Supplemental Materials Molecular Biology of the Cell

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SUPPORTING INFORMATION

for

Coordinate action of distinct sequence elements localizes checkpoint kinase Hsl1 to the septin collar at the bud neck in *Saccharomyces cerevisiae*

Gregory C. Finnigan,¹ Sarah M. Sterling,¹ Angela Duvalyan,¹ Elizabeth N. Liao,¹ Aspram Sargsyan,¹ Galo Garcia III,^{1†} Eva Nogales,^{1,2,3} and Jeremy Thorner¹*

¹Division of Biochemistry, Biophysics and Structural Biology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202 USA;

²Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720 USA;

³Howard Hughes Medical Institute

Strain	Genotype	Reference
BY4741	MATa leu2 Δ ura3 Δ met15 Δ his3 Δ	(Brachmann et al., 1998)
GFY-42	BY4741 cdc10∆::CDC10::mCherry::SpHIS5	(Finnigan et al., 2015b)
GFY-1507 ^a	BY4742 <i>msn5</i> ∆::Kan ^R	Life Technologies, Inc.
GFY-1506 ^a	BY4742 los1∆::Kan ^R	Life Technologies, Inc.
GFY-1537 ^b	MAT a ade2-101 his3-11,15 trp1-901 ura3-52 cse1-1	This study
	+ pGF-IVL774	
GFY-1539 ^b	MATα ade2-1 LYS2 leu2-3,112 trp1-1 can1-100	This study
	ura3-1 his3-11,15 xpo1::LEU2 + pKW457 [pRS313-	
	xpo1-1ts] + pGF-IVL774	
GFY-1541 ^c	BY4741 <i>hsl1</i> ∆:: <i>Kan^R</i>	This study
GFY-39	BY4741 shs1∆::Hyg ^R	This study
GFY-1451	BY4741 shs1∆::Hyg ^R hsl1∆::Kan ^R	This study
GFY-1120 ^d	BY4741 cdc11∆::cdc11(G29D)::mCherry::Nat ^R +	This study
	pJT1520	
GFY-1450	BY4741 cdc11∆::cdc11(G29D)::mCherry::Nat ^R	This study
	hsl1∆::Kan ^R + pJT1520	
GFY-1430 ^e	BY4741 cdc12∆::cdc12(K392N; ∆393-	This study
	407)::mCherry::SpHIS5 + pJT1622	
GFY-1449	BY4741 cdc12∆::cdc12(K392N; ∆393-	This study
	407)::mCherry::SpHIS5 hsl1∆::Kan ^R + pJT1622	
GFY-1156	BY4741 cdc10∆::CDC10::mCherry::SpHIS5	This study
	hsl1∆::Kan ^R	
GFY-768 ^f	BY4741 cdc11∆::CDC11::mCherry::SpHIS5	(Finnigan et al., 2015b)
GFY-1157	BY4741 cdc11∆::CDC11::mCherry::SpHIS5	This study
	hsl1∆::Kan ^R	
GFY-104 ^g	BY4741 cdc12∆::cdc12(K392N; ∆393-	(Finnigan et al., 2015a)
	407)::mCherry::Kan ^R + pJT1622	
GFY-	BY4741 cdc10∆::CDC10::mCherry::SpHIS5	This study
1561 ^{h,i}	hsl1∆::HSL1(1-1518)::HA₃::Nat ^R	
GFY-1562 ^h	BY4741 cdc10∆::CDC10::mCherry::SpHIS5	This study
	hsl1∆::hsl1(1-1043; 1201-1518)::HA₃::Nat ^R	

Table S1. Yeast strains used to obtain the data in Figures S1 to S7.

GFY-1563 ^h	BY4741 cdc10∆::CDC10::mCherry::SpHIS5 hsl1∆::	This study
	hsl1(1-950; 1044-1518)::HA₃::Nat ^R	
GFY-1564 ^h	BY4741 cdc10∆::CDC10::mCherry::SpHIS5 hsl1∆::	This study
	hsl1(1-950; 1201-1518)::HA₃::Nat ^R	
GFY-1565 ^h	BY4741 cdc10∆::CDC10::mCherry::SpHIS5 hsl1∆::	This study
	hsl1(1-950; 1358-1518)::HA₃::Nat ^R	
GFY-1566 ^h	BY4741 cdc10∆::CDC10::mCherry::SpHIS5 hsl1∆::	This study
	hsl1(1-1043; 1358-1518)::HA₃::Nat ^R	

^aThese strains are clonal isolates obtained from the genome deletion collection ($MAT\alpha$) and confirmed using multiple diagnostic PCRs.

