Figure S1

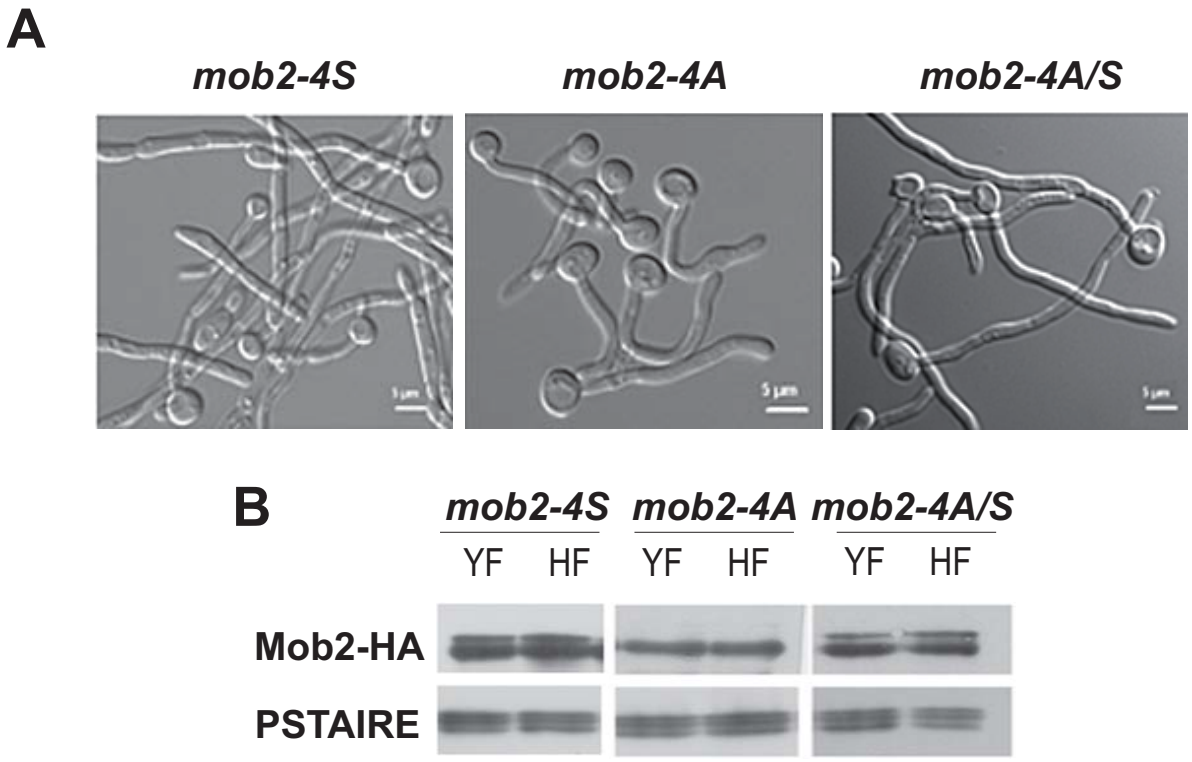
A
mob2-4S
mob2-4A
mob2-4A/S
B
mob2-4S
mob2-4A
mob2-4A/S
YF
HF
YF
HF
YF
HF
Mob2-HA
PSTAIRE

