Current Biology, Volume *21*

Supplemental Information

SIN-Inhibitory Phosphatase Complex

Promotes Cdc11p Dephosphorylation and

Propagates SIN Asymmetry in Fission Yeast

N. Sadananda Singh, Nan Shao, Janel R. McLean, Mayalagu Sevugan, Liping Ren, Ting Gang Chew, Andrea Bimbo, Reetu Sharma, Xie Tang, Kathleen L. Gould, and Mohan K. Balasubramanian

Supplemental Inventory

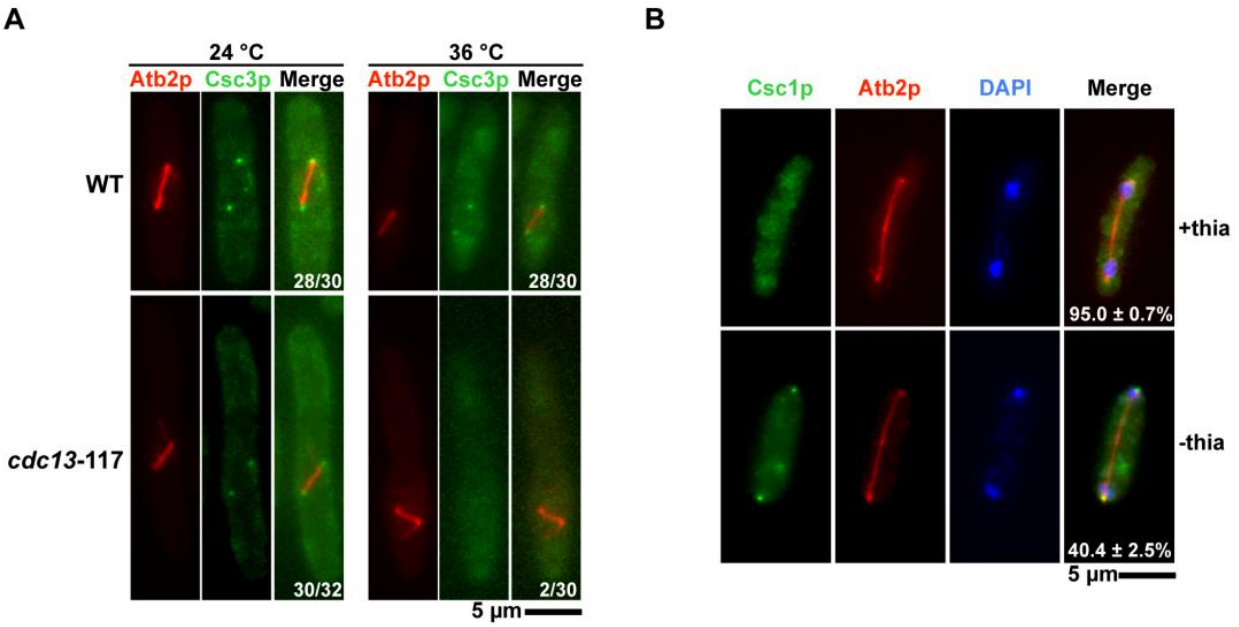

Figure S1. Localization of Csc1p to SPB Depends on Active Cyclin-Dependent Kinase

(A) Loss of Csc1 localization to SPB on Cdc13p inactivation: Wild-type and *cdc13*-117 cells expressing Csc3p-GFP and mCherry-Atb2p were arrested at metaphase by overproduction of Mad2p. Arrested cells were then shifted to 36°C, to inactivate Cdc13p in the *cdc13*-117 mutant. Cells at 24°C and 36°C were fixed and the localization of Csc3p in cells with short spindles quantitated. The proportion of cells showing the displayed pattern is indicated at the bottom of the image.

(B) Prolonged maintenance of Csc1p on SPB upon Cdc13p overexpression: A non-degradable form of Cdc13p (Cdc13pΔ81) was overexpressed in *csc1-GFP mCherry-atb2* cells and the localization of Csc1p on SPB was observed in cells with a mitotic spindle of atleast 5.2 µm. The percentage of cells with Csc1p on SPB was determined (n=50x3).

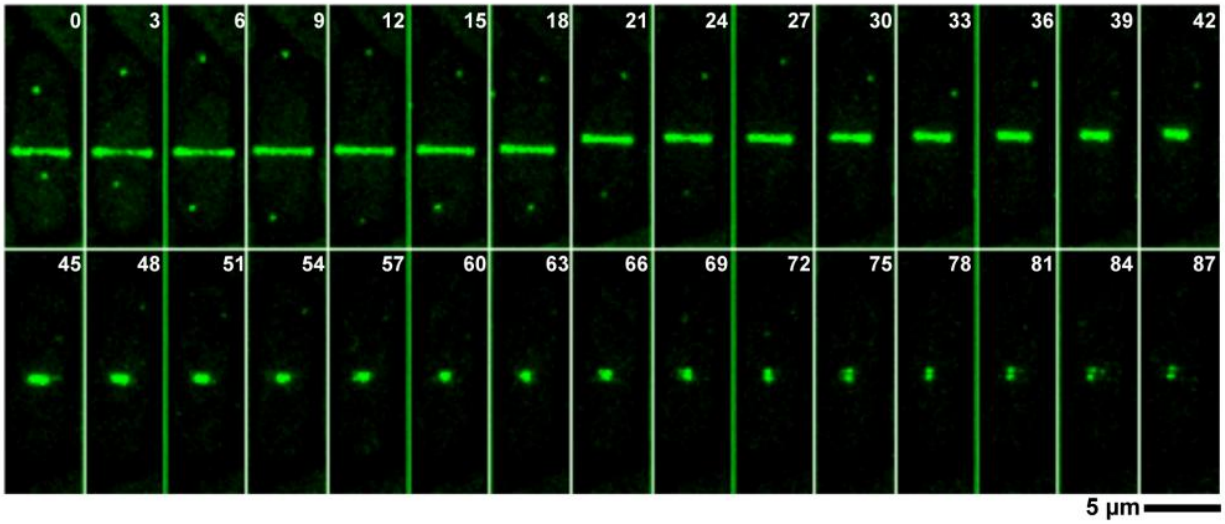

Figure S2. Cdc7p Is Lost from Both SPBs upon or before Completion of Septation in *csc1*Δ Cells

*csc1*Δ cells expressing Rlc1p-GFP, Cdc7p-GFP and Pcp1p-mCherry were imaged by time-lapse microscopy.