^bStrains KWY125 and KWY121 (Strahl et al., 2005) were transformed with plasmid pGF-IVL774 and selected on SD-URA plates. Spontaneous white colonies were selected as clonal isolates (although the nature of the adenine pathway mutation(s) were not identified). Strain KWY125 was confirmed as cold sensitive (11-12°C) while strain KWY121 was confirmed as heat sensitive (37°C).

^cThis strain is a clonal isolate from the haploid deletion collection (LIfe Technologies Inc., now ThermoFisher Scientific, Inc.) and confirmed as both haploid (*MATa*) and deleted for the *HSL1* gene using multiple diagnostic PCRs both upstream and downstream of the locus and specific primers to the *Kan^R* cassette. Subsequent strains deleted for *HSL1* were created using chromosomal DNA from this strain as a template to amplify a *hsl1*Δ::*Kan^R* fragment with flanking UTR sequence.

^dThis strain was generated by swapping the *Kan^R* marker from strain GFY-121 to *Nat^R*.

^eThis strain was created by swapping the *Kan^R* marker from strain GFY-104 to *SpHIS5*.

^fThis strain is derived from GFY-58 and was generated in the presence of a WT *CDC11*expressing *URA3*-marked plasmid, pJT1520. Two rounds of selection on media containing 5-FOA was used to remove the covering vector.

^gThe mCherry tag is placed in-frame after the K391N mutation within the *cdc12-6* allele (Finnigan et al., 2015a).

^hThese strains were constructed by integrating the tagged *HSL1* allele at the endogenous *HSL1* locus in strain GFY-1156 using overlapping PCR fragments of the entire *HSL1* cassette (first generated on plasmids pGF-IVL787 through pGF-IVL792) including the C-terminal epitope tag and drug resistance marker.

ⁱFor clarity, the residues present within the resulting Hsl1 protein are presented for each construct, rather than the residues deleted.

Plasmid	Description	Reference
pGF-	pRS315 prCDC11::HSL1(1-1518)::eGFP::Kan ^R	This study
IVL521 ^{a,b}		
pGF-IVL524	pRS315 <i>prCDC11::hsl1(1358-1518)::eGFP::Kan^R</i>	This study
pGF-IVL667	pRS315 <i>prCDC11::hsl1(1-1143; 1201-1518)::eGFP::Kan^R</i>	This study
pGF-IVL775	pRS315 <i>prCDC11::hsl1(1-950; 1044-1518)::eGFP::Kan^R</i>	This study
pGF-IVL776	pRS315 <i>prCDC11::hsl1(1-950; 1201-1518)::eGFP::Kan^R</i>	This study
pGF-IVL777	pRS315 <i>prCDC11::hsl1(1-950; 1358-1518)::eGFP::Kan^R</i>	This study
pGF-IVL778	pRS315 <i>prCDC11::hsl1(1-1044; 1358-1518)::eGFP::Kan^R</i>	This study
pGF-IVL536	pRS315 prCDC11::hsl1(611-950; 1245-1518)::eGFP::Kan ^R	This study
pGF-IVL652 ^c	pRS315 prCDC11::hsl1(611-950 K775A E776A	This study
	N777A)::eGFP::Kan ^R	
pGF-IVL653 ^d	pRS315 prCDC11::hsl1(611-950 R828A	This study
	L831A)::eGFP::Kan ^R	
pGF-IVL654	pRS315 prCDC11::hsl1(611-950 K775A E776A N777A	This study
	R828A L831A)::eGFP::Kan ^R	
pGF-IVL655	pRS315 prCDC11::hsl1(611-950; 1245-1518 K775A E776A	This study
	N777A)::eGFP::Kan ^R	
pGF-IVL656	pRS315 prCDC11::hsl1(611-950; 1245-1518 R828A	This study
	L831A)::eGFP::Kan ^R	
pGF-IVL657	pRS315 prCDC11::hsl1(611-950; 1245-1518 K775A E776A	This study
	N777A R828A L831A)::eGFP::Kan ^R	
pGF-IVL612	pRS315 prCDC11::hsl1(611-950)::eGFP::Kan ^R	This study
pGF-IVL672 ^e	pRS315 prCDC11::hsl1(611-710)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL774 ^f	pRS316 prCDC11::HSL1(1-1518)::eGFP::Nat ^R	This study
pGF-	pRS315 prCDC11::CDC11::eGFP::Hyg ^R	This study
preIVL51		
pGF-IVL650 ^g	pRS315 prCDC11::eGFP::HSL7::SpHIS5	This study
pGF-IVL672 ^h	pRS315 prCDC11::hsl1(611-710)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL673	pRS315 prCDC11::GST::MBP::eGFP::SpHIS5	This study
pGF-IVL701	pRS315 prCDC11::hsl1(611-710 R635A R636A K645A	This study
	H648A K649A R653A K654A)::GST::MBP::eGFP::Kan ^R	