Figure S2

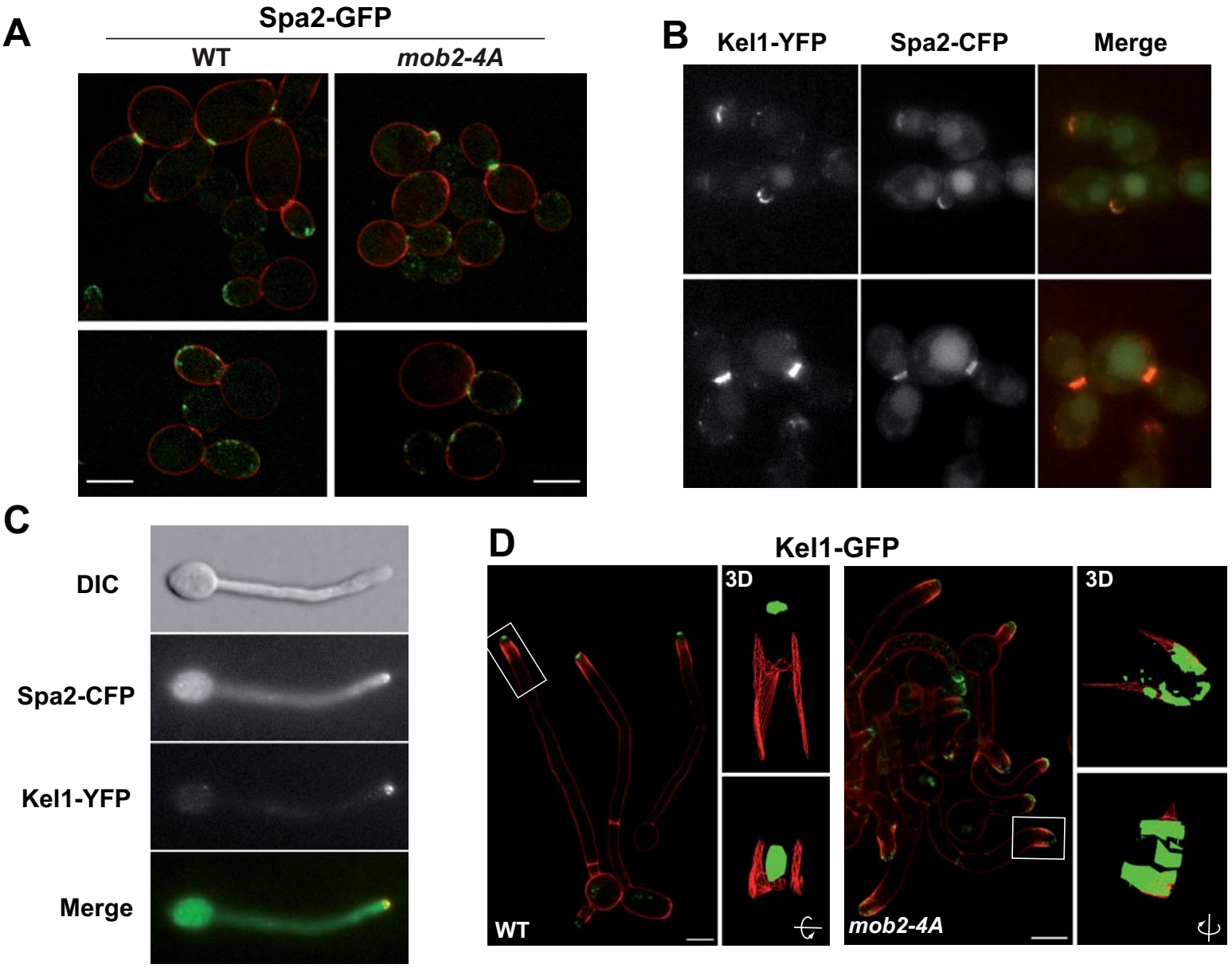
A
Spa2-GFP
WT
mob2-4A
B
Kel1-YFP
Spa2-CFP
Merge
C
DIC
Spa2-CFP
Kel1-YFP
Merge
D
Kel1-GFP
3D
3D
WT
mob2-4A