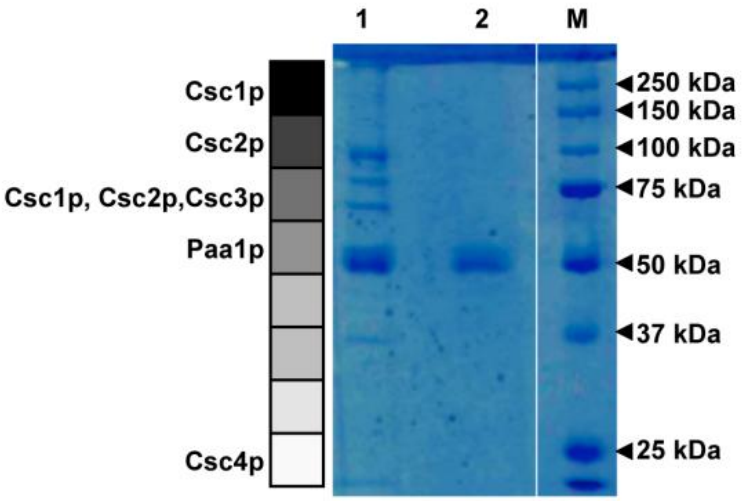

Figure S3. Immunoaffinity Purification of Csc1p

Lysates from wild-type cells expressing Csc1p-Myc_{13} were generated and incubated with antibodies against the Myc-epitope, immune complexes separated by SDS-PAGE, and stained with Coomassie Blue. A similar gel was segmented into 8 pieces as indicated by the grey scale boxes and subjected to mass spectrometric analysis to identify the proteins. The proteins identified from such gel slices are indicated besides the corresponding boxes. Lane 1 contains immune complex generated from wild-type cells expressing Csc1p-Myc_{13}, while Lane 2 contains immune complexes generated from a wild-type strain which did not express the Myc_{13}-tagged version of Csc1p.

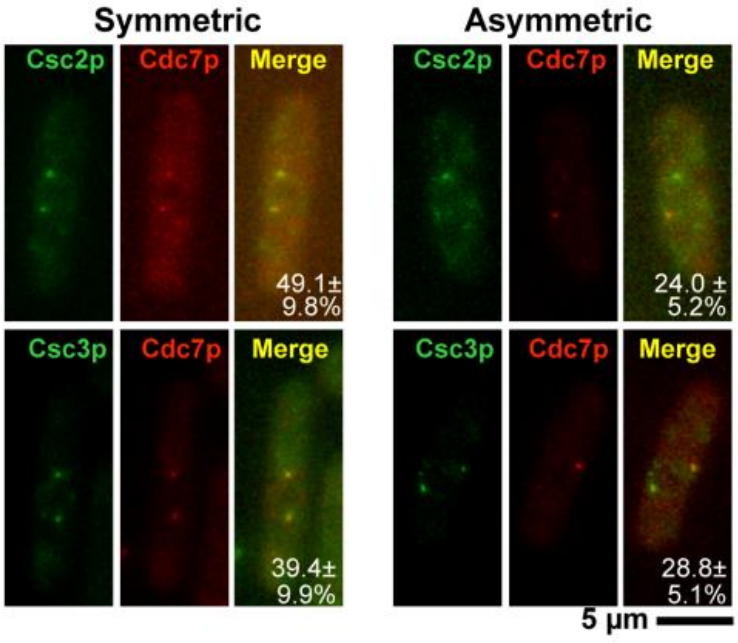

Figure S4. Csc2p and Csc3p Show a Transient Asymmetry at the SPB before Leaving the SPB

Wild-type cells expressing Cdc7p-mCherry and either Csc2p-GFP or Csc3p-GFP were grown at 24°C, fixed and then imaged to estimate the proportion of cells with symmetric localization of Csc2p and Csc3p and those with an asymmetric localization of these two proteins (n=50x3).

Supplemental Table 1. Proteins identified in Csc3-TAP and Paa1-TAP purifications

ORF	Seq. Cov. (%)	TSC	Csc3-TAP TSC	Csc3-TAP Exp1	Csc3-TAP Exp2	Paa1-TAP	Protein	Description
SPBC1773.01	**73**	**928**	**878**	**478**	**400**	**50**	**Csc3**	**striatin homolog**
SPBC3H7.13	50	106	100	45	55	6	Csc1	FHA domain protein (previously Far10)
SPBC27B12.04c	26	90	81	37	44	9	Csc2	conserved eukaryotic protein
SPAC9.09	54	177	60	49	11	117	Met26	homocysteine methyltransferase
SPAC1F3.06c	25	70	58	29	29	12	Spo15	sporulation protein Spo15
SPAC9E9.09c	47	65	51	28	23	14		aldehyde dehydrogenase
SPBC19C2.07	57	71	43	31	12	28	Fba1	fructose-bisphosphate aldolase
SPBC660.16	31	53	41	34	7	12		phosphogluconate dehydrogenase, decarboxylating
SPBC21C3.08c	40	50	38	34	4	12		ornithine aminotransferase
SPBC28F2.03	47	45	37	30	7	8	Ppi1/Cyp2	cyclophilin family peptidyl-prolyl cis-trans isomerase
SPAC26A3.05	15	46	34	13	21	12	Chc1	clathrin heavy chain
SPAC110.04c	36	51	34	27	7	17	Pss1	heat shock protein
SPAC2C4.10c	50	43	33	15	18	10	Csc4	sequence orphan
SPAC222.12c	36	36	31	18	13	5	Atp2	F1-ATPase beta subunit
SPBC2G5.05	26	30	29	20	9	1		transketolase
SPCC736.15	37	35	28	13	15	7		protein kinase inhibitor associated with endocytosis
SPBC9B6.04c	36	26	26	5	21	0	Tuf1	mitochondrial translation elongation factor EF-Tu
SPCPB16A4.03c	38	31	25	24	1	6	Ade10	IMPcyclohydrolase/phosphoribosylaminoimidazolecarboxamideformyltransferase
SPBC336.10c	51	44	25	16	9	19	Tif512	translation initiation factor eIF5A
SPBC56F2.09c	25	27	25	10	15	2	Arg5	arginine specific carbamoyl-phosphate synthase non catalytic subunit
SPBC14F5.03c	17	36	24	8	16	12	Kap123	karyopherin
SPBC8D2.18c	39	29	24	17	7	5		adenosylhomocysteinase
SPBC1A4.08c	30	27	24	10	14	3	Cct3	chaperonin-containing T-complex gamma subunit
SPCC24B10.21	63	35	23	22	1	12	Tpi1	triosephosphate isomerase
SPAC9.07c	30	23	23	8	15	0	Rbg1	GTPase
SPAP8A3.09c	**79**	**2013**	**22**	**7**	**15**	**1991**	**Paa1**	**protein phosphatase regulatory subunit**
SPBC215.05	39	43	19	19	0	24	Gpd1	glycerol-3-phosphate dehydrogenase
SPBC17D1.06	18	19	19	6	13	0	Dbp3	ATP-dependent RNA helicase
SPCC794.12c	17	23	18	18	0	5	Mae2	malic enzyme
SPBC1604.05	26	23	18	16	2	5	Pgi1	glucose-6-phosphate isomerase
SPAC1006.07	43	66	18	12	6	48		translation initiation factor eIF4A
SPAC19A8.15	26	29	18	10	8	11	Trp2	tryptophan synthase
SPBC146.14c	14	24	18	8	10	6	Sec26	coatomer beta subunit
SPAC22H10.04	24	22	18	8	10	4	Ppa3	protein phosphatase type 2A
SPCC1322.13	19	24	17	7	10	7	Ade6	phosphoribosylaminoimidazole carboxylase
SPAC1635.01	26	20	17	7	10	3		voltage-dependent anion-selective channel