Table S2. Plasmids used to obtain the data in Figures S1 to S7.

pGF-IVL750	pRS315	This study
	H648A K649A R653A K654A R663A R664A K672A R673A	
	K683A R684A)::GST::MBP::eGFP::Kan ^R	
pGF-IVL756	pRS315 prCDC11::hsl1(711-950)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL753	pRS315 prCDC11::hsl1(744-950)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL754	pRS315 prCDC11::hsl1(800-950)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL755	pRS315 prCDC11::hsl1(850-950)::GST::MBP::eGFP::Kan ^R	This study
pGF-IVL821	pRS315 prCDC11::hsl1(611-710 T665A L666A N668A	This study
	S669A S671A K672A R673A S674A L675A Y676A S677A	
	S680A I681A S682A K683A R684A S685A N687A	
	L688A)::GST::MBP::eGFP::Kan ^R	

^aFor clarity, rather than present the deleted residues, the numbering system for alleles of *HSL1* present the amino acid resides still present within the final protein sequence.

^bFor variants of Hsl1 that include the *CDC11* promoter and are C-terminally tagged with *eGFP::Kan^R*, the following construction strategy was utilized to aid in plasmid generation. First, a parent vector (pGF-IVL520) was generated that contained *prCDC11::eGFP::Kan^R* with a unique *NotI* restriction site between the promoter sequence and the first codon of eGFP. *In vivo* ligation and homologous transformation was used to gap repair the linearized parent vector to insert the fragment(s) of *HSL1* sequence. Additionally, due to the large size of the *HSL1* gene, multiple PCRs were often generated containing a significant amount (several hundred bases) of homology to link adjacent fragments.

^cThe identified KEN box motif K-E-N-X₃-E/D/N (Burton and Solomon, 2001) had residues K775 E776 and N777 mutated to Ala.

^dThe identified destruction (D) box motif $R-X_2-L-X_4-N/D/E$ (Burton and Solomon, 2001) had residues R828 and L831 mutated to Ala.

^eThe GST and MBP tags were amplified from vectors pJT4649 and pJT3239, respectively. Additionally, the MBP tag has the amino acid sequence "NSSSARL" appended to its C-terminus.

¹This plasmid was built using *in vivo* ligation and homologous recombination; *pRS316::prCDC11* (pGF-V106) was used as the parental vector. The Hsl1 sequence also contains a point mutation (W923R) found within a poorly conserved region (Figure S2) that does not alter its bud neck localization.

^gThe parental vector for this plasmid was *pRS315::prCDC11* (pGF-V201).

^hThe GST and MBP tags were amplified from vectors pJT4649 and pJT3239, respectively. Additionally, the MBP tag has the amino acid sequence "NSSSARL" appended to its C-terminus.