Figure S3

A

```
scSpa2  RDIFHYYVSLKTFFEVTGE--NRDRSNSTRAQKARAKLLKLSSSQFYELSTDVSDELQRR  70
        +D+  +Y  LK F  ++ +  +R +SNS+RAQ+AR KLLKLSS+QF ELSTDV DEL+RR
caSpa2  KDLVQHYKVLKQFLAISDDQQSRSKSNSSRAQRAREKLLKLSSAQFKELSTDVYDELRRR  63

scSpa2  IGEDANQPDYLLPKANFHMKRNQARQKLANLSQTRFNDLLDDILFEIKRRGFDKDLDAPR  130
        I E  ++PDYLLPK++FH KRNQARQKLA+L QTRF DL+ DI +EI+RR   +  +
caSpa2  IDESRSEPDYLLPKSSFHPKRNQARQKLASLPQTRFKDLVADISYEIERRDLHVERQSQH  123

scSpa2  PPLPQ----------------------------------------PMKQEVSKDSDDTA  140
                                          *          +EV ++ +  +
caSpa2  SHTTSMSSNGSQFQHERKSSLASSHHRNDSANGYHSRSASHHLNDFAATKEVDEEKESDS  183

scSpa2  RTSTNSSSVTQVA-PN-------VSVQPSLVIPKMASIDWSSEEEEEEQVKEKPNEPEGK  201
        R   N++S   +  PN       + +QPS V+P  A++DWSS++E +++           +
caSpa2  RDDLNNTSSKNITMPNAEASNQSIGIQPSQVVPTKANLDWSSDDEGDDE----------Q  233

scSpa2  QTSMDEKKEAKPALNPIVTDSDLPDSQ  228
        +   +EK + K   +P  T ++   +Q
caSpa2  EEEEEEKGKVKNISDPKHTQAEQHQNQ  261
```

B

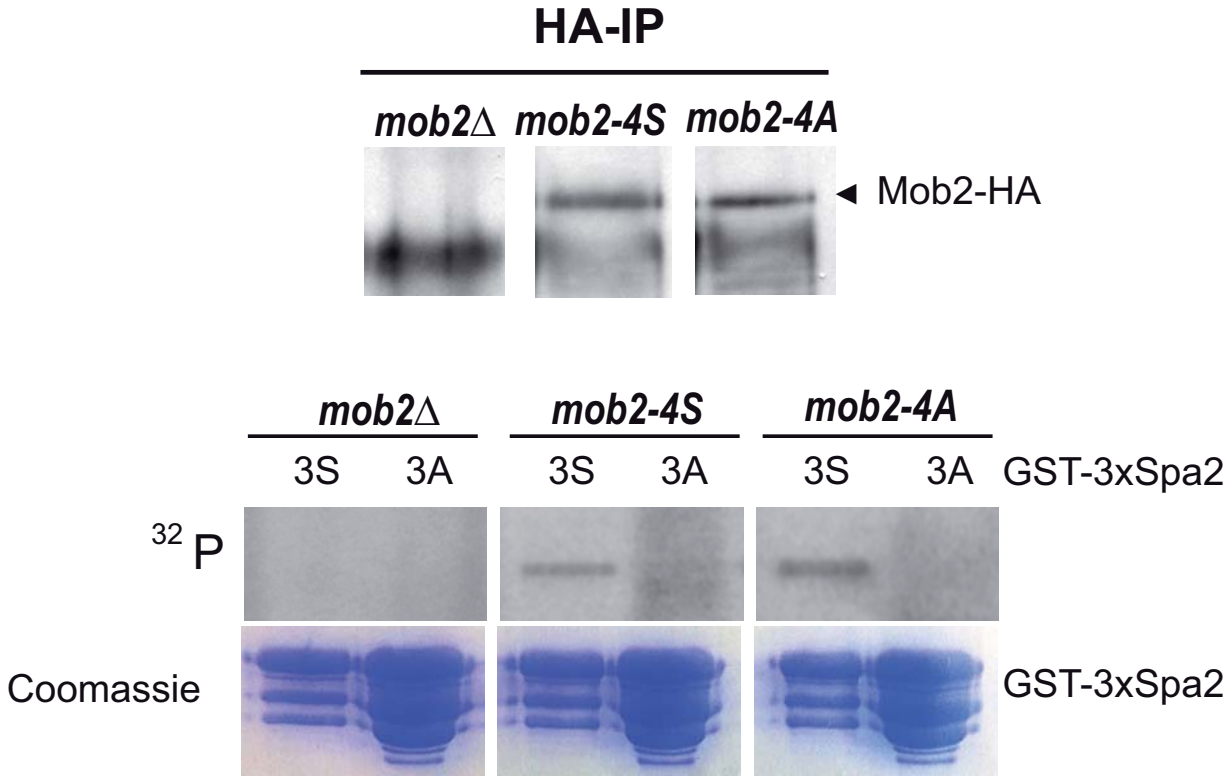

Figure S4

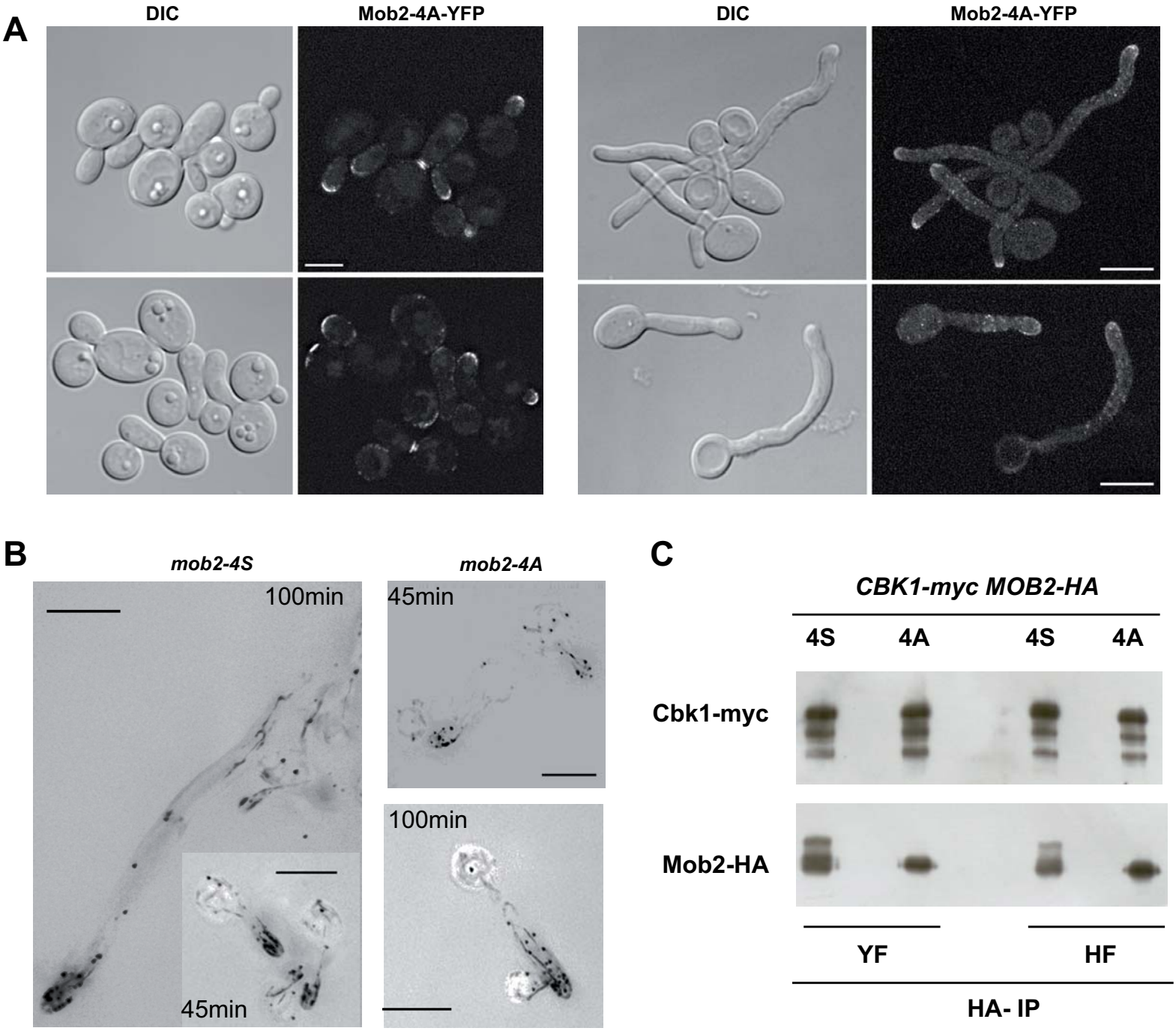
A
DIC
Mob2-4A-YFP
DIC
Mob2-4A-YFP
B
mob2-4S
mob2-4A
100min
45min
45min
100min
C
CBK1-myc MOB2-HA
4S
4A
4S
4A
Cbk1-myc
Mob2-HA
YF
HF
HA- IP