ORF	Seq. Cov. (%)	TSC	Csc3-TAP TSC	Csc3-TAP Exp1	Csc3-TAP Exp2	Paa1-TAP	Protein	Description
SPCC1020.06c	56	82	17	16	1	65	Tal1	transaldolase
SPAC1F12.02c	54	28	17	14	3	11	P23Fy	translationally controlled tumor protein homolog
SPAC3C7.08c	13	22	17	5	12	5	Elf1	AAA family ATPase
SPAP8A3.07c	22	18	16	16	0	2		phospho-2-dehydro-3-deoxyheptonate aldolase
SPBC215.09c	44	28	16	15	1	12	Erg10	acetyl-CoA C-acetyltransferase
SPAC6B12.15	27	22	16	14	2	6	Cpc2	RACK1 homologue
SPBC428.02c	27	21	16	14	2	5	Eca39	branched chain amino acid aminotransferase
SPAC3A11.12c	38	32	16	8	8	16	Rpt5	19S proteasome regulatory subunit
SPAC29A4.15	38	43	16	7	9	27		cytoplasmic serine-tRNA ligase
SPAC1834.02	15	34	16	7	9	18	Aro1	pentafunctional aromatic polypeptide
SPCC757.09c	30	22	16	8	8	6	Rnc1	RNA-binding protein that suppresses calcineurin deletion
SPAC20G8.06	8	18	16	7	9	2	Not1	CCR4-Not complex subunit
SPBC646.09c	18	24	16	5	11	8	Int6	eIF3e subunit homolog
SPCC1450.04	39	36	15	14	1	21	Tef5	translation elongation factor EF-1 beta subunit
SPAC23C11.09	18	34	15	11	4	19	Ala1	mitochondrial and cytoplasmic alanine-tRNA ligase
SPBC26H8.07c	21	15	15	9	6	0	Nda3	tubulin beta
SPBC16A3.15c	15	19	15	5	10	4	Nda2	tubulin alpha 1
SPAC23C11.05	40	16	14	14	0	2		inorganic pyrophosphatase
SPCC645.14c	41	52	14	13	1	38	Sti1	chaperone activator
SPBC1703.13c	18	16	14	7	7	2		mitochondrial inorganic phosphate transporter
SPBC13A2.04c	11	15	14	4	10	1		PTR family peptide transporter
SPCC1840.03	11	22	14	5	9	8	Sal3	karyopherin
SPCC576.03c	47	21	13	13	0	8	Tpx1	thioredoxin peroxidase
SPBPJ4664.04	14	21	13	9	4	8		coatomer alpha subunit
SPAC21E11.08	17	25	13	7	6	12	Lcb2	serine palmitoyltransferase
SPBC56F2.08c	9	13	13	6	7	0		RNA-binding protein
SPBC18E5.06	30	17	13	7	6	4	Rps21	40S ribosomal protein S21
SPBC20F10.01	27	16	13	5	8	3	Gar1	snoRNP pseudouridylase complex protein
SPAC1D4.04	26	14	13	4	9	1	Cct2	chaperonin-containing T-complex beta subunit
SPBC8E4.01c	12	14	13	3	10	1		inorganic phosphate transporter
SPBC215.08c	11	12	12	12	0	0	Arg4	arginine specific carbamoyl-phosphate synthase
SPBC1703.10	56	30	12	5	7	18	Ypt1	GTPase
SPBC800.05c	11	14	12	3	9	2	Atb2	tubulin alpha 2
SPCC364.07	15	11	11	10	1	0		D-3 phosphoglycerate dehydrogenase
SPAC10F6.06	61	24	11	9	2	13	Vip1	RNA-binding protein
SPBC776.03	25	11	11	9	2	0		homoserine dehydrogenase
SPBC337.05c	25	14	11	6	5	3	Cct8	chaperonin-containing T-complex theta subunit

ORF	Seq. Cov. (%)	TSC	Csc3-TAP TSC	Csc3-TAP Exp1	Csc3-TAP Exp2	Paa1-TAP	Protein	Description
SPAC4F8.07c	18	11	11	11	0	0	Hxk2	hexokinase 2
SPCC1322.14c	14	13	11	7	4	2	Vtc4	vacuolar transporter chaperone
SPAC20G8.05c	13	14	11	6	5	3	Cdc15	cell division control protein
SPAC20G8.09c	8	11	11	4	7	0		N-acetyltransferase
SPBC21D10.12	20	15	11	3	8	4	Hob1	BAR adaptor protein
SPAC1F12.07	40	34	10	8	2	24		phosphoserine aminotransferase
SPAC9E9.07c	37	15	10	6	4	5	Ypt2	GTPase
SPBC29A10.01	14	11	10	5	5	1	Ccr1	NADPH-cytochrome p450 reductase
SPCC777.09c	25	10	10	10	0	0	Arg1	acetylornithine aminotransferase
SPAPB17E12.05	42	34	10	5	5	24	Rpl3703	60S ribosomal protein L37
SPBC3D6.02	21	46	9	9	0	37	But2	But2 family protein
SPAC1F5.02	22	28	9	9	0	19		protein disulfide isomerase
SPAC56F8.05c	37	21	9	9	0	12	Mug64	BAR domain protein
SPCC645.08c	19	24	9	7	2	15	Snd1	RNA-binding protein
SPBC8D2.06	9	17	9	7	2	8	Irs1	cytoplasmic isoleucine-tRNA ligase
SPBC16G5.05c	20	12	9	5	4	3		MSP domain
SPAC1783.05	7	9	9	6	3	0	Hrp1	ATP-dependent DNA helicase
SPCC550.11	11	13	9	5	4	4		karyopherin
SPAC24H6.04	23	17	8	7	1	9	Hxk1	hexokinase 1
SPBC12C2.06	34	30	8	6	2	22		ATP-dependent RNA helicase
SPAC17G8.06c	7	9	8	6	2	1		dihydroxy-acid dehydratase
SPBC106.06	20	13	8	5	3	5	Cct4	chaperonin-containing T-complex delta subunit
SPAC27E2.03c	27	30	8	8	0	22		GTP binding protein
SPAC977.14c	22	8	8	8	0	0		aldo/keto reductase, unknown biological role
SPAC25B8.12c	15	8	8	8	0	0		nucleotide-sugar phosphatase
SPAC19B12.01	7	10	8	5	3	2		TPR repeat protein, TTC27 family
SPAC26F1.07	20	7	7	7	0	0		glucose 1-dehydrogenase
SPAC3C7.14c	18	7	7	7	0	0	Obr1	ubiquitinated histone-like protein
SPBC30D10.13c	33	23	7	6	1	16	Pdb1	pyruvate dehydrogenase e1 component beta subunit
SPACUNK4.07c	18	30	7	5	2	23	Cta4	P-type ATPase, calcium transporting
SPBC119.01	25	20	7	5	2	13	Rpn3	19S proteasome regulatory subunit
SPCC16C4.07	14	11	7	4	3	4	Scw1	RNA-binding protein
SPBC1703.07	34	45	6	5	1	39		ATP citrate synthase subunit 1
SPBC3D6.15	31	73	6	2	4	67	Rps2501	40S ribosomal protein S25
SPBC18E5.02c	16	17	6	1	5	11		serine palmitoyltransferase complex subunit
SPAC22A12.16	44	64	6	5	1	58		ATP-citrate synthase subunit 2
SPAC1486.04c	10	24	6	5	1	18	Alm1	medial ring protein

ORF	Seq. Cov. (%)	TSC	Csc3-TAP TSC	Csc3-TAP Exp1	Csc3-TAP Exp2	Paa1-TAP	Protein	Description
SPBC1778.06c	28	39	5	5	0	34	Fim1	fimbrin
SPBC17D11.07c	14	27	5	3	2	22	Rpn2	19S proteasome regulatory subunit
SPAC1834.04	18	15	5	0	5	10	Hht1	histone H3 h3.1
SPAC17H9.14c	20	16	5	4	1	11		protein disulfide isomerase
SPCC306.09c	16	17	5	3	2	12	Cap1	adenylyl cyclase-associated protein
SPAC6F6.15	42	20	4	3	1	16	Ypt5	GTPase
SPAC222.14c	17	15	4	3	1	11	Sey1	GTP binding protein
SPAC22A12.07c	11	14	4	0	4	10	Ogm1	protein O-mannosyltransferase
SPAC6F12.10c	15	28	4	4	0	24	Ade3	phosphoribosylformylglycinamidine synthase
SPACUNK4.10	28	16	3	3	0	13		hydroxyacid dehydrogenase
SPCC1682.05c	32	32	3	1	2	29	Srp68	signal recognition particle subunit
SPAC26F1.03	25	20	3	3	0	17	Pda1	pyruvate dehydrogenase e1 component alpha subunit
SPAC30C2.08	29	19	3	3	0	16		conserved fungal protein
SPAC27F1.07	28	36	2	2	0	34		dolichyl-diphospho-oligosaccharide-protein glycosyltransferase
SPBC3B9.01	32	20	2	2	0	18		Hsp70 nucleotide exchange factor
SPAC23G3.11	24	17	2	2	0	15	Rpn6	19S proteasome regulatory subunit
SPAC22H12.01c	41	15	2	0	2	13	Mug35	sequence orphan
SPAC3C7.11c	18	59	2	2	0	57	Cnx1	calnexin
SPBC1921.05	15	15	2	2	0	13	Ape2	aminopeptidase
SPAC9E9.04	42	20	1	1	0	19		bcap family homolog
SPAPYUK71.03c	9	15	1	1	0	14		C2 domain protein
SPBC365.13c	18	12	1	1	0	11	Hba1	Ran GTPase binding protein
SPBC12C2.08	14	15	1	0	1	14	Dnm1	dynamin
SPAC607.05	12	11	1	0	1	10	Rpn9	19S proteasome regulatory subunit
SPBC16H5.07c	84	522	1	1	0	521	Ppa2	serine/threonine protein phosphatase
SPAC823.15	62	369	1	1	0	368	Ppa1	minor serine/threonine protein phosphatase
SPCC1322.16	24	22	1	1	0	21	Phb2	prohibitin
SPAC1142.04	11	15	1	1	0	14		Noc complex subunit Noc2 family
SPCC188.02	54	906	0	0	0	906	Par1	protein phosphatase regulatory subunit
SPAC227.07c	56	337	0	0	0	337	Pab1	protein phosphatase regulatory subunit
SPAC1782.05	44	64	0	0	0	64		phosphotyrosyl phosphatase activator homolog
SPAC31F12.01	33	63	0	0	0	63	Zds1	zds family protein phosphatase type A regulator
SPCC338.15	24	25	0	0	0	25		dolichyl-di-phosphooligosaccharide-protein glycotransferase subunit
SPAC3H8.04	28	24	0	0	0	24		chromosome segregation protein
SPAC6F12.12	20	24	0	0	0	24	Par2	protein phosphatase regulatory subunit
SPBC365.12c	14	19	0	0	0	19	Ish1	LEA domain protein
SPAC18G6.06	28	18	0	0	0	18	Utp11	U3 snoRNP-associated protein

ORF	Seq. Cov. (%)	TSC	Csc3-TAP TSC	Csc3-TAP Exp1	Csc3-TAP Exp2	Paa1-TAP	Protein	Description
SPAC22F8.05	12	17	0	0	0	17		alpha,alpha-trehalose-phosphate synthase
SPCC1393.12	43	16	0	0	0	16		sequence orphan
SPAC1705.03c	9	15	0	0	0	15		conserved fungal family
SPAC1786.02	12	14	0	0	0	14		phospholipase
SPBC3F6.02c	18	14	0	0	0	14		3 beta-hydroxysteroid dehydrogenase/delta 5-->4-isomerase
SPBC582.07c	17	13	0	0	0	13	Rpn7	19S proteasome regulatory subunit
SPAPB1A10.08	17	12	0	0	0	12		sequence orphan
SPBC3E7.02c	49	12	0	0	0	12	Hsp16	heat shock protein
SPCC1183.02	21	12	0	0	0	12		glutathione S-transferase
SPCC1235.14	12	12	0	0	0	12	Ght5	hexose transporter
SPAC2G11.07c	14	11	0	0	0	11	Ptc3	protein phosphatase 2C
SPAC4G9.11c	17	11	0	0	0	11	Cmb1	cytosine-mismatch binding protein 1
SPBP4H10.17c	14	11	0	0	0	11		carboxyl methyl esterase

Key:
Baits are bold
components of the SIP-complex are highlighted in yellow
TSC = total spectral counts
Exp = experiment

Table S2. Localization Dependencies of the SIP-Complex Components

	*csc1*Δ	*csc2*Δ	*csc3*Δ	*Csc4*Δ	*paa1* L566P	*ppa3*Δ
Csc1-GFP	-	x	x	x	NA	x
Csc2-GFP	x	-	x	x	NA	x
Csc3-GFP	x	x	-	x	x	x
Paa1-GFP	x^p	x^p	x^p	x^p	-	x^p

(-) Not relevant, (x) does not localize to SPB(s), (NA) not analyzed, (x^p)-partial localization in some cells.