FIGURE S1. Checkpoint kinase Hsl1 is recruited to the bud neck in a septin-dependent manner. (A) Overnight cultures (grown at 25°C) of the indicated genotypes (yeast strains BY4741, GFY-1541, GFY-39, GFY-1451, GFY-1120, GFY-1450, GFY-1430 and GFY-1449) were spotted in five-fold serial dilution onto rich medium plates and grown at the indicated temperatures for 2 days (37°C) or 3 days (25°C, 30°C), then imaged. All strains were selected twice on synthetic complete medium containing 5-FOA to counter-select for the WT covering plasmids [at room temperature, for strains harboring the cdc11(G29D) or cdc12-6 alleles] prior to being cultured for the growth assays. Absence of Shs1 has non-lethal, but readily detectable, effects on septin collar structure and function (Garcia et al., 2011; Finnigan et al., 2015b). The cdc11(G29D) allele is compromised for its G interface-mediated association with Cdc12 and is inviable at elevated temperature (Weems et al., 2014; Finnigan et al., 2015b). The cdc12-6 allele causes rapid dissolution of the entire septin collar and is also inviable at elevated temperature (Finnigan et al., 2015a; Johnson et al., 2015). (B) Exponentially-growing cultures (30°C) of the indicated genotype which express a mCherry-tagged copy of Cdc10 (GFY-42 and GFY-1156) or Cdc11 (GFY-768 and GFY-1157) from the corresponding endogenous locus were visualized by fluorescent microscopy. Yeast were grown on 5-FOA twice as in (A) prior to culturing. Dotted white lines, cell periphery. Scale bar, 2 µM. Arrowheads, cells with elongated morphology. (C) Yeast expressing an mCh-tagged derivative of the cdc12-6^{ts} allele (GFY-104) were transformed with vectors expressing either Cdc11-GFP (pGF-preIVL51, left) or HsI1-GFP (pGF-IVL521, right) and selected on SD-LEU+5-FOA medium twice to remove the WT CDC12-expressing vector. Cultures were grown to exponential phase at 25°C and shifted to the restrictive temperature (37°C) for 1 h prior to collecting cells and imaging by fluorescent microscopy. Representative images are presented and all were scaled identically as in (B). Scale bar, 2 µM. (D) Cells from different stages of the cell division cycle in a strain expressing both Cdc10mCherry (GFY-42) and HsI1-GFP (from vector pGF-IVL521) were imaged as in (B). Scale bar, 2 μM.

Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	<pre>(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)</pre>	MTGHVSKTSHVPKGRPSSLAKKAAKRAMAKVNSNPKRASGHERVVQSVNDATERLSOPDSTVSVATKS-SKRKSRDTVGPWKLGKTLGK MSVLIPHQQQRLRASSAAKKAAWNANNKTVNIVSPHNNVNYVKRSPKTNSNHLDRUTKSVNDATERLSOPDSTVSVATKS-SKRKSRDTVGPWKLGKTLGK MNEYKPTVLNTAASAARNAMARTNERVAPGMSGPTKAVNKNDRHLERVIESVHDATERLSOPDSFNS-ASINTKS-SKRRSRDTVGPWKLGKTLGK MPAPMGRRYATTSQRSSNVAKKAARNAMKVPGTANTNVKTAVNTDTNPTAGVTQNQLDRVVQSVNDATERLSOPESAFSISTTTKS-SKRRSRDTVGPWKLGKTLGK MPVPMYANRGQRPSSVAKKAARNAMFKAAGTAVATRP-STTSKISQQUDRVVQSVNDATERLSOPESAMSGSTTTKS-SKRKSRDTVGPWKLGKTLGK MPVPMYANRGQRPSSVAKKAARNAMFKAAGTAVATRP-STTSKISQQUDRVVQSVNDATERLSOPESAMSGSTTTKS-SKRKSRDTVGPWKLGKTLGK MPSSVIRKPRLKKN-TTLAQSAAKNAMAKKESIDKKPVKYVPKDSNAKTNAHLERVIQSVNDATERLSOPSSTSTVTKS-SKRKSRDTVGPWKLGKTLGK MPSSVIRKPRLKKN-TTLAQSAAKNAMLRVSGLDQDFTSNLSNTFPTVTHERVIQSVSDATERLSOPSSTMT(21)NNSKNKNKRTRDTVGPWKLGKTLGK MVSVNKYRSSSOHRGVRTSGSINSGSIT (85-369)	(89) (101) (96) (109) (97) (109) (100) (102)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(90) (102) (97) (110) (98) (110) (101) (103)	GSSGRVRLAKNMETