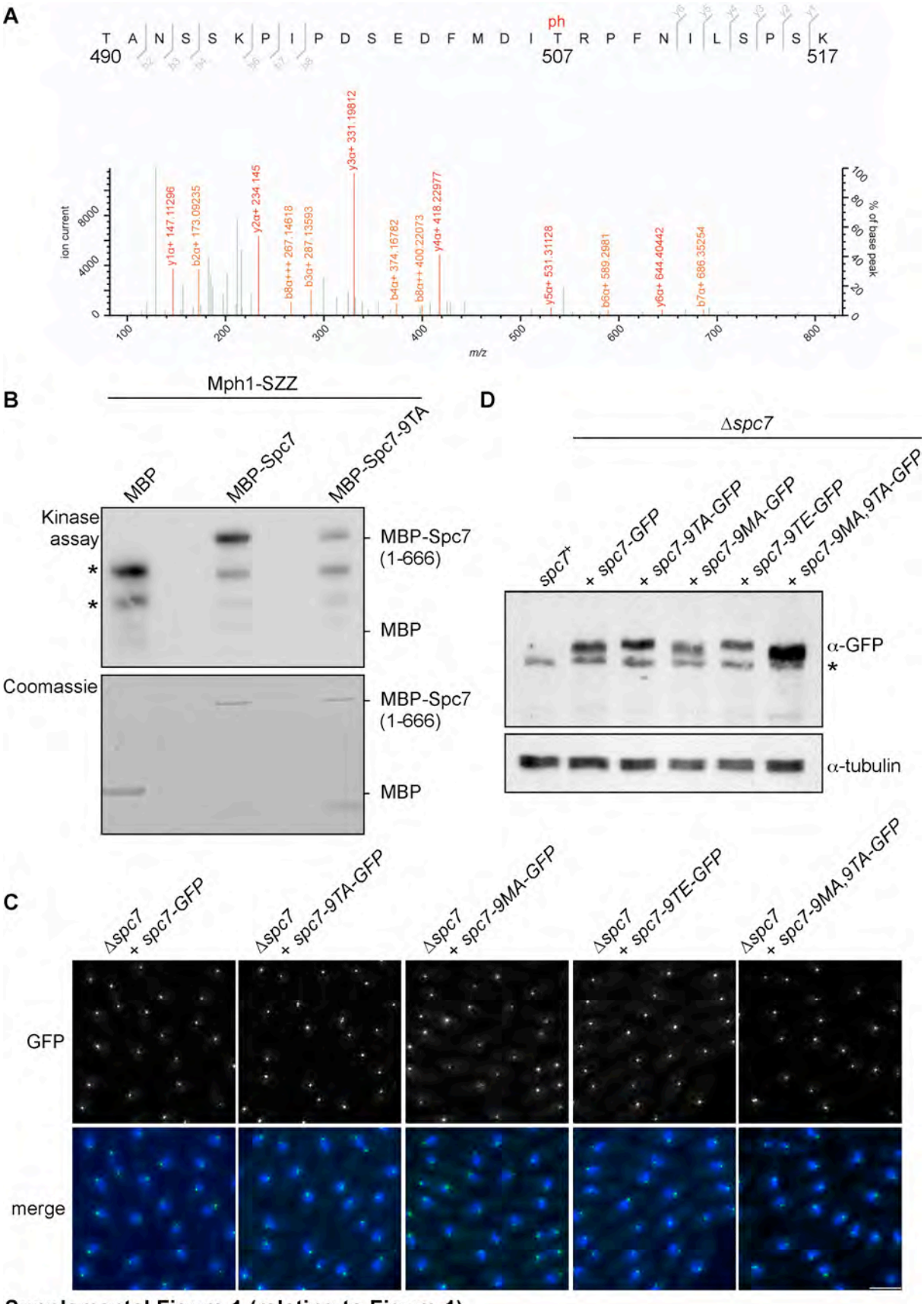
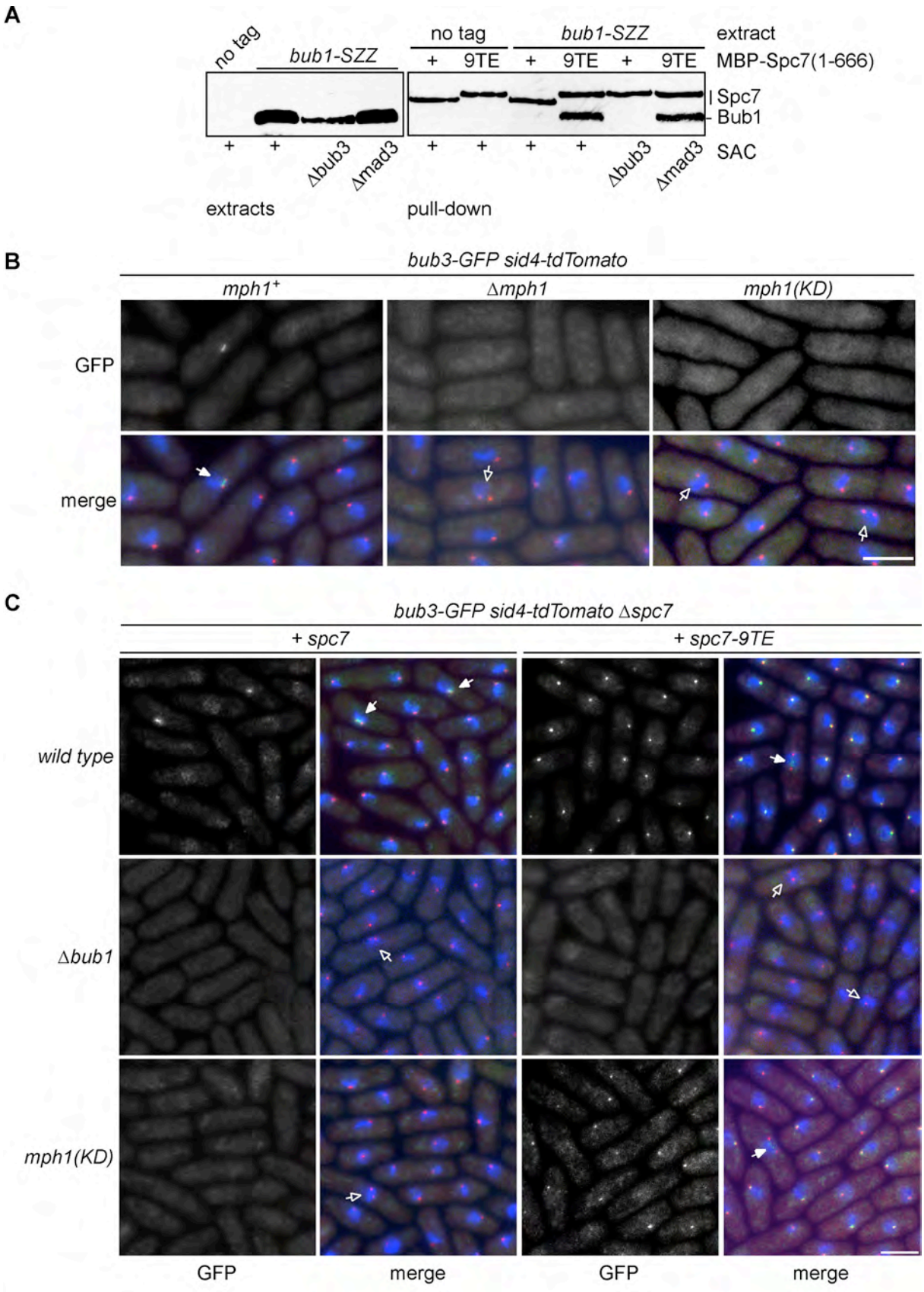
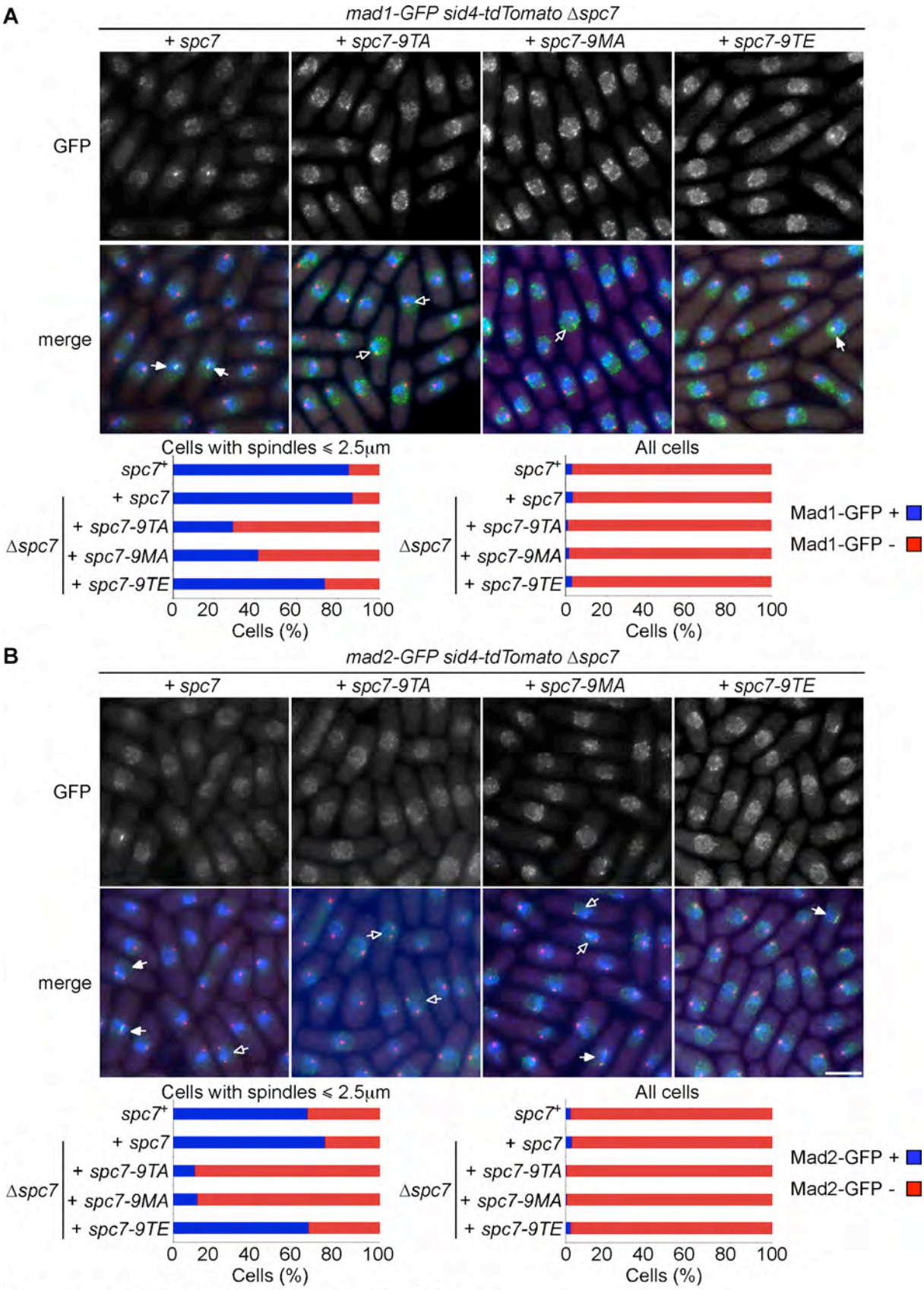
Supplemental Figures