Table S3. Percentage of SIN-Hyperactivated Cells in the Csc Double-Deletion Mutants

	*csc1*Δ (1.8±0.5)	*csc2*Δ (0.9±0.3)	*csc3*Δ (1.4±0.5)	*csc4*Δ (0.5±0.5)	*ppa3*Δ (0.1±0.1)
*csc2*Δ(0.9±0.3)	4.6±0.8				
*csc3*Δ (1.4±0.5)	1.3±0.3	3.6±0.8			
*csc4*Δ(0.5±0.5)	1.5±0.4	1.3±0.5	1.3±0.1		
*ppa3*Δ (0.1±0.1)	1.4±0.3	0.4±0.3	1.5±0.7	1.6±0.7	
paa1-L566P (4.5±0.6)	2.0±0.6	6.4±0.8	5.8±0.4	4.8±1	4.8±2.6

Table S4: Strains and plasmids used in this study

MBY102	*ade6*-210 *ura4*-D18 *leu1*-32 h^+	Lab stock
MBY4336	*csc1Δ:: ura4*$^+$ *leu1*-32 *ura4*-D18 h^-	This study
MBY5886	*csc1Δ:: ura4*$^+$*leu1*-32 *ura4*-D18 h^+	This study
MBY286	*cdc16*-116 *leu1*-32 *ura4*-D18 *ade6*-M210 h^+	[13]
MBY2415	*cdc7-GFP::ura4*$^+$ *ade6*-21x *leu1*-32 *ura4*-D18 *his3*-D1 h^+	Lab stock
MBY604-1	*sid1-GFP:: ura4*$^+$ *ade6*-210 *leu1*-32 h^+	Lab stock
MBY6193	*kanMX-nmt1-csc1-GFP:: ura4*$^+$ *ura4*-D18	This study
MBY4310	*csc1-GFP:: ura4*$^+$ *leu1*-32 *ura4*-D18 h^-	This study
MBY5856	*mCherry-atb2::hph leu1*-32 *ura4*-D18 h^-	
MBY6964	*csc1-GFP:: ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18 h^-	This study
MBY6965	*csc1-GFP:: ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18 h^+	This study
MBY6968	*cdc25*-22 *csc1::gfp-ura4+ mCherry-atb2::hph leu1*-32 *ura4*-D18 h^-	This study
MBY7085	*sid4*-SA1 *csc1-GFP:: ura4*$^+$*mCherry-atb2:hph* h^+	This study
MBY7016	*cdc25*-22 *csc1-GFP:: ura4*$^+$ *cdc7-mCherry:kanMx*	This study
MBY7357	*nda3*-KM311 *csc1-GFP:: ura4*$^+$ *mCherry-atb2::hph*	This study
MBY6569	*csc1Δ::ura4*$^+$ *cdc7-GFP:: ura4*$^+$ *mCherry-atb2::hph leu1-32 ura4*-D18	This study
MBY5887	*sid1-GFP:: ura4*$^+$ *csc1Δ::ura4+ leu1*-32	This study
MBY4627	*cdc16*-116 *csc1Δ::ura4*$^+$	This study
MBY6361	*csc3-GST::kanMx csc1-myc*$_{13}$*::KanMx ade6*-210 *ura4*-D18 *leu1*-32	This study
MBY6363	*csc2-GST::KanMx csc1- myc*$_{13}$*::KanMx ade6*-210 *ura4*-D18 *leu1*-32	This study
MBY6332	*paa1-GST::kanMx csc1- myc*$_{13}$*::kanMx ade6*-210 *ura4*-D18 *leu1*-32	This study
MBY6911	*csc3-GFP::KanMx mCherry-atb2::hph leu1*-32 *ura4*-D18 h^+	This study
MBY 7353	*csc2-GFP::kanMx mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7329	*paa1-GFP::kanMx mCherry-atb2::hph ade6*-210 *ura4*-D18 *leu1*-32 h^+	This study
MBY6336	*csc3Δ::kanMx ade6*-210 *ura4*-D18 *leu1*-32 h^+	This study
MBY6339	*csc2Δ::KanMx ade6*-210 *ura4*-D18 *leu1*-32 h^+	This study
MBY6341	*csc4Δ:: ura4*$^+$ *ade6*-210 *leu1*-32 *ura4*-D18 h^+	This study
MBY6386	*csc2Δ::kanMx cdc16*-116 *ade6*-210 *ura4*-D18 *leu1*-32	This study
MBY6385	*csc3Δ::kanMx cdc16*-116 *ade6*-210 *ura4*-D18 *leu1*-32	This study
MBY6389	*csc4Δ::ura4*$^+$ *cdc16*-116 *ade6*-210 *ura4*-D18 *leu1*-32	This Study
MBY6643	*paa1*-L566P-*his5+::ura4*$^+$ *his5Δ ura4D*-18 *leu1*-32 h^+	This study
MBY7206	*cdc16*-116 *paa1*-L566P-*his5+::ura4*$^+$ *ura4*-D18 *leu1*-32	This study
MBY6471	*csc2Δ::kanMx cdc7-GFP::ura4*$^+$ *mcherryatb2::hph*	This study

==========

Table S4 contd.