Figure S1. Reintroduction of Cdk consensus phosphorylation serine residues rescues the phenotype of the *mob2·4A* mutant. (A) DIC images of hyphal cultures of the JC613 (*MOB2-4S-HA/mob2*Δ), JC785 (*mob2-4A-HA/mob2*Δ) and JC843 (*MOB2-4A/S-HA/mob2*Δ) strains 4 hours after serum induction. (B) The same strains were grown under yeast- (YF) and hypha-inducing conditions (HF) for 2 hours. Protein extracts were analyzed by Western blot, using anti-HA and anti-PSTAIRE antibodies.

Figure S2. Colocalization of Spa2 and Kel1 in yeast and hyphal cells. (A) Spa2-GFP localization in yeast cultures of wild-type (JC961) and *mob2-4A* (JC921) strains. Spa2-GFP is shown in green, while calcofluor is shown in red. Kel1 and Spa2 colocalize in yeast (B) and hyphae (C). The JC1011 strain (*KEL1/KEL1-YFP SPA2/SPA2-CFP*) was grown in yeast and hypha-inducing conditions and analyzed with a Nikon microscope. Images of each channel and the merged image are shown. Spa2-CFP was loaded in the green channel and Kel1-YFP in the red channel. (D) Localization of Kel1-YFP in wild-type (JC1000) and *mob2-4A* (JC958) cells 150 min after hyphal induction. Kel1-GFP is shown in green, while calcofluor is shown in red. To the right of the images, 3D models generated using softWoRx from the regions indicated by rectangles are shown. The fluorescent signal is shown in green, while calcofluor is represented as a red wire frame. The second frame of each 3D model was generated by rotating the first image 90º on the indicated axis.

Figure S3. (A) Partial amino acid sequence alignment of *S. cerevisiae* (sc) and *C. albicans* (ca) Spa2. Rectangles show the Spa2 direct repeat elements (SDR1 and 2). Cbk1 consensus phosphorylation sites are denoted in yellow. Asterisk: S163, residue phosphorylated *in vivo* (Beltrao et al, 2009). (B) Mob2 associated kinase activity *in vitro*. Mob2 was immunoprecipitated from extracts of *MOB2-HA* (JC599), *mob2-4A-HA* (JC785) and *mob2*Δ (JC502) cells grown in hypha-inducing conditions and used in an *in vitro* kinase assay with GST-3xSpa2$^{138-163}$ as substrate (lanes labeled as 3S). Substitution of residues S^{143}, S^{153} and S^{163} to Ala abolished ^{32}P labeling of the GST-3xSpa2 protein (lanes labeled as 3A). Protein levels of GST-3xSpa2 and Mob2-HA in the kinase reactions were confirmed by Coomassie staining and Western blotting respectively.

Figure S4. *mob2-4A* mutant characterization. (A) Localization of Mob2-4A-YFP (JC957) under yeast and hypha induction. (B) Negative images showing Alexa-phalloidin staining of wild-type (JC613) and *mob2-4A* (JC785) strains grown as hyphae (C) Protein extracts from wild-type *MOB2-HA* (JC413) and *mob2-4A-HA* (JC964) yeast and hyphal cultures were immunoprecipitated using anti-HA antibodies and probed by anti-myc and anti-HA antibodies.

Supplemental Table 1. Yeast strains used in this study

Strain[a]	Genotype	Source
BWP17	*ura3Δ::imm434/ ura3Δ::imm434::hisG/his::hisG arg4::hisG/ arg4::hisG*	Wilson *et al*., 1999
LCR6	*CDC28/CDC28-myc::ARG4*	Li *et al*., 2008
	cdc28Δ::ARG4/URA3-pMET3-cdc28as	Yue Wang
JC369	*CBK1-YFP-URA3/CBK1*	This study
JC413	*CBK1-myc-HIS1/CBK1 MOB2-HA-URA3/MOB2*	This study
JC482	*MOB2-myc::HIS1/MOB2*	This study
JC493	*MOB2-myc::HIS1/ mob2Δ::URA3*	This study
JC502	*mob2Δ::ARG4/mob2Δ::HIS1*	This study
JC524	*CBK1-YFP-URA3/CBK1 mob2Δ::ARG4/mob2Δ::HIS1*	This study
JC599	*MOB2-HA-URA3/mob2Δ::ARG4*	This study
JC613	*mob2-4S-HA-URA3/mob2Δ::ARG4*	This study
JC620	*mob2-4E-HA-URA3/mob2Δ::ARG4*	This study
JC645	*CBK1-myc-HIS1/cbk1Δ::URA3*	This study
JC672	*CBK1-YFP-ARG4/cbk1Δ::HIS1*	This study
JC785	*mob2-4A-HA-URA3/mob2Δ::ARG4*	This study
JC843	*mob2-4A/S-HA-HIS1mob2Δ::ARG4*	This study
JC871	*MOB2-YFP-ARG4/MOB2*	This study
JC877	*cdc28Δ::ARG4/URA3-pMET3-cdc28as MOB2-HA-SAT1*	This study
JC895	*MOB2-YFP-ARG4/mob2Δ::URA3*	This study
JC921	*mob2-4A::URA3/mob2Δ::ARG4 SPA2-GFP-HIS1/SPA2*	This study
JC957	*mob2-4A-YFP-HIS1/mob2Δ::ARG4*	This study
JC958	*mob2-4A-HA-URA3/mob2Δ::ARG4 KEL1-GFP-HIS1/KEL1*	This study
JC961	*mob2-4S::URA3/mob2Δ::ARG4 SPA2-GFP-HIS1/SPA2*	This study
JC964	*mob2-4A-HA-URA3/mob2Δ::ARG4 CBK1-myc-HIS1/CBK1*	This study
JC1000	*KEL1-YFP-HIS1/KEL1*	This study
JC1011	*KEL1-YFP-HIS1/KEL1 SPA2-CFP-URA3/SPA2*	This study
JC1031	*KEL1-YFP-HIS1/kel1Δ::ARG4*	This study

[a] The strains constructed in this study are all derivates of BWP17

Supplemental Table 2. Primers used in this study.

Deletion and gene disruption	
Name	Sequence 5'-3'
S1*CBK1*	TTGAAAATAAAAGAAACTGAAAAGTAATAGCCACCAAGAGACTTTAATAACTGATTTTCTCGAGATACCTTAAAAGATCAAATAG AGTTTTATAGATCTTGAAGCTTCGTACGCTGCAGGTC
S2*CBK1*	TAGTGTGGTACGAGTAGAACCTTCATTATAAAACATGAAAAAACAATCATTAAATATAGAGAGTGTTGGGACGAACAACTATACA TAGTTATTATTTTACTCTGATATCATCGATGAATTCGAG
S1*KEL1*	TTTCCCACGAGAAGAAATTTTTAATATAAATTTTTCTTCACCGGTTTGTTTATCAACTAACCTCCACCCCCCCCTCACTTCAAAAT TTGGATAATAATTTGAAGCTTCGTACGCTGCAGGTC
S2*KEL1*	CCGTTTGATAGTTCCCCAGGATAATAACTCGAATTCAGAGACGTAAACATTACATATTATATAAAAAATTAAAATATACACACAAA CACACACATATAAATCTGATATCATCGATGAATTCGAG
S1*MOB2*	GGAAAAGAGAAAGAAGAGGAAAGAAAAAATATAACAGGAATTGACAGAACAGGTGTTATAGATTACAGCCTTTTTTCATTAACC AACTATATTAATTAGCGAAGCTTCGTACGCTGCAGGTC
S2*MOB2*	ATACACGTACTATACTATACTATTCAATATATACACTAAACTCAACAATTCAAGGCACTAAATAAGATCAATCTCGTAGTCTTGG CAACATATAGCTTGTCTGATATCATCGATGAATTCGAG
Tagging	
Name	Sequence 5'-3'
S1*CBK1myc*	TGCTAAGAATGGAGGCGGCAGAAAGAATCCAAAGGAAGATTTACCATTTATTGGATACACTTATTCTAGATTTGATTATTTGACA AGAAAGAATGCG TTACGGATCCCCGGGTTAATTAA
S2*CBK1myc*	ATAATGCATAAACAATAACATCATCCGGCTGTACTACCATTCCAAATGACCACCTATTGTTGATACATGTATGATAAGAAAAGGT TGGCATGCTTTGTTGGAATTCCGGAATATTTATGAGAA
S1*MOB2HA*	GTCAAAGAGTTCAATTTGATTGATAGAACTGAAATGGAACCGTTGTTACCTTTGTTACCTTTGATAGAGAATTTTGAACAACAAG GAAAAATCACCCAAGCAAGCAAAACGCGTTATCCATATGATGTTCC
S2*MOB2HA*	CTATAGCATGGTAAGCGATAGATTTAGATACACAATTCAATTAGTTGCATTCAAACCAAGAAGTATGTATCATGCATTGCAATTG CAATTCACACACAACAAACGCCAGGGTTTTCCCAGTCACGACG
S1*MOB2myc*	GTCAAAGAGTTCAATTTGATTGATAGAACTGAAATGGAACCGTTGTTACCTTTGATAGAGAATTTTGAACAACAAGGAAAAATCAC CAAGCAAACGGATCCCCGGGTTAATTAA
S2*MOB2myc*	CTATAGCATGGTAAGCGATAGATTTAGATACACAATTCAATTAGTTGCATTCAAACCAAGAAGTATGTAGATTCATGCATTGCAATT CACAACAAACGGAATTCCGGAATATTTATGAGAA
S1*CBK1XFP*	TTGAAAATAAAAGAAACTGAAAAGTAATAGCCACCACCAAGAGACTTTAATAAATGATTTTCTCGAGATACCTTAAAAGATCAAA TAGAGTTTTATAGATCTTGAAGCTTCGTACGCTGC
S2*CBK1XFP*	TAGTGTGGTACGAGTAGAACCTTCATTATAAAACATGAAAAAACAATCATTAAATATAGAGAGTGTTGGGACGAACAACTATACA TAGTTATTATTTTACTCTGATATCATCGATGAATTCGAG
S1*KEL1XFP*	AGACTTGGAAGCTGACTTGTATATATTGAAACAAGAAAGAGATCAATTAAAAGACAATGTCACTTCGTTGCAAAAACAACTTTAT TTAGCTCAGAATCAAGGTGCTGGCGCAGGTGCTTC
S2*KEL1XFP*	GAACAACCAAGTCCAGTCAACATGACTTCGAATCAACCATTCTCGTTTTTTACATACCAGAACACCAACACGTCCCCGCCAGTC CCATTGGATGACGTCTGATATCATCGATGAATTCGAG
S1*MOB2XFP*	TCAATTTGATTGATAGAACTGAAATGGAACCGTTGTTACCTTTGATAGAGAATTTTGAACAACAAGGAAAAATCACCCAAGCAAG CAAATAGTAAACAAGGGTGCTGGCGCAGGTGCTTC
S2*MOB2XFP*	TTAATTCAATAAAAAATTTAAAAAGAAACTATAGCATGGTAAGCGATAGATTTAGATACACAATTCAATTAGTTGCATTCAAACCA AGAAGTATGTAGATAGCACCTGCGCCAGCCCCTGCGC
S2*MOB2HA/ XFP*	AACTGTACTTACCAACGAAAACAACAAAAAAAAGGGGAAATAAATTGACCAGCAGTTTATTTTCTTTTTGCTTGATCTAAAAGAA AAGAATTTAGAACCGTCTGATATCATCGATGAATTCGAG
S1*SPA2XFP*	TATTGCCAAATGTACTAAAGAATTAGTCAAGACGGTGGAAGAAGCTAGTCTTAAAGAAGATATTGCTTATCTTGATGCTAGAATA AGTCAAAATCTTGAAGGTGCTGGCGCAGGTGCTTC
S2*SPA2XFP*	TAAATTCACAGCATCATCAAAAATTCATGTCCAAATACATTCGATAATTCCTACAAATACAATTATAATATCAAAATAATAAAATTA TTACATATATACTATATCATCGATGAATTCGAG
Mob2 site-directed mutagenesis	
Name	Sequence 5'-3'
M1*MOB2*	CCCCTCGAGGTCGACGGTATCGATAAGCTTGGGCGGGTATCCATTAAGCAAGGG