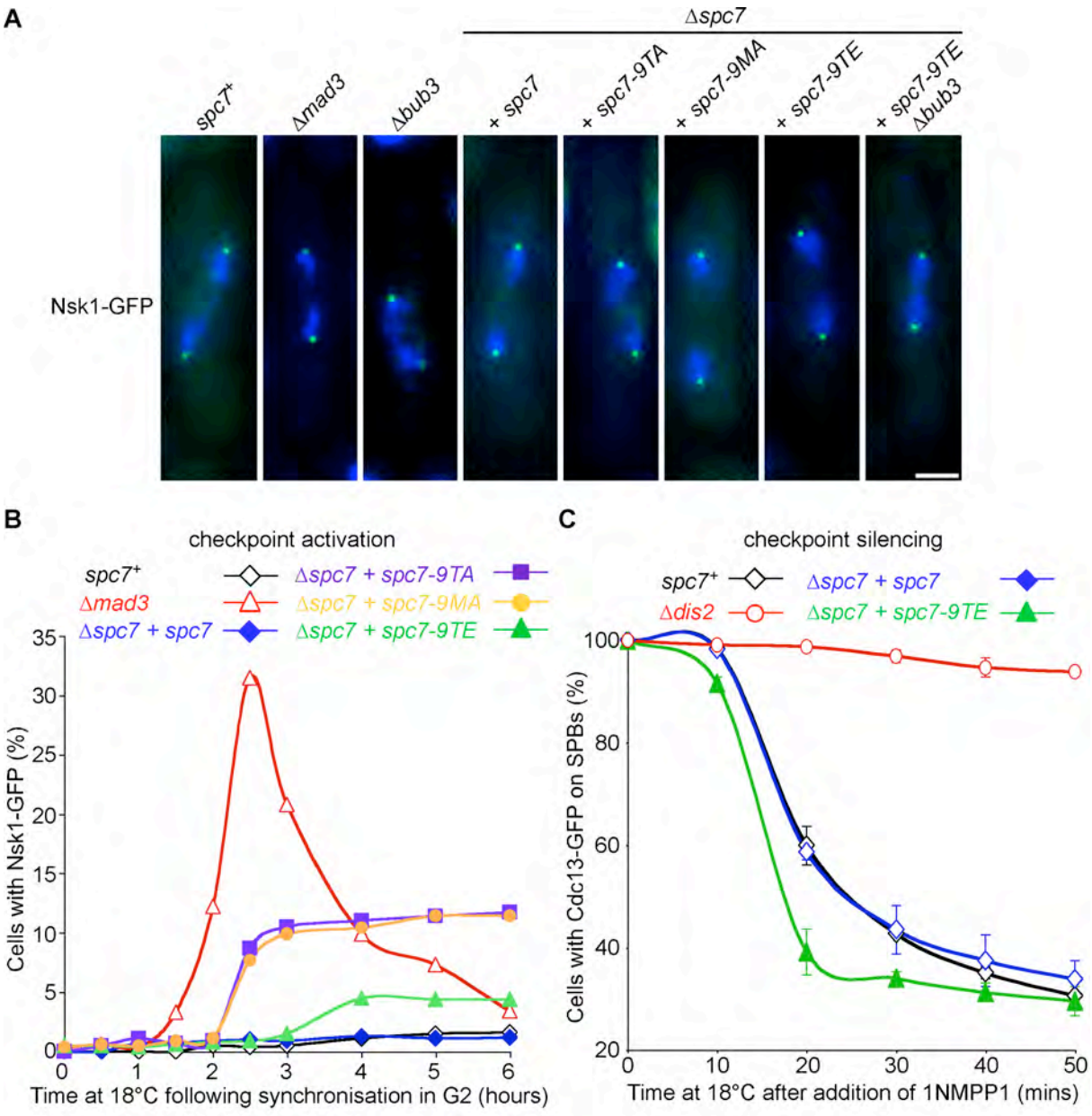
Supplemental Figure 1 (relating to Figure 1)



Supplemental Figure 2 (relating to Figure 3)



Supplemental Figure 3 (relating to Figure 3)



Supplemental Figure 4 (relating to Figure 4)

Supplemental Figure Legends

Supplemental Figure 1 (relating to Figure 1). Phosphorylation of Spc7 by Mph1 does not influence Spc7 localisation or expression

(A) MS/MS spectrum from mass spectrometric analyses of MBP-Spc7(1-666). Example of MS/MS spectra used to identify and assign phosphorylation to this MELT containing phosphopeptide (T507) from the in vitro Mph1 kinase assay. Fragment ions containing the peptide's N- (b-ions) or C- (y-ions) termini are labelled and matched with 10 ppm or better. Significant fragmentation has occurred allowing peptide sequencing and supports the hypothesis that T507 is modified, although S500 would also be a possible phosphorylation site. (B) Mph1 kinase was incubated with MBP, MBP-Spc7 or MBP-Spc7-9TA fusion proteins. Kinase assay (top panel) and coomassie stained gel of input proteins are shown (bottom panel). Asterisks indicate Mph1 autophosphorylation. (C) Mutation of the MELT motifs does not influence Spc7 localisation. Log phase cultures of *Δspc7 spc7-GFP, Δspc7 spc7-9TA-GFP, Δspc7 spc7-GFP-9MA-GFP, Δspc7 spc7-9TE-GFP* and *Δspc7 spc7-9MA,9TA-GFP* cells, in which *spc7* is expressed from its own promoter integrated at the *lys1* locus, were grown in rich medium at 30°C followed by fixation and analysed by fluorescence microscopy. Bar, 5μm. (D) Mutation of the MELT motifs does not influence Spc7 expression. Total cell extracts were prepared from log phase cultures of the cells in (C) grown in rich medium at 30°C. Proteins were separated by SDS-PAGE and western blot and probed with anti-GFP and anti-tubulin antibodies. Asterisk denotes a non-specific band that reacts with the anti-GFP antibody.