MBY6926	*paa1*-L566P-*his5+::ura4*$^+$ *mCherry-atb2::hph cdc7-GFP::ura4+*	This study
MBY7358	*csc3*Δ*::kanMx cdc7-GFP::ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY6571	*csc4*Δ*::ura4*$^+$ *cdc7-gfp::ura4 mCherry-atb2::hph ura4*-D18 *leu1*-32	This study
MBY7352	*ppa3*Δ*::kanMx cdc7-GFP::ura4*$^+$ *mCherry-atb2::Hph leu1*-32 *ura4*-D18 *h*$^-$	This study
MBY6647	*cdc11- myc*$_{13}$*::kanMx ade6*-M210 *ura4*-D18 *leu1*-32 *h*$^-$	[14]
MBY6772	*cdc11-myc::kanMx csc1*Δ*::ura4+ leu1*-32 *ura4*-D18 *h*$^+$	This study
MBY603-1	*cdc7-3xHA::ura4*$^+$ *leu1*-32 *h*$^+$	Lab stock
MBY5717	*csc1*Δ*::ura4*$^+$ *cdc7-3xHA::ura4*$^+$ *leu1*-32 *ura4*-D18	This study
MBY6648	*cdc11*-136 *ura4*-D18 *leu1*-32 *h*$^-$	Lab stock
MBY7007	*cdc11*-136 *csc4*Δ*::ura4*$^+$ *ura4*-D18 *leu1*-32	This study
MBY7008	*cdc11*-136 *csc1*Δ*::ura4*$^+$ *ura4*-D18 *leu1*-32	This study
MBY7345	*csc3-3xTAP::kanMx ade6*-210 *ura4*-D18 *leu1*-32 *h*$^+$	This study
KGY10015	*paa1*-linker-*TAP::kan*^{R} *ade6*-M210 *leu1*-32 *ura4*-D18 *h*$^-$	This study
MBY6169	*csc1-myc*$_{13}$*::kanMx ura4*-D18 *leu1*-32	This study
MBY6935	*cdc7-mCherry:kanMx leu1*-32 *ura4*-D18 *ade6*-210 *h*$^-$	This study
MBY7415	*ppa3*Δ*::kanMx ura4*-D18 *leu1*-32 *h*$^-$	This study
MBY7723	*cdc7-GFP::ura4*$^+$ *csc1*Δ*::ura4*$^+$ *rlc1-3GFP::KanMx*	This study
MBY7654	*cdc13*-117 *csc3-GFP::KanMx mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7642	*csc3-GFP::kanMx cdc7-mCherry::kanMx leu1*-32 *ura4*-D18 *ade6*-210	This study
MBY7355	*csc2-GFP::kanMx cdc7-mCherry::kanMx leu1*-32 *ura4*-D18 *ade6*-210	This study
MBY7650	*kanMx*::*nmt81-ppc89 csc1-GFP::ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7656	*cdc16*-116 *csc1-GFP::ura4*$^+$ mCherry-atb2::hph leu1-32 ura4-D18	This study
MBY7543	*h*$^+$*/h*$^-$*ppc89*Δ*::ura4*$^+$*/ppc89*$^+$*ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32*	Rosenberg [15]
MBY7657	*h+/h-ppc89*Δ*::ura4+/ppc89+ade6*-M210*/ade6*-M216 *csc3-GFP::kanMx mCherry-atb2::hph ura4*-D18	This study
MBY7447	*ppa1*Δ*::ura4*$^+$ *leu1*-32 *ura4*-D18 *h*$^-$	[16]
MBY7448	*ppa2*Δ*::ura4*$^+$ *leu1*-32 *ura4*-D18 *h*$^-$	[16]
MBY7658	*ppa1*Δ*::ura4*$^+$ *ppa3*Δ*::kanMx ura4*-D18 *leu1*-32	This study
MBY7659	*ppa2*Δ*::ura4*$^+$ *ppa3*Δ*::kanMx ura4*-D18 *leu1*-32	This study
MBY7482	*ppa1*Δ*::ura4*$^+$ *cdc7-mCherry:kanMx leu1*-32 *ura4*-D18 *ade6*-M210 *h*$^+$	This study
MBY7483	*ppa2*Δ*::ura4*$^+$ *cdc7-mCherry:kanMx leu1*-32 *ura4*-D18 *ade6*-210 *h*$^+$	This study
MBY7348	*nda3*-KM311 *csc3-3XTAP::kanMx ura4*-D18	This study
MBY7644	nda3-KM311 *cdc11-myc*$_{13}$*::kanMx csc1*Δ*::ura4*$^+$	This study

Table S4 contd.

MBY7324	*kanMx-nmt1-csc1-GFP::ura4*$^+$*cdc7-mCherry::kanMx*	This study
MBY7660	*kanMX-nmt1-csc1-GFP::ura4*$^+$*myc*$_{13}$*-sid1::ura4*$^+$ *ura4*-D18	This study
MBY6380	*csc2*Δ*::kanMx csc1*Δ*::ura4*$^+$ *ade6*-M210 *ura4*-D18 *leu1*-32	This study
MBY6378	*csc3*Δ*::ura4*$^+$*csc1*Δ*::ura4*$^+$*ade6*-M210 *ura4*-D18 *leu1*-32	This study
MBY7661	*csc1*Δ*::ura4+ csc4*Δ*::ura4*$^+$ *leu1*-32 *ura4*-D18	This study
MBY7476	*ppa3*Δ*::kanMx csc1*Δ*::ura4*$^+$ *ura4*-D18 *leu1*-32 *h*$^+$	This study
MBY7478	*csc1*Δ*::ura4*$^+$ *paa1*-L566P-*his5+:ura4*$^+$ *ura4*-D18 *leu1*-32 *h*$^+$	This study
MBY7662	*csc2*Δ*::KanMx csc3*Δ*::kanMx ura4*-D18	This study
MBY7663	*csc2*Δ*::KanMx csc3*Δ*::kanMx ura4*-D18	This study
MBY7663	*csc2*Δ*::KanMx csc4*Δ*::ura4*$^+$ *ura4*-D18 *leu1*-32	This study
MBY7664	*csc2*Δ*::KanMx ppa3*Δ*::kanMx ura4*-D18 *leu1*-32	This study
MBY7208	*paa1*-L566P-*his5+:ura4*$^+$ *csc2*Δ*::KanMx ura4*-D18 *leu1*-32	This study
MBY7665	*csc4*Δ*:: ura4+ csc3*Δ*::kanMx ura4*-D18 *leu1*-32	This study
MBY766	*csc3*Δ*::kan ppa3*Δ*::kanMx ura4*-D18	This study
MBY6927	*paa1*-L566P*::his5+-ura4*$^+$ *csc3*Δ*:: kan ura4*-D18 *h*$^+$	This study
MBY7667	*csc4*Δ*::ura4*$^+$ *ppa3*Δ*::kanMx ura4*-D18 *leu1*-32	This study
MBY7091	*paa1-* L566P-*his5+:ura4*$^+$ *csc4*Δ*::ura4*$^+$ *ura4*-D18 *leu1-32 h*$^+$	This study
MBY7668	*ppa3*Δ*::kanMx paa1*-L566P-*his5+:ura4*$^+$ *ura4*-D18 *leu1*-32	This study
MBY7669	*csc2-GFP::kanMx mcherry-atb2::hph csc1*Δ*::ura4*$^+$ *ura4*-D18 *h*$^+$	This study
MBY7670	*csc1*Δ*::ura4*$^+$ *csc3-GFP::KanMx mCherry-atb2::hph leu1*-32 *ura4*-D18 *h*$^-$	This study
MBY	*csc1*Δ*::ura4*$^+$ *paa1-GFP::KanMx*	This study
MBY7671	*csc2*Δ*::KanMx csc1-GFP::ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7672	*csc2*Δ*::KanMx csc3-GFP::KanMx mCherry-Atb2::hph leu1*-32 *ura4*-D18	This study
MBY7673	*csc2*Δ*::KanMx paa1-GFP::kanMx ura4*-D18	This study
MBY6899	*csc3*Δ*::kanMx csc1-GFP::ura4*$^+$ *ura4*-D18 *leu1*-32 *h*$^+$	This study
MBY7675	*csc3*Δ*::kanMx csc2-GFP::kanMx mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7676	*csc3*Δ*::kanMx paa1-GFP::kanMx ura4*-D18	This study
MBY7677	*ppa3*Δ*::kanMx csc1-GFP::ura4*$^+$ *mCherry-atb2::hph leu1*-32 *ura4*-D18 *h*$^-$	This study
MBY7455	*ppa3*Δ*::kanMx csc2-GFP::kan mCherry-atb2::hph ura4*-D18 *leu1*-32 *h*$^-$	This study

Table S4 contd.

