

Table S1. Yeast strains and plasmids

Yeast strains	Genotype/construction	Source or reference
A11993	W303 <i>MATa ade2-1 leu2-3 ura3 his3-11,15 can1-100 GAL psi+ HA-BFA1 cdc15::CDC15-as1(L99G)::URA3</i>	D'Aquino et al., 2005
CLY169	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 cdc5-10</i>	Pereira et al., 2002
ESM356-1	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1</i>	Pereira et al., 2001
ESM709	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[spc72^{Δ176-230} LEU2] Δspc72::kanMX6</i>	Usui et al., 2003
ESM888	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δkar9::klTRP1</i>	Pereira et al., 2000
ESM899	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δbfa1::kanMX6 Δkar9::klTRP1</i>	Pereira et al., 2000
ESM900	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbub2::kanMX6 Δkar9::klTRP1</i>	Pereira et al., 2000
ESM917	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[ts-act5 LEU2] Δact5::klTRP1 Δkar9::HIS3MX6</i>	This study
ESM1008	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6</i>	Pereira et al., 2000
ESM1278	<i>MATa cdc15-1 ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	Pereira et al., 2002
ESM2154	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δkar9::klTRP1 Δkin4::hphNT1</i>	Pereira and Schiebel, 2005
ESM2197	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 spc72-7 Δkin4::hghNT</i>	This study
ESM2204	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KIN4-GFP-hghNT1 SPC42-eqFP611-natNT2</i>	Pereira and Schiebel, 2005
ESM2263	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δkin4::hghNT1</i>	Pereira and Schiebel, 2005
ESM2492	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 CDC5-GFP-kanMX6 SPC42-eqFP611-hghNT1</i>	This study
ESM2497	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 spc72-7 KIN4-GFP-hghNT1 SPC42-eqFP611-natNT2</i>	This study
K699	W303 <i>MATa ade2-1 trp1-1 can1-100 leu2-3, -112 his3-11, -15 ura3</i>	K. Nasmyth
KCY2	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[nud1-2 LEU2] Δnud1::kanMX6</i>	Gruneberg et al., 2000
KAY12	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbub2::URA3</i>	Pereira et al., 2000
MGY96	Expresses <i>GST-CDC5^{ΔN70 S165D T238D}</i>	S. Sedgwick
MGY97	Expresses <i>GST-CDC5^{ΔN70 N209A}</i>	S. Sedgwick
SHM1314	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 HIS3-pGal1-3HA-KIN4</i>	This study
SHM1326	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 SPC42-eqFP611-kanMX6 natNT2-P_{GALI}-GFP-KIN4-pr-LEU2</i>	This study
SHM1327	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 SPC42-eqFP611-kanMX6 natNT2-P_{GALI}-GFP-KIN4-pr-SS-LEU2</i>	This study
SHM1346	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δkin4B::HIS3MX6::[KIN4-3HA-hphNT1 LEU2]</i>	This study
SHM1347	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δkin4B::HIS3MX6::[KIN4^{T209A}-3HA-hphNT1 LEU2]</i>	This study
SHM1378	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δdyn1::klTRP1 CDC5-GFP-hphNT1 SPC42-eqFP611-kanMX6</i>	This study
SHM1447	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δbfa1::hphNT1 HIS3-P_{GALI}-3HA-KIN4</i>	This study
SHM1448	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[SPC72 LEU2] Δspc72::kanMX6 KIN4-GFP-hphNT1 SPC42-eqFP611-natNT2</i>	This study
SHM1449	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[spc72^{Δ176-230} LEU2] Δspc72::kanMX6 KIN4-GFP-hphNT1 SPC42-eqFP611-natNT2</i>	This study
SHM1512	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1::[spc72^{Δ176-230} LEU2] Δspc72::kan Δkar9::TRP1</i>	This study
SHM1515	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1</i>	This study
SHM1550	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 Δkin4::HIS3MX6</i>	This study
SHM1551	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 Δbub2::HIS3MX6</i>	This study
SHM1570	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 Δbfa1::HIS3MX6</i>	This study
SHM1575	W303 <i>MATa ade2-1 leu2-3 ura3 his3-11,15 can1-100 GAL psi+ HA-BFA1 cdc15::CDC15-as1(L99G)::URA3 pGal1-KIN4-LEU2</i>	This study
SHM1584	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 KIN4-pr-LEU2</i>	This study
SHM1585	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 KIN4-pr-SS-LEU2</i>	This study
SHM1586	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1 LEU2]</i>	This study
SHM1587	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1^{2A} LEU2]</i>	This study
SHM1589	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 Δbfa1::HIS3::[BFA1 LEU2]</i>	This study
SHM1590	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δdyn1::klTRP1 Δbfa1::HIS3::[BFA1^{2A} LEU2]</i>	This study
SHM1597	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1-3HA-klTRP1 LEU2]</i>	This study
SHM1598	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1^{2A}-3HA-klTRP1 LEU2]</i>	This study
SHM1605	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 HIS3-P_{GALI}-3HA-KIN4 Δbfa1::hphNT1::[BFA1 LEU2]</i>	This study
SHM1606	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 HIS3-P_{GALI}-3HA-KIN4 Δbfa1::hphNT1::[BFA1^{2A} LEU2]</i>	This study
SHM1617	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 kan-P_{GALI}-GST-KIN4</i>	This study
SHM1618	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 Δkin4::HIS3MX6::[kan-P_{GALI}-GST-KIN4^{T209A} LEU2]</i>	This study
SHM1620	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1-GFP-hphNT1 LEU2]</i>	This study
SHM1621	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1^{2A}-GFP-hphNT1 LEU2]</i>	This study
SHM1748	<i>MATa ura3-52::[mCherry-Tub1 URA3] his3Δ200 trp1Δ63 leu2Δ1 SPC42-4xhcRed-kanMX6</i>	This study
SHM1749	<i>MATa ura3-52::[mCherry-Tub1 URA3] his3Δ200 trp1Δ63 leu2Δ1 SPC42-4xhcRed-kanMX6 Δkin4::HIS3MX6::[KIN4-SPB LEU2]</i>	This study
SHM1753	<i>MATa ura3-52::[mCherry-Tub1 URA3] his3Δ200 trp1Δ63 leu2Δ1 SPC42-4xhcRed-kanMX6 Δkin4::HIS3MX6::[KIN4-SPB LEU2] Δbfa1::klTRP1</i>	This study
SHM1760	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 Δbfa1::kanMX6::[BFA1^{2A}-3HA-klTRP1 LEU2] HIS3-P_{GALI}-CDC5</i>	This study
SHM1761	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 SPC42-4xhcRed-kanMX6 Δkin4::HIS3MX6::[KIN4-SPB-GFP-klTRP1 LEU2]</i>	This study
YPH499	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1</i>	Sikorski and Hieter, 1989
YMK205	<i>MATa ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 spc72-7</i>	Knop and Schiebel, 1998

Plasmids

pAK11	pRS306 carrying <i>mCherry-Tub1</i>	Khmelinskii et al., 2007
pBJ781	pRS305 carrying temperature-sensitive- <i>act5</i>	Muhua et al., 1998
pCL43	pGEX-5x-1 carrying <i>CDC5</i>	This study
pGEX-5x-1	<i>E.coli</i> expression vector carrying GST under the control of the lacZ promoter	GE Healthcare
pGP68	pGEX-5x-1 carrying <i>SPC72</i> ¹⁻²⁷¹	Knop and Schiebel, 1998
pHM152	pRS305 carrying <i>KIN4</i> ⁶¹⁴⁻⁸⁰⁰ - <i>pr</i>	This study
pHM153	pRS305 carrying <i>KIN4</i> ⁶¹⁴⁻⁸⁰⁰ - <i>pr-SS</i>	This study
pHM164	pRS305 carrying <i>KIN4</i>	This study
pHM165	pRS305 carrying <i>KIN4</i> ^{T209A}	This study
pHM169	pRS305 carrying <i>KIN4-SPC72</i> ¹⁷⁷⁻⁶²²	This study
pHM191	pGEX-5x-1 carrying <i>SPC72</i> ^{Δ1-98}	This study
pHM224-21	pRS305 carrying <i>BFA1</i>	This study
pHM225-29	pRS305 carrying <i>BFA1</i> ^{S150A S180A}	This study
pHM229-11	pRS305 carrying <i>BFA1</i> ^{S150A S180A S490A}	This study
pHM238-10	pMal-2c carrying <i>BFA1</i> ^{S150A S180A S490A}	This study
pHM240-3	pMal-2c carrying <i>BFA1</i> ^{S150A S180A}	This study
pMal-2c	<i>E.coli</i> expression vector carrying MBP under the control of the tac promoter	New England Biolabs, Inc.
pMal-2c-BFA1	pMal-2c carrying <i>BFA1</i>	S. Sedgwick
pMal-2c-TEM1	pMal-2c carrying <i>TEM1</i>	S. Sedgwick
pMK298	pGEX-5x-1 carrying <i>NUD1</i> ¹⁻⁴³⁵	This study
pSM413	pGEX-4T-1 carrying <i>SPC72</i> ¹⁻⁶²²	This study
pSM447	pRS316 carrying <i>SPC72</i>	Knop and Schiebel, 1998
pTH158	pMal-2c carrying <i>BUB2</i>	Höfken et al., 2004
pTU48-1	pGEX-5x-1 carrying <i>SPC72</i> ^{Δ176-230}	Usui et al., 2003

K. Nasmyth, University of Oxford, Oxford, UK. S. Sedgwick, National Institute for Medical Research, London, UK. NatNT2 encodes the *Streptomyces noursei* *nat1* gene. *klTRP1* encodes the *Kluyveromyces lactis* *TRP1* gene. *hphNT1* encodes the *E. coli* *hph* gene.

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