Supplemental Figure 2 (relating to Figure 3). Phosphorylation of Spc7 stimulates the binding and recruitment of the Bub1-Bub3 complex to kinetochores

(A) Interaction of Spc7-9TE with Bub1 requires Bub3. MBP-Spc7 (+) or MBP-Spc7-9TE (9TE) fusion proteins were incubated in extracts of untagged wild cells (no tag), *bub1-SZZ* cells or the same cells lacking Bub3 (*Δbub3*) or Mad3 (*Δmad3*). Interacting proteins were precipitated on amylose beads, separated by SDS-PAGE and subjected to western blot with anti-PAP antibody. Note that the anti-PAP antibody cross-reacts with the MBP-Spc7 proteins. (B) Log phase cultures of *bub3-GFP sid4-tdTomato* cells either wild type ($mph1^+$), lacking Mph1 (*Δmph1*) or defective in Mph1 kinase activity (*mph1(KD)*) were fixed and imaged. Representative images are shown. Cells with mitotic spindles less than 2.5μm exhibiting localised Bub3-GFP (closed arrowheads) or lacking Bub3-GFP localisation (open arrowheads) are highlighted. Bar, 5μm. (C) Log phase cultures of *bub3-GFP sid4-tdTomato Δspc7 spc7*$^+$ and *bub3-GFP sid4-tdTomato Δspc7 spc7-9TE* cells either wild type, (*wild type*) lacking Bub1 (*Δbub1*) or defective in Mph1 kinase activity (*mph1(KD)*) were fixed and imaged. Cells with mitotic spindles less than 2.5μm exhibiting localised Bub3-GFP (closed arrowheads) or lacking Bub3-GFP localisation (open arrowheads) are highlighted. Bar, 5μm.

Supplemental Figure 3 (relating to Figure 3). Phosphorylation of the Spc7 MELT motifs is required for recruitment of Mad1 and Mad2 during mitosis

Log phase cultures of *Δspc7 spc7, Δspc7 spc7-9TA, Δspc7 spc7-9MA* and *Δspc7 spc7-9TE* expressing either (A) *mad1-GFP sid4-tdTomato* or (B) *mad2-GFP sid4-tdTomato* were fixed. The proportion of pre-anaphase mitotic cells (SPBs less than or equal to 2.5μm apart) with (A) Mad1 or (B) Mad2 foci (closed arrowheads; blue bars) and without (open arrowheads; red bars) was assessed (n=3; left panels). The relative proportion of all cells with (A) Mad1 or (B)

Mad2 foci (blue bars) or without (red bars) was also quantified from the same populations (right panels). Bar, 5μm.

Supplemental Figure 4 (relating to Figure 4). Ectopic recruitment of Bub1 and Bub3 to Spc7 aids spindle checkpoint silencing.

(A) Nsk1 localisation is unaltered in *spc7-MELT* mutants. Log phase cultures of *wild type* (*spc7*$^{+}$), *Δmad3, Δbub3, Δspc7 spc7, Δspc7 spc7-9TA, Δspc7 spc7-9MA, Δspc7 spc7-9TE* and *Δspc7 spc7-9TE Δbub3* cells expressing *nda3-KM311 ark1-as3 nsk1-GFP* were fixed and imaged. Representative images of anaphase B cells are shown. Bar, 2μm. (B) Phosphorylation of Spc7 MELT motifs is required for maintenance of the spindle checkpoint. Log phase cultures of *wild type* (*spc7*$^{+}$), *Δmad3*, *Δspc7 spc7, Δspc7 spc7-9TA, Δspc7 spc7-9MA,* and *Δspc7 spc7-9TE* cells expressing *nda3-KM311 ark1-as3 nsk1-GFP* were synchronised in early G2 by lactose gradient centrifugation and incubated at 18°C for the times indicated. The cells were fixed and the percentage of cells with spindle pole associated Nsk1 was assessed. (C) Ectopic recruitment of Bub1 and Bub3 to Spc7 aids spindle checkpoint silencing. Log phase cultures of wild type, *Δdis2*, *Δspc7 spc7* and *Δspc7 spc7-9TE* cells expressing *nda3-KM311 ark1-as3 cdc13-GFP* were synchronized in prometaphase by incubation at 18°C for 6 hours. At time zero 5μM 1NMPP1 was added and at the times indicated cells were fixed and the percentage of cells with spindle pole associated Cdc13-GFP was assessed. 200-300 cells were analysed for each treatment condition and for each time point with error bars representing standard deviation from three independent experiments. Data are presented as the percentage of cells with spindle pole associated Cdc13 relative to that at time zero

Supplemental Table 1. Genetic interactions of *spc7-MELT* mutants

	Δspc7 + spc7	*Δspc7 + spc7-9TA*	*Δspc7 + spc7-9MA*	*Δspc7 + spc7-9TE*	*Δspc7 + spc7-9MA,9TA*
wild type	++	++	++	++	++
Δdam1	++	-	-	-	-
Δdis2	++	-	-	+	-
Δklp5	++	-	-	++	-
Δdis1	++	-	-	++	-
Δbub1	++	++	++	++	++
Δbub3	++	++	++	++	++
Δmad2	++	++	++	++	++
Δmad3	++	++	++	++	++
Δmph1	++	++	++	++	++
Δdam1 Δmad3	++	-	-	-	-
Δdis2 Δmad3	++	-	-	+	-
Δklp5 Δmad3	++	-	-	++	-
Δdis1 Δmad3	++	-	-	++	-