MBY7456	*ppa3*Δ*::kanMx csc3-GFP::Kan mCherry-atb2::hph leu1*-32 *ura4*-D18 *h*$^-$	This study
MBY7678	*ppa3*Δ*::kanMx paa1-GFP::kanMx mCherry-atb2::hph ura4*-D18 *leu1*-32 *h*$^-$	This study
MBY7679	*paa1*-L566P-*his5+::ura4*$^+$ *csc2-gfp::KanMx mCherry-atb2::hph leu1*-32 *ura4*-D18	This study
MBY7696	*csc1-GFP::ura4*$^+$ *mCherry-atb2::hph csc4*Δ*::ura4+ leu1*-32 *ura4*-D18	This study
MBY7698	*csc2-GFP::kanMx mCherry-atb2::hph csc4*Δ*::ura4*$^+$ *leu1*-32 *ura4*-D18	This study
MBY7700	*csc3-GFP::KanMx mCherry-atb2::hph csc4*Δ*::ura4*$^+$ *leu1*-32 *ura4*-D18	This study
MBY7702	*paa1-GFP::kanMx mCherry-atb2::hph csc4*Δ*::ura4*$^+$ *ade6*-210 *leu1*-32 *ura4*-D18	This study
MBY7721	*nda3*-KM311 *cdc11-myc*$_{13}$*::kanMx ade6-M21x ura4*-D18 *leu1*-32	This study
MBY7644	*nda3*-KM311 *cdc11-myc*$_{13}$*::kanMx csc1*Δ*::ura4*$^+$ *ura4*-D18 *leu1*-32	This study

plasmids

pCDL1529	pREP41-*GST*	This study
pCDL514	pREP1-*mad2*	Lab stock
pCDL1553	pREP41-*csc1-GST*	This study
pCDL834	pREP41-cdc13Δ81	Lab stock

Supplemental Experimental Procedures

Yeast Strains and Methods

The yeast strains used in this study are listed in Table S4. Standard fission yeast media, growth conditions and manipulations were employed as previously described [1]. Induction of gene expression from the *nmt* promoter [2] was achieved by growing the cells in thiamine containing medium (repressing condition), washed three times in medium lacking thiamine and grown for another 16 hr or as indicated in the medium lacking thiamine. All experiments involving temperature sensitive strains were done at permissive temperature 24°C and the restrictive temperature of 36°C unless otherwise indicated. Standard genetic and recombinant DNA methods were used as described previously [1, 3]. Yeast transformations were carried out using lithium acetate method [4].

Gene Deletion and Tagging

PCR-based gene targeting method was used to generate the deletion mutants as well as to tag the genes with GFP or GST or Myc_{13} or 3xHA. Deletion mutants of *csc1* and *csc4* were generated by replacing the coding region with an *ura4*$^+$ cassette [4] while *csc2*, *csc3* and *ppa3* were replaced with a kanamycin resistance cassette. The tagged derivatives *csc1-myc*$_{13}$*, csc2-GST, csc2-GFP, csc3-GFP, csc3-GST, paa1*-GST, *paa1-GFP* and *csc3-3xTAP* were generated using *myc*$_{13}$*::kanMx*, *GST::kanMx*, *GFP::kanMx* and *3xTAP::kanMx* cassettes as previously described [5] while *csc1-GFP* was generated by using *GFP::ura4*$^+$ cassette [4]. The native promoter of *csc1* was replaced with the thiamine repressible *nmt1* promoter in wild type and *csc1-GFP* to generate the *nmt1-csc1* and *nmt1-csc1-GFP* strains respectively. Similarly, the native promoter of *ppc89* was replaced with the *nmt81* promoter to generate the ppc89 shut off strain. Further, the temperature sensitive *paa1*-L566P mutant was isolated using the fragment switch method as described earlier [6].

Immunoprecipitation and Mass spectrometry of Csc1p-Myc_{13}

Immunoprecipitation was performed as described earlier [7] except using NP40 buffer (1% NP-40, 150 mM NaCl, 2 mM EDTA, 6 mM NA_2HPO_4, 4 mM NaH_2PO_4). Briefly, cell extracts were prepared by glass bead disruption and solubilized in NP40 buffer containing complete protease inhibitors (Roche Diagnostics) and 1.5 µg/ml of PMSF. Cell extracts were then clarified by centrifugation at 14,000 rpm for 10 min at 4°C. To immunoprecipitate protein complex, 500 µl of soluble protein was incubated with 5 µl of the appropriate antibodies for 1–2 h at 4°C. Protein A-Sepharose beads (100 µl, Amersham Biosciences) were then added to the antigen-antibody immunocomplex and incubated for 45 min at 4°C. After six washes with NP40 buffer, the protein A-immune complex was treated with 1mM DTT, followed by iodoacetamide to block the cysteine residues and the beads were resuspended in SDS-PAGE loading buffer and heated at 95°C for 5 min. The Protein A-Sepharose beads were spun down at 14,000 rpm for 5 min and the proteins were resolved on 10% SDS-PAGE. The gel was then cut into 8 bands followed by in-gel trypsin digestion. The resulting peptides in each of the bands were analyzed by mass spectrometry to identify the proteins.

MS analysis of Csc3p-3xTAP and Paa1p-3xTAP

The proteins were purified as described previously and TCA precipitated followed by digestion with trypsin (Promega). The resultant peptides were subjected to 2D LC-MS/MS (liquid chromatography-tandem mass spectrometry) analysis on a Thermo LTQ as previously described [8, 9]. Thermo RAW files were converted to MZML files using Scansifter (software developed in-house at the Vanderbilt University Medical Center). Spectra with less than 20 peaks were excluded from our analysis. The *S. pombe* database (http://www.sanger.ac.uk, October 2009) was searched with the Myrimatch algorithm [10] v1.6.75 on a high performance computing cluster (Advanced Computing Center for Research & Education at Vanderbilt University). We added contaminant proteins (*e.g.* keratin, IgG) to the complete *S. pombe* database and reversed and concatenated all sequences to allow estimation of false discovery rates (10186 entries). Myrimatch parameters were as follows: strict tryptic cleavage; modification of methionine (oxidation, dynamic modification, +16 Da), S/T/Y (phosphorylation, dynamic modification, +80 Da) and cysteine (carboxamidomethylation, static modification, +57 Da) was allowed; precursor ions were required to be within 0.6 *m/z* of the peptide monoisotopic mass; fragment ions were required to fall within 0.5 *m/z* of the expected monoisotopic mass. IDPicker[11, 12] v2.6.165 was used to filter peptide matches with the following parameters: max. FDR per result 0.05, max. ambiguous IDs per result 2, min. peptide length per

result 5, min. distinct peptides per protein 3, min. additional peptides per protein group 1, minimum number of spectra per protein 3, indistinct modifications M 15.994 Da, C 57.05 Da and distinct modifications S/T/Y 80 Da. IDPicker results were processed in Excel (Microsoft®) to generate protein identification lists. Proteins identified as contaminants (e.g. keratin) or non-specific background (i.e. identified in no TAP tag negative controls or in over 50% of other unrelated TAP/LC-MS/MS analyses performed in our laboratory) were removed from the final protein list. Proteins listed in Supplemental Table 1 are the top 100 hits from Csc3-TAP or Paa1-TAP (ordered by Csc3-TAP).

