 5'-cattcgcgaaagtaccgca-3'
down-check 5'-cctgttcgctcaatccttg-3'

Unique primers to confirm deletion:

upch-uni 5'-gtcgttagaacgcggctaca-3'
dwch-uni 5'-tctgggcctccatgctgctgg-3'

Note: restriction sites used for cloning are underlined.

Table S2. Plasmids used in this study.

p100	<i>pCloneNat1-Δcdc2 (XbaI)</i>
p101	<i>pCloneHyg1-cdc2-wt (BclI)</i>
p102	<i>pCloneHyg1-cdc2-as1 (F84G, BclI)</i>

p103	<i>pCloneNat1-Δcdc7 (XbaI)</i>
p104	<i>pCloneHyg1-cdc7-wt (NheI)</i>
p105	<i>pCloneHyg1-cdc7-as1 (L85G, NheI)</i>
p106	<i>pCloneHyg1-cdc7-as2 (L85A, NheI)</i>

p107	<i>pCloneNat1-Δcdk9 (XbaI)</i>
p108	<i>pCloneHyg1-cdk9-wt (StuI)</i>
p109	<i>pCloneHyg1-cdk9-as1 (T120G, StuI)</i>

p110	<i>pCloneNat1-Δcka1 (XbaI)</i>
p111	<i>pCloneHyg1-cka1-wt (BlpI)</i>
p112	<i>pCloneHyg1-cka1-as1 (F117G, BlpI)</i>
p113	<i>pCloneHyg1-cka1-as2 (F117A, BlpI)</i>

p114	<i>pCloneNat1-Δcrk1 (XbaI)</i>
p115	<i>pCloneHyg1-crk1-wt (XcmI)</i>
p116	<i>pCloneHyg1-crk1-as1 (L87G, XcmI)</i>
p117	<i>pCloneHyg1-crk1-as2 (L87A, XcmI)</i>

p118	<i>pCloneNat1-Δhsk1 (XbaI)</i>
p119	<i>pCloneHyg1-hsk1-wt (AccI)</i>
p120	<i>pCloneHyg1-hsk1-as1 (L173G, AccI)</i>
p121	<i>pCloneHyg1-hsk1-as2 (L173A, AccI)</i>

p122 *pCloneNat1- Δ ksg1 (XbaI)*

p123 *pCloneHyg1-ksg1-wt (AfeI)*

p124 *pCloneHyg1-ksg1-as1 (L177G, AfeI)*

p125 *pCloneNat1- Δ nak1 (XbaI)*

p126 *pCloneHyg1-nak1-wt (BsiWI)*

p127 *pCloneHyg1-nak1-as1 (M88G, BsiWI)*

p128 *pCloneHyg1-nak1-as2 (M88A, BsiWI)*

p129 *pCloneNat1- Δ orb6 (XbaI)*

p130 *pCloneHyg1-orb6-wt (SwaI)*

p131 *pCloneHyg1-orb6-as1 (M170G, SwaI)*

p132 *pCloneNat1- Δ pat1 (XbaI)*

p133 *pCloneHyg1-pat1-wt (PpuMI)*

p134 *pCloneHyg1-pat1-as1 (L95G, PpuMI)*

p135 *pCloneHyg1-pat1-as2 (L95A, PpuMI)*

p136 *pCloneNat1- Δ plo1 (XbaI)*

p137 *pCloneHyg1-plo1-wt (BclI)*

p138 *pCloneHyg1-plo1-as1 (L117G, BclI)*

p139 *pCloneNat1- Δ ppk37 (XbaI)*

p140 *pCloneHyg1-ppk37-wt (XcmI)*

p141 *pCloneHyg1-ppk37-as1 (M537G, XcmI)*

p142 *pCloneHyg1-ppk37-as2 (M537A, XcmI)*

p143 *pCloneNat1- Δ prp4 (XbaI)*

p144 *pCloneHyg1-prp4-wt (SfuI)*

p145	<i>pCloneHyg1-prp4-as1 (F238G, SfuI)</i>
p146	<i>pCloneHyg1-prp4-as2 (F238A, SfuI)</i>
<hr/>	
p147	<i>pCloneNat1-Δshk1 (XbaI)</i>
p148	<i>pCloneHyg1-shk1-wt (Bst1107I)</i>
p149	<i>pCloneHyg1-shk1-as1 (M460G, Bst1107I)</i>
p150	<i>pCloneHyg1-shk1-as2 (M460A, Bst1107I)</i>
<hr/>	
p151	<i>pCloneNat1-Δsid1 (NheI)</i>
p152	<i>pCloneHyg1-sid1-wt (PaeI)</i>
p153	<i>pCloneHyg1-sid1-as1 (M84G, PaeI)</i>
p154	<i>pCloneHyg1-sid1-as2 (M84A, PaeI)</i>
<hr/>	
p155	<i>pCloneNat1-Δsid2 (XbaI)</i>
p156	<i>pCloneHyg1-sid2-wt (BsaBI)</i>
p157	<i>pCloneHyg1-sid2-as1 (M285G, BsaBI)</i>
p158	<i>pCloneHyg1-sid2-as2 (M285A, BsaBI)</i>

Note: restriction enzymes to linearize the plasmids and corresponding gate-keeper residues are listed.

Table S3. Primers used to create integrative plasmids bearing mutant kinase alleles.

<i>cdc2</i>	cdc2-promotor	atatc <u>tcgaga</u> agtctttgacagttttacaatt
	cdc2-terminator	atat <u>ggatcct</u> gcttttgtaaaaagaacgg
	cdc2-sense (F84G)	gctgaatcaaagttgatcttgtt <u>ggg</u> agtttttagacatgg
	cdc2-anti-sense (F84G)	ccatgtctaaaaact <u>cccca</u> acaagatacaactttgattcagc
<i>cdc7</i>	cdc7-promotor	atata <u>tcgag</u> agctctatcctatttcaaaaacgac
	cdc7-terminator	atata <u>ggatc</u> caaagcagcaaattaatagcc
	cdc7-sense (L85G)	ttatcagacgaacgattctctatgcattatt <u>ggg</u> gagtagctgatttc
	cdc7-anti-sense (L85G)	gaaaatcacgtact <u>ccca</u> ataatgcatagagaatcggtcgtctgata
	cdc7-sense (L85A)	ttatcagacgaacgattctctatgcattatt <u>gcg</u> gagtagctgatttc
	cdc7-anti-sense (L85A)	gaaaatcacgtact <u>ccg</u> caataatgcatagagaatcggtcgtctgata
<i>cdk9</i>	cdk9-promotor	atatt <u>gtac</u> attgttcccgcgatgtaaaacatg
	cdk9-terminator	atat <u>ggatc</u> ctgactctgttttggatgacg
	cdk9-sense (T120G)	gtcgtggttctatatataggt <u>ggg</u> tccctatatggatcatgaccttt
	cdk9-anti-sense (T120G)	aaaggtcatgatccatataagg <u>acc</u> ccatataatagaccacgac
<i>cka1</i>	cka1-promotor	atatc <u>tcgag</u> gcgataataatattattacaataactcg
	cka1-terminator	atat <u>ggatc</u> cctaagggtgctctctaattaaggct
	cka1-sense (F117G)	cgataactaatattttatagagtaaaacaccatctttaatt <u>ggg</u> gaatttggtgacaatattgatttc
	cka1-anti-sense (F117G)	gaaatcaatattgtcaacaaatt <u>ccca</u> aataaagatggtgtttactctataaaatattagtatcg
	cka1-sense (F117A)	cgataactaatattttatagagtaaaacaccatctttaatt <u>gcg</u> gaatttggtgacaatattgatttc
	cka1-anti-sense (F117A)	gaaatcaatattgtcaacaaatt <u>ccg</u> caataaagatggtgtttactctataaaatattagtatcg
<i>crk1</i>	crk1-ATG	atatc <u>tcgag</u> caagtgacgtatgtgaagg
	crk1-terminator	atat <u>ggatc</u> cagatgaaaataagagtgtcc

crk1-sense (L87G)	gtccaacctcaacatcatc ggg gaattcctggacagtgat
crk1-anti-sense (L87G)	atcactgtccaggaatt ccc gatgatggtgaggttgac
crk1-sense (L87A)	gtccaacctcaacatcatc cg gaattcctggacagtgat
crk1-anti-sense (L87A)	atcactgtccaggaatt cgc gatgatggtgaggttgac

hsk1 hsk1-promotor	atatg tcgac gacatgaaatcttaatcttttccat
hsk1-terminator	atatagatcttaaggttttgggtaagggtc
hsk1-sense (L173G)	atgaagatcaggtagctgtagt gg accctactacgagcatac
hsk1-anti-sense (L173G)	gtatgctcgtagtagg gtcca actacgagtacctgatcttcat
hsk1-sense (L173A)	atgaagatcaggtagctgtagt gc accctactacgagcatac
hsk1-anti-sense (L173A)	gtatgctcgtagtagg gtgca actacgagtacctgatcttcat

ksg1 ksg1-promotor	atatg tcgac gaaaataacttggttgacaa
ksg1-terminator	atatagatctgtaaccacccttagtatgca
ksg1-sense (L177G)	ctttccaagatgctcacaatttgatgtt ggg agtcttgctcgaatg
ksg1-anti-sense (L177G)	catttcgagcaagact cca acaaaatacaaatgtgagcatcttggaag

nak1 nak1-ATG	atatg tcgac gaaaataaactgcttcttcg
nak1-terminator	atatagatctc gac gggttgatgctttg
nak1-sense (M88G)	gctcttatttgtaggtactaatttatggattatag ggg attattgcatggaggt
nak1-anti-sense (M88G)	acctccatgacaataat ccct tataatccataaattagtagctaccaataagagc
nak1-sense (M88A)	gctcttatttgtaggtactaatttatggattatag cg attattgcatggaggt
nak1-anti-sense (M88A)	acctccatgacaataat cgc tataatccataaattagtagctaccaataagagc