(++) viability is indistinguishable from *wild type*

(+) slow growth phenotype

(-) inviable

Supplemental Table 2. Strains used in this study

Strain no.	Genotype	Source
PR109	*h⁻*	*P. Russell*
SI218	*h⁻ ark1-as3:hygR ura4+ ade6-M216*	*S. Hauf*
SP78	*h⁻ mis12-GFP:LEU2*	*M.Yanagida*
JM3483	*h⁺ fta3-mRFP:hygR*	*X.He*
JM3725	*h⁺ sid4-tdtomato:hygR his2-245*	*I. Hagan*
JM2952	*h⁻ dam1::hygR*	*our stocks*
JM2114	*h⁻ dis2::ura4*	*M.Yanagida*
JM4243	*h⁺ dis1::hygR*	*our stocks*
JM3007	*h⁻ klp5::ura4*	*T.Toda*
JM2323	*h⁻ bub1::ura4*	*our stocks*
JM2325	*h⁻ bub3::ura4*	*our stocks*
AE148	*h⁻ mad2::ura4*	*T. Matsumoto*
JM4137	*h⁺ mad3::ura4*	*our stocks*
JM4217	*h⁺ mad3::hygR*	*our stocks*
YJZ4	*h⁻ mph1-SZZ:leu1⁺ ade6-210*	*our stocks*
YJZ24	*h⁻ mph1-D459A-SZZ:leu1⁺ ade6-210*	*our stocks*
RA1645	*h⁺ ade6-210 his3-D1 arg3-D4*	*our stocks*
SJ636	*h⁻ bub1-SZZ:KanMX6*	*our stocks*
AS406	*h⁾ bub1-SZZ:KanMX6 bub3::ura4*	*our stocks*
AS408	*h⁾ bub1-SZZ::KanMX6 mad3::ura4*	*our stocks*
JM5746	*h⁺ spc7::natR lys1::spc7:kanR*	*our stocks*
JM6053	*h⁺ spc7::natR lys1::spc7-9TA:kanR*	*this study*
JM6055	*h⁺ spc7::natR lys1::spc7-9TE:kanR*	*this study*
JM6611	*h⁺ spc7::natR lys1::spc7-9MA:kanR*	*this study*
JM6057	*h⁺ spc7::natR lys1::spc7-9MA,9TA:kanR*	*this study*
JM6462	*h⁻ spc7::natR lys1::spc7:ura4*	*this study*
JM6464	*h⁻ spc7::natR lys1::spc7-9TA:ura4*	*this study*
JM6466	*h⁺ spc7::natR lys1::spc7-9TE:ura4*	*this study*
JM6468	*h⁺ spc7::natR lys1::spc7-9MA:ura4*	*this study*
JM6687	*h⁺ spc7::natR lys1::spc7-9MA,9TA:ura4*	*this study*
JM6673	*h⁻ spc7::natR lys1::spc7-GFP:ura4*	*this study*
JM6675	*h⁻ spc7::natR lys1::spc7-9TA-GFP:ura4*	*this study*
JM6677	*h⁻ spc7::natR lys1::spc7-9TE-GFP:ura4*	*this study*
JM6892	*h⁻ spc7::natR lys1::spc7-9MA-GFP:ura4*	*this study*
JM6679	*h⁻ spc7::natR lys1::spc7-9MA,9TA-GFP:ura4*	*this study*
JM6983	*h⁻ spc7::natR lys1::spc7-GFP:ura4 bub1-3HA:kanMX6*	*this study*
JM6685	*h⁻ spc7::natR lys1::spc7-9TA-GFP:ura4 bub1-3HA:kanMX6*	*this study*
JM6687	*h⁻ spc7::natR lys1::spc7-9TE-GFP:ura4 bub1-3HA:kanMX6*	*this study*
JP503	*h⁻ ade6-M210 Ch16(ade6-M216)*	*J-P. Javerzat*
JM7047	*h⁾ mph1::ura4 ade6-M210 Ch16(ade6-M216)*	*our stocks*
JP501	*h⁻ bub1::LEU2 ade6-M210 Ch16(ade6-M216 bub1::ura4)*	*J-P. Javerzat*
JM6948	*h⁻ bub3::kanMX6 ade6-M210 Ch16(ade6-M216)*	*our stocks*
JM6202	*h⁻ spc7::natR lys1::spc7:kanR ade6-M210 Ch16(ade6-M216)*	*this study*
JM6204	*h⁻ spc7::natR lys1::spc7-9TA:kanR ade6-M210 Ch16(ade6-M216)*	*this study*
JM6206	*h⁻ spc7::natR lys1::spc7-9TE:kanR ade6-M210 Ch16(ade6-M216)*	*this study*

JM6748	*h+ spc7::natR lys1::spc7-9MA:kanR ade6-M210 Ch16(ade6-M216)*	*this study*
JM6208	*h+ spc7::natR lys1::spc7-9MA,9TA:kanR ade6-M210 Ch16(ade6-M216)*	*this study*
JM2589	*h- cdc13-117:cdc13-GFP(LEU2)*	*our stocks*
JM3029	*h- cdc13-117:cdc13-GFP(LEU2) dam1::kanMX6*	*our stocks*
JM6327	*h- spc7::natR lys1::spc7:kanMX6 cdc13-117:cdc13-GFP(LEU2)*	*this study*
JM6329	*h- spc7::natR lys1::spc7-9TA:kanMX6 cdc13-117:cdc13-GFP(LEU2)*	*this study*
JM6331	*h- spc7::natR lys1::spc7-9TE:kanMX6 cdc13-117:cdc13-GFP(LEU2)*	*this study*
JM6765	*h- spc7::natR lys1::spc7-9MA:ura4 cdc13-117:cdc13-GFP(LEU2)*	*this study*
JM6333	*h- spc7::natR lys1::spc7-9MA,9TA:kanMX6 cdc13-117:cdc13-GFP(LEU2)*	*this study*
JM2589	*h- cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*our stocks*
JM3768	*h? cdc13-117:cdc13-GFP(LEU2) dam1::kanMX6 mad3::ura4*	*our stocks*
JM6454	*h? spc7::natR lys1::spc7: kanMX6 cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*this study*
JM6456	*h? spc7::natR lys1::spc7-9TA:kanMX6 cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*this study*
JM6458	*h? spc7::natR lys1::spc7-9TE:kanMX6 cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*this study*
JM6932	*h? spc7::natR lys1::spc7-9MA:kanMX6 cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*this study*
JM6460	*h? spc7::natR lys1::spc7-9MA,9TA:kanMX6 cdc13-117:cdc13-GFP(LEU2) mad3::hygR*	*this study*
JM6442	*h- nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6*	*our stocks*
JM6963	*h- nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6 mad3::ura4*	*this study*
JM6512	*h- nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6 dis2::ura4*	*our stocks*
JM6979	*h- nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6 bub3::ura4*	*this study*
JM6504	*h+ spc7::natR lys1::spc7:ura4 nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6*	*this study*
JM6506	*h+ spc7::natR lys1::spc7-9TA:ura4 nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6*	*this study*
JM6508	*h+ spc7::natR lys1::spc7-9TE:ura4 nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6*	*this study*
JM7045	*h+ spc7::natR lys1::spc7-9TE:ura4 nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6 bub3::ura4*	*this study*
JM6749	*h+ spc7::natR lys1::spc7-9MA:ura4 nda3-KM311 ark1-as3:hygR nsk1-GFP:kanMX6*	*this study*
JM6608	*h- bub1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6914	*h? bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1::ura4*	*this study*
JM6916	*h? bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1(D459A):LEU2*	*this study*
JM6653	*h+ spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6655	*h? spc7::natR lys1::spc7-9TA:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6657	*h- spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6774	*h? spc7::natR lys1::spc7-9MA:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM7027	*h? spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR nda3-KM311*	*this study*
JM7029	*h? spc7::natR lys1::spc7-9TA:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR nda3-KM311*	*this study*
JM7031	*h? spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6*	