Pull-down and Immunoblotting

Protein extracts for the pull-down experiments were isolated as described above for the immunoprecipitation. The extract was then incubated for 1–2 h at 4°C with 20 µl of pre-swollen glutathione-sepharose 4B (Amersham Biosciences) beads equilibriated with NP40 buffer for GST tagged proteins and with IgG- sepharose (GE Healthcare) beads for 3xTAP tagged prteins. The beads were then washed six times with NP40 buffer and resuspended in SDS-PAGE loading buffer. The proteins were resolved on 8% SDS-PAGE, electroblotted onto a PVDF membrane and detected using anti-Myc antibodies (SIGMA).

To check the phosphorylation level of Cdc11p, the cells were grown to early log phase at the permissive temperature and arrested at prometaphase by incubation at 18°C for four hours. The cells were then released at 32°C for 50 min and lysed in the lysis buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 1% Triton X-100) containing 1 mM PMSF, 1 mM benzamidine and complete EDTA-free phosphatase inhibitor (GE Healthcare) by glass bead disruption method. The lysate was boiled in 1X SDS-PAGE loading buffer for 5 min and clarified by centrifugation at 13,000 rpm for 5 min. The supernatant was used for analyzing the level of Cdc11p phosphorylation. For phosphatase treatment, the extract was diluted 10 times and ~60 µg of the extract was treated with 40 units of the calf intestinal alkaline phosphatase (CIP) in the prescribed buffer system (New England Biolabs). The phosphorylation level was analyzed using western blot analysis by resolving the proteins on 8% SDS-PAGE.

Microscopy

Septum/cell wall and DNA were stained with aniline blue (Sigma) and 4′, 6-diamidino-2-phenylindole (DAPI), respectively. For immunofluorescence studies, cells were fixed with 3.7% formaldehyde for 20 min and permeabilised using PBS (3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 8% w/v NaCl and 0.2% w/v KCl) containing 1% triton-X100 for 2 min. Cells were washed three times with PBS and resuspended in PBS containing 1.2M sorbitol and 1.5 mg/ml lysing enzyme and 0.75 mg/ml zymolyase were added to protoplast the cells. The cells were then washed three times with PBS and blocked with PBAL buffer (PBS containing 1% albumin) for 1 hour at room temperature. Appropriate primary antibodies were added and incubated overnight at 4°C. The cells were then washed three times with PBAL and appropriate alexa 488 or alexa 594 labelled secondary antibodies were added. It was then incubated for 2 hours at room temperature, washed three times with PBAL and analyzed for the fluorescence signals. Images were captured using an Olympus IX71 microscope equipped with a Photometrics CoolSNAP ES camera. All images were processed with MetaMorph 6.1. GFP or mCherry tagged proteins were either observed directly in live cells or cells fixed for 6 min with methanol using the Olympus IX71 microscope equipped with a Photometrics CoolSNAP ES camera or spinning disk confocal microscope.

Supplemental References

1. Moreno, S., Klar, A., and Nurse, P. (1991). Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol *194*, 795-823.
2. Maundrell, K. (1993). Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene *123*, 127-130.
3. Sambrook, J., Fritsch, E.F., and Miniatis, T. (1989). Molecular Cloning. A laboratory manual.
4. Keeney, J.B., and Boeke, J.D. (1994). Efficient targeted integration at *leu1-32* and *ura4-294* in *Schizosaccharomyces pombe*. Genetics *136*, 849-856.
5. Bahler, J., Wu, J.Q., Longtine, M.S., Shah, N.G., McKenzie, A., 3rd, Steever, A.B., Wach, A., Philippsen, P., and Pringle, J.R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast *14*, 943-951.
6. Tang, X., Huang, J., Padmanabhan, A., Bakka, K., Bao, Y., Tan, B.Y., Cande, W.Z., and Balasubramanian, M.K. (2011). Marker reconstitution mutagenesis: a simple and efficient reverse genetic approach. Yeast *28*, 205-212.
7. Ge, W., Chew, T.G., Wachtler, V., Naqvi, S.N., and Balasubramanian, M.K. (2005). The novel fission yeast protein Pal1p interacts with Hip1-related Sla2p/End4p and is involved in cellular morphogenesis. Mol Biol Cell *16*, 4124-4138.
8. McDonald, W.H., Ohi, R., Miyamoto, D.T., Mitchison, T.J., and Yates, J.R., 3rd (2002). Comparison of three directly coupled HPLC MS/MS strategies fo identification of proteins from complex mixtures: single-dimension LC-MS/MS, 2-phase MudPIT, and 3-phase MudPIT. . Int. J. Mass Spectrom. *219*, 245-251.
9. Roberts-Galbraith, R.H., Chen, J.S., Wang, J., and Gould, K.L. (2009). The SH3 domains of two PCH family members cooperate in assembly of the Schizosaccharomyces pombe contractile ring. J Cell Biol *184*, 113-127.
10. Tabb, D.L., Fernando, C.G., and Chambers, M.C. (2007). MyriMatch: highly accurate tandem mass spectral peptide identification by multivariate hypergeometric analysis. J Proteome Res *6*, 654-661.
11. Zhang, B., Chambers, M.C., and Tabb, D.L. (2007). Proteomic parsimony through bipartite graph analysis improves accuracy and transparency. J Proteome Res *6*, 3549-3557.
12. Ma, Z.Q., Dasari, S., Chambers, M.C., Litton, M.D., Sobecki, S.M., Zimmerman, L.J., Halvey, P.J., Schilling, B., Drake, P.M., Gibson, B.W., et al. (2009). IDPicker 2.0: Improved protein assembly with high discrimination peptide identification filtering. J Proteome Res *8*, 3872-3881.
13. Minet, M., Nurse, P., Thuriaux, P., and Mitchison, J.M. (1979). Uncontrolled septation in a cell division cycle mutant of the fission yeast Schizosaccharomyces pombe. J Bacteriol *137*, 440-446.
14. Morrell, J.L., Tomlin, G.C., Rajagopalan, S., Venkatram, S., Feoktistova, A.S., Tasto, J.J., Mehta, S., Jennings, J.L., Link, A., Balasubramanian, M.K., et al. (2004). Sid4p-Cdc11p assembles the septation initiation network and its regulators at the S. pombe SPB. Curr Biol *14*, 579-584.
15. Rosenberg, J.A., Tomlin, G.C., McDonald, W.H., Snydsman, B.E., Muller, E.G., Yates, J.R., 3rd, and Gould, K.L. (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. Mol Biol Cell *17*, 3793-3805.
16. Kinoshita, N., Ohkura, H., and Yanagida, M. (1990). Distinct, essential roles of type 1 and 2A protein phosphatases in the control of the fission yeast cell division cycle. Cell *63*, 405-415.