orb6 orb6-promotor	atatg tcgac gtgaaattgtcaacgttttgcc
orb6-terminator	atatagatctggtgagttttacaagccaatg
orb6-sense (M170G)	gctttcaagattcactgtatctttacttgatt ggg gagttttaccaggcg

	orb6-anti-sense (M170G)	cgcttggtaaaaact ccca atcaagtaaagatacagtgatcttgaaaagc
<i>pat1</i>	pat1-promotor	atatctc gagc gattgtgttccttctcatcc
	pat1-terminator	atat ggatcc ggtgatacaaatgactgcatgc
	pat1-sense (L95G)	aagacgccatttatgtcgtt ggcc agattgtccgaatgg
	pat1-anti-sense (L95G)	ccattcggacaatact ggcca acgacataaatggcgtctt
	pat1-sense (L95A)	aagacgccatttatgtcgtt ggcc agattgtccgaatgg
	pat1-anti-sense (L95A)	ccattcggacaatact gggca acgacataaatggcgtctt
<i>plol</i>	plol- promotor	atatctc gag ttaggggaacttctgaca
	plol-terminator	atat ggatcc ggaattagttataagtactttaaagca
	plol-sense (L117G)	ttatagattgtttgaagattcgcgaatattacttaatt ggg gagctttgtgaacataaatc
	plol-anti-sense (L117G)	gatttatgttcacaaagct ccca attaagtaaatctcgaatctcaaaacaatctataa
<i>ppk37</i>	ppk37-promotor	atatctc gag attccgtaggtaaattgcctcc
	ppk37-terminator	atat ggatc ctatctgaacacatcggctcagc
	ppk37-sense (M537G)	gacacgtggtgcgctgtt ggg gacttttgtccaga
	ppk37-anti-sense (M537G)	tctgggacaaaagt ccca acagcgcaccacgtgtc
	ppk37-sense (M537A)	gacacgtggtgcgctgtt ggg gacttttgtccaga
	ppk37-anti-sense (M537A)	tctgggacaaaagt cgca acagcgcaccacgtgtc
<i>prp4</i>	prp4-promotor	atatctc gag atccattttaataatcctcgc
	prp4-terminator	atat ccggg aatggtaactttgaataccgc
	prp4-sense (F238G)	gcacaaaaatcatctttgcatggt ggg tgagatgtaagtcttaatcttcgg
	prp4-anti-sense (F238G)	ccgaagattaagacttaacatctc acc caccatgcaaagatgattttgtgc
	prp4-sense (F238A)	gcacaaaaatcatctttgcatggt ggc tgagatgtaagtcttaatcttcgg
	prp4-anti-sense (F238A)	ccgaagattaagacttaacatctc agc caccatgcaaagatgattttgtgc

shk1	shk1-promotor	atata <u>agctt</u> cctaactgattagtgaaattaatataagat
	shk1-terminator	atag <u>gatcctt</u> ccttttagtttttataatgca
	shk1-sense (M460G)	taaatcagagttgtggatggtag <u>ggg</u> gaatacatgcgcggtg
	shk1-anti-sense (M460G)	caccgcgatgtattc <u>ccct</u> accatccacaactctgattta
	shk1-sense (M460A)	taaatcagagttgtggatggtag <u>cg</u> gaatacatgcgcggtg
	shk1-anti-sense (M460A)	caccgcgatgtattc <u>cgct</u> accatccacaactctgattta

sid1	sid1-promotor	atat <u>ctcgag</u> agtagattgccaattaaattg
	sid1-terminator	atag <u>gatcca</u> acttacaagtacgacgttca
	sid1-sense (M84G)	cggatacacattatggatttt <u>gggg</u> gagcatatggatggagg
	sid1-anti-sense (M84G)	cctccatccatagctc <u>cccc</u> aaaatccataatgtgtatccg
	sid1-sense (M84A)	cggatacacattatggatttt <u>ggcg</u> gagcatatggatggagg
	sid1-anti-sense (M84A)	cctccatccatagctc <u>cg</u> ccaaaatccataatgtgtatccg

sid2	sid2-ATG	atat <u>ctcgag</u> atatgtcaccagttgaagg
	sid2-terminator	atatt <u>gtacatt</u> gcagatagtgctatttcc
	sid2-sense (M285G)	ttcaggatacttcaaacatttatctcgct <u>ggg</u> gaattgttctctggtg
	sid2-anti-sense (M285G)	caccaggaacaaatt <u>cccc</u> agcgagataaatgtttgaagtatcctgaa
	sid2-sense (M285A)	ttcaggatacttcaaacatttatctcgct <u>cg</u> gaattgttctctggtg
	sid2-anti-sense (M285A)	caccaggaacaaattc <u>cg</u> cagcgagataaatgtttgaagtatcctgaa