	sid4-tdtomato:hygR nda3-KM311	*this study*
JM7078	*h+ spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 fta3-tdtomato:hygR pREP3X-mad2*	*this study*
JM7080	*h? spc7::natR lys1::spc7-9TA:ura4 bub1-GFP:kanMX6 fta3-tdtomato:hygR pREP3X-mad2*	*this study*
JM7082	*h- spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6 fta3-tdtomato:hygR pREP3X-mad2*	*this study*
JM7084	*h? spc7::natR lys1::spc7-9MA:ura4 bub1-GFP:kanMX6 fta3-tdtomato:hygR pREP3X-mad2*	*this study*
JM6879	*h- bub3-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6918	*h? bub3-GFP:kanMX6 sid4-tdtomato:hygR mph1::ura4*	*this study*
JM6920	*h? bub3-GFP:kanMX6 sid4-tdtomato:hygR mph1(D459A):LEU2*	*this study*
JM6896	*h? spc7::natR lys1::spc7:ura4 bub3-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6898	*h? spc7::natR lys1::spc7-9TA:ura4 bub3-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6900	*h? spc7::natR lys1::spc7-9TE:ura4 bub3-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6902	*h? spc7::natR lys1::spc7-9MA:ura4 bub3-GFP:kanMX6 sid4-tdtomato:hygR*	
JM6605	*h- mad1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6871	*h? spc7::natR lys1::spc7:ura4 mad1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6873	*h? spc7::natR lys1::spc7-9TA:ura4 mad1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6875	*h? spc7::natR lys1::spc7-9TE:ura4 mad1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6877	*h? spc7::natR lys1::spc7-9MA:ura4 mad1-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6606	*h- mad2-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6647	*h? spc7::natR lys1::spc7:ura4 mad2-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6649	*h? spc7::natR lys1::spc7-9TA:ura4 mad2-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6651	*h? spc7::natR lys1::spc7-9TE:ura4 mad2-GFP:kanMX6 sid4-tdtomato:hygR*	*this study*
JM6771	*h- spc7::natR lys1::spc7-9MA:ura4 mad2-GFP:LEU2 sid4-tdtomato:hygR*	*this study*
JM6951	*h? spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1::ura4*	*this study*
JM6953	*h? spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1::ura4*	*this study*
JM6955	*h? spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1(D459A):LEU2*	*this study*
JM6957	*h? spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR mph1(D459A):LEU2*	*this study*
JM6959	*h? spc7::natR lys1::spc7:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR bub3::ura4*	*this study*
JM6961	*h? spc7::natR lys1::spc7-9TE:ura4 bub1-GFP:kanMX6 sid4-tdtomato:hygR bub3::ura4*	*this study*

All strains are *leu1-32 ura4-D18* unless otherwise stated.

Supplemental Table 3. Oligonucleotides used in this study

Spc7 KpnI FW
TTATGGTACCATTGGCCCTTTTTGTCAGTTGAA

Spc7 BamHI RV
CGCGGATCCGGCTGTATTTGTTCCAAACAGTAAGACAAGC

Spc7 BssHII FW
TTGGCGCGCATATTAATGGGAATGATTAGCTATGCT

Spc7 BssHII RV
TTGGCGCGCGCGCATTACGGGTTTAACA

Spc7(1-666) FW gateway
GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCCAACATCGCCTCGTCGC

Spc7(1-666) Rev gateway
GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAATTCAAAGTTGAAATTGATTTT
CG

Pic1-IN box FW gateway
GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGATTATTCGGATGATTCAGATGA

Pic1-IN box Rev gateway
GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATAAAAAACCCATGTTTTTCTTA
TAGTTATC

CNT A (central core primer)
AACAATAAACACGAATGCCTC

CNT B (central core primer)
ATAGTACCATGCGATTGTCTG

OTR A (outer repeat primer)
CACATCATCGTCGTACTACAT

OTR B (outer repeat primer)
GATATCATCTATATTTAATGACTACT

Fbp A (euchromatic control primer)
AATGACAATTCCCCACTAGCC

Fbp B (euchromatic control primer)
ACTTCAGCTAGGATTCACCTGG

Supplemental Experimental Procedures

Plasmid and strain construction

Wild type and mutant alleles of *spc7* were introduced into the *lys1* locus by cloning a *SphI*/*BamHI* fragment from pIRT2u-Spc7 into the *SphI* and *BamHI* sites of pLYS1K or pLYS1U. pLYS1K-Spc7 and pLYS1U-Spc7 or mutant alleles thereof were digested with *NotI* and transformed into wild type cells. Integration at the *lys1* locus was confirmed by PCR. To create mutant *spc7* alleles a 1.4Kb fragment of *spc7* flanked by unique restriction sites *MscI* and *AgeI* was synthesised by GeneArt (Life Technologies™) with methionine residues 254, 335, 363, 392, 419, 450, 504, 526, 549 or threonine residues 257, 338, 366, 395, 422, 453, 507, 529, 552 mutated to alanine or glutamic acid. The fragment was excised from the GeneArt vector pMA-RQ using *MscI* and *AgeI* and cloned into pLYS1K-Spc7 or pLYS1U-Spc7. A full list of strains can be found in Supplemental Table 2. A list of oligonucleotides can be found in Supplemental Table 3.