M2A*MOB2*	AGCAGACGTGTATGCAGCTGAGCCTTGTGCACCTTTTGAAGATAATTTTGAAGGGGCAAACTTGGTAGGTGCTTGTGTTC
M2E*MOB2*	TTCAGACGTGTATGCAGCTGAGCCTTGTGCACCTTTTGAAGATAATTTTGAAGGTTCAAACTTGGTAGGTTCTTGTGTTC
M3A*MOB2*	GCACAAGGCTCAGCTGCATACACGTCTGCTCCTACAAAGC
M3E*MOB2*	GCACAAGGCTCAGCTGCATACACGTCTGAACCTACAAAGCGTAGC
M4A*MOB2*	GTAGTCTGTATGGAAGAACGCTTCAGCGGTGCTACACTTC
M4E*MOB2*	GTAGTCTGTATGGAAGAACGCTTCAGCGGTTCTACACTTC
M5A*MOB2*	GAAGTGTAGCACCGCTGAAGCGTTCTTCCATACAGACTAC
M5E*MOB2*	GAAGTGTAGAACCGCTGAAGCGTTCTTCCATACAGACTAC
M6*MOB2*	GGTGGCGGCCGCTCTAGAACTAGTGGATCCCTTGACAATTGCCTCGCTGGAGG
Integration	
Name	Sequence 5'-3'
G1*CBK1XFP*	ACAAACCATGCAAACATGGAG
G1*CBK1*	CACTTCAAAGCAATTTGAGAATACGCCTCC
G2*CBK1*	GGTCATTCAAGGGTGAGCAAG
G1*KEL1*	CCATTTGGGAACCCCTAAAGTC
G4*KEL1*	GCCGCTGTATGTGCCACCAG
G1*MOB2*	CATGACGTAATGGGCCCAACATTATCCAG
G2B*MOB2*	CTTGACAATTGCCTCGCTGGAGG
MYC2	CACCGTCGAGTCCGTTCAAGTC
X2Ca*ARG4*	AATGGATCAGTGGCACCGGTG
X3Ca*ARG4*	GCTCTTGGTGGTACTGCTAAAAGTGCCG
X2Ca*HIS1*	CAACGAAATGGCCTCCCCTACCACAG
X3Ca*HIS1*	GACGAATTGAAGAAAGCTGGTGCAACCG
X2Ca*URA3*	GTGTTACGAATCAATGGCACTACAGC
X3Ca*URA3*	GGAGTTGGATTAGATGATAAAGGTGATGG
X2*SAT1*	GCACACACTACTTAATATACACAG
X3*SAT1*	GTGAAGTGTAAGGGGGAG
Quantitative RT-PCR	
Name	Sequence 5'-3'
ADE2 forward	TGTTGTCACATCTTCCATGC
ADE2 reverse	ATTCCCACCAATGGAGATTC
CHT3 forward	CTTCTAGAGCCGCTGGATCA
CHT3 reverse	GCTCCAACCAGCTGAAACAT
SCW11 forward	GTCCGGCTCAGCCAACTACT
SCW11 reverse	GGATGATGCGGTTGTTGTTC
***MOB2* cloning**	
Name	Sequence 5'-3'
E1*MOB2* (BamHI)	GCGCGGATCCATGTCTTTTTTAAATACTATACGTG
E2*MOB2* (HindIII)	GCGCAAGCTTCTATTTGCTTGCTTGGGTGATTTTT