GFP-tagging of *spc7* alleles

A fragment containing the GFP gene was excised from pFA6a-GFP(S65T):kanMX6 using *BamHI* and *BssHII*. A 310bp *KpnI*/*BamHI* fragment of Spc7 and ~200bp *BssHII* fragment of 3'UTR was amplified from the pLYS1U-Spc7 vectors. The fragments were ligated and cloned into the *KpnI* and *BssHII* sites of pLYS1U-Spc7, pLYS1U-Spc7-9TA, pLYS1U-Spc7-9TE, pLYS1U-Spc7-9MA and pLYS1U-Spc7-9MA,9TA to create the following plasmids: pLYS1U-Spc7-GFP, pLYS1U-Spc7-9TA-GFP, pLYS1U-Spc7-9TE-GFP, pLYS1U-Spc7-9MA-GFP and pLYS1U-Spc7-9MA,9TA-GFP. Constructs were verified by sequencing. Integration was performed as previously described.

Cloning of recombinant MBP-Spc7 and MBP-Pic1-IN box fusion proteins

To generate MBP-Spc7 fusion proteins a gene fragment encoding Spc7 residues 1-666 was amplified from pLYS1K-Spc7, pLYS1K-Spc7-9TA and pLYS1K-Spc7-9TE plasmids, respectively, using high fidelity polymerase (Roche Diagnostics GmbH) and primers Spc7(1-666) FW gateway and Spc7(1-666) Rev gateway. The PCR products were cloned into pDONR201 donor vector using Gateway BP Clonase II enzyme mix (Invitrogen) and then transferred to pHGGWA Gateway-based destination vector [1] using Gateway LR Clonase II enzyme mix (Invitrogen) according to the manufacturer's instructions. Gene fragment encoding Pic1 residues 925-1018 (containing IN-box) was PCR amplified from pDONR201-Pic1 using primers Pic1-IN box FW gateway and Pic1-IN box Rev gateway. pHMGWA-Pic1-IN box was generated using gateway system as described above.

Expression and purification of MBP-Spc7 fusion proteins

To express and purify MBP-fusion proteins expression vectors were transformed into *E.coli* BL21-RIL cells. 1L culture (1:100 dilution of an overnight culture containing 100μg/ml ampicilin) was grown in LB medium at 37°C. The culture was induced with 0.5mM IPTG (at OD_{600}=0.4-0.8) and shifted to 18°C for 8-15 hours. Cells were pelleted, frozen and ground in a mortar under liquid nitrogen Cell powder was resuspended in ice-cold lysis buffer PBS containing 0.5% Tween-20, 0.5M NaCl, 1mM Pefabloc, 10µg/ml LPC and complete mini EDTA-free Protease Inhibitor Cocktail Tablets (Roche Applied Science). After sonication with 20s pulse, 20s interval for three cycles (Sonics, Vibra-Cell), DNAse and lysozyme were added and the lysate was incubated at room temperature for 15-20minutes and then centrifuged for 15min at 17 500rpm. Supernatant was filtered through a 25mm GD/X syringe filters (Whatman, pore size 1.6µM). Clarified lysate was passed through Amylose Resin slurry (New England BioLabs) three times. After two washes with PBS containing 0.05%

Tween 20, 0.25M KCl, 0.5mM DTT and one wash without Tween-20, recombinant MBP-fusion proteins were eluted according to the manufacturer's instructions.

MBP-Spc7 binding assays

Recombinant MBP-Spc7(1-666) (wild type and mutant versions) were incubated with amylose resin (NEB) in PBS containing 0.5% Tween-20, 0.15M NaCl, 1mM Pefabloc, 10μg/ml LPC and complete mini EDTA-free Protease Inhibitor Cocktail Tablets for 30 min at 4°C and washed three times in lysis buffer (50mM Hepes pH7.6, 75mM KCl, 1mM $MgCl_2$, 1mM EGTA, 0.1 % Triton-X100) before incubation in cell extracts. Log phase *bub1-SZZ* cells frozen pellets were resuspended in the lysis buffer containing 1mM Na_3VO_4 0.1µM microcystin, 1mM pefablock, 10µg/ml LPC, complete mini EDTA-free protease inhibitor cocktail tablets, and then immediately bead beat twice for 20s with a 30s incubation on ice between the pulses. The lysate was centrifuged at 14 000 rpm for 15min at 4°C then incubated with MBP-Spc7(1-666) fusion proteins bound to amylose resin (NEB). Following incubation for 15min at 4°C beads were washed three times in lysis buffer. Proteins were eluted by boiling in 2× SDS-PAGE sample buffer. Samples were analyzed by SDS-PAGE and western blotting using antibodies at 1:1000 dilution in PBS plus 0.05% Tween-20 containing 4% dried skimmed milk. SZZ-tagged proteins were detected with PAP antibody (Sigma-Aldrich).

Mph1 purification from fission yeast

Cycling cells expressing either Mph1WT-SZZ or Mph1 KD-SZZ were grown in 4 × YES to an OD_{595} below 10. Cells were then pelleted, washed once with ice-cold water, frozen and ground in a mortar under liquid nitrogen. Cell powders were resuspended in one cell powder weight of 2× Hyman lysis buffer (100mM bis-Tris propane, 200mM KCl, 10mM EGTA, 10mM EDTA, 20% glycerol, protease inhibitors 1mM pefablock, 10µg/ml LPC and complete mini EDTA-free Protease Inhibitor Cocktail Tablets). TritonX-100 was added to 1% final

concentration and the cell suspension sonicated for 30s (Sonics, Vibra-Cell). Lysed cells were centrifuged for 10min, 4500 rpm at 4°C to remove cell debris. The remaining supernatants were filtered through a 25mm GD/X syringe filters (Whatman, pore size 2.6μm) and a 25mm GD/X syringe filters (Whatman, pore size 1.6μM). Clarified lysates were then incubated with IgG dynabeads (Invitrogen) for 30min rolling at 4°C, washed 3 times with 15ml 1× Hyman buffer and 3 times with 15ml 1× Hyman buffer with the addition of 1mM DTT and 0.1 % Tween-20 using a magnet (MagnaBot, Promega). The purified proteins were cleaved off the beads using 100 units AcTEV protease (Invitrogen) overnight rotating at 4°C. Supernatants were transferred to new tubes and IgG-coupled Dynabeads washed once with 1ml 1× Hyman buffer containing 1mM DTT and 0.1% Tween-20. The supernatants containing the cleaved proteins were incubated with S-protein agarose beads (Novagen) for 4 hours at 4°C and coupled beads washed five times with 1× Hyman buffer. Purified proteins were stored at -80°C.

***In vitro* Mph1 kinase assay**

Purified Mph1 kinase coupled to S-protein agarose beads was washed twice with 1× kinase buffer (50mM Hepes pH7.6, 50mM KCl, 10mM $MgCl_2$, 10mM $MnCl_2$, 25mM β-glycerophosphate, 0.5mM DTT). 25μl of kinase reaction buffer (12.5μl 2x kinase buffer, 20μM ATP and 50μCi [γ-^{32}P] ATP) containing either 3μg of recombinant MBP-Spc7(1-666) or 3μg MBP was added to the beads and incubated for 30min at 30°C. Reactions were stopped by adding 4× gel loading buffer. Samples were run on NuPAGE Novex 4-12 % Bis-Tris gel. The gel was stained with Instant*Blue* (Expedeon) then dried onto Whatman paper and exposed to film.

Mass Spectrometry methods

Proteins (4µg) were electrophoresed through NuPAGE 4-12% Bis-Tris gels (Invitrogen) in MOPS buffer (Invitrogen). Proteins were stained with InstantBlue. Bands were excised and digested with trypsin as previously described [2, 3] with the following modifications: reduction in 10mM DTT for 30min at 37°C, alkylation with 55mM iodoacetamide for 20min at room temperature in the dark, and digestion with 13ng/µl trypsin (proteomics grade, Sigma) overnight at 37°C. Peptides were desalted and concentrated using StageTips [4, 5] and analyzed using an LTQ Orbitrap Velos (Thermo Fisher Scientific) coupled with a nano-ACQUITY UPLC system (Waters) and a 250mm long self-packed analytical column with a self-assembled particle frit [4]. Peptides were loaded at 0.7µl/min and separated at a flow rate of 0.3µl/min. Mobile phase A consisted of water, 0.1% formic acid; Mobile phase B consisted of acetonitrile, 0.1% formic acid. A linear gradient was increased from 5% to 35% acetonitrile in 0.1% formic acid to elute peptides. Mass spectra were recorded at 100,000 resolution. The ten peaks with the highest intensity were selected in each cycle for higher energy collisional activated dissociation (HCD) with 40% normalized collision energy and detected by the Orbitrap at 7,500 resolution. Dynamic exclusion was set to 90s and repeat count was one. Raw files were processed using MaxQuant software (version 1.1.1.36) [6] with phosphorylation as variable modification.

Chromatin immunoprecipitation (ChIP) analysis

Chromatin immunoprecipitations were carried out as described [7], incorporating the following modifications. Mid-log *nda3-KM311* cells were either metaphase-arrested for 6 hr at 18°C or allowed to process through cell cycle by incubation at permissive temperature 32°C. The cells were then fixed with 1% paraformaldehyde at 18°C for 30min. Cells were spheroplasted at 10^8 cells/ml in PEMS (100mM piperazine-N,N'-bis(2-ethane-sulfonic acid) (pH 7.0), 1mM EDTA, 1mM $MgCl_2$, 1.2M sorbitol) plus 0.4mg Zymolyase 100T/ml for

30min at 37°C. Cells were then washed twice in PEMS, and pellets were frozen at -80°C. Protein A beads (Dynal) were coated with 4μg of anti-GFP antibody (Molecular Probes) by incubating in PBS–0.02% Tween-20 for 1h at 4°C and then washed twice with PBS–0.02% Tween-20 and once with lysis buffer before being used to immunoprecipitate the protein from the crude lysate without preclearing. After washing, the beads were mixed with Chelex-100 resin (Bio-Rad), boiled and treated with Proteinase K for 30min at 55°C [8]. Proteinase K was then inactivated by boiling the samples at 100°C for 10min. The samples were centrifuged and the supernatant containing co-precipitated DNA was collected to perform PCR reaction.

Mini-chromosome loss assay

Mini-chromosome loss in cells bearing the Ch16 (*ade6-M216*) mini-chromosome and *ade6-M210* allele was assayed as previously described [9].

Western blotting and co-immunoprecipitation

For western blotting of total cell extracts, cells were lysed in 20% trichloroacetic acid (TCA) and precipitated proteins solubilised in SDS sample buffer. GFP-tagged proteins were detected with Sheep anti-GFP antibodies as previously described. α-tubulin was detected with anti-Tat1 antibodies (a gift from Keith Gull). SZZ-tagged proteins were detected with PAP antibody (Sigma-Aldrich). *In vivo* co-immunoprecipitation experiments were carried out as described in [10]. HA-tagged proteins were detected using anti-HA (clone 3F10, Eurogentec).

Fluorescence microscopy

Fluorescence imaging of cells expressing GFP- or tdTomato-tagged proteins was performed on a Nikon TE-2000 inverted microscope with a 100x 1.49 N.A. objective lens equipped with a Photometrics Coolsnap-HQ2 liquid cooled CCD camera (Photometrics, Tucson, AZ). Images were collected and analysed using Metamorph (version 7.5.2.0 MAG Biosystems

Software). Exposure times of 1s were used for GFP and tdTomato and 0.25s for DAPI. Pre-anaphase mitotic index was analysed as described in [10]. Checkpoint activation and silencing assays are described in the figure legends.

Supplemental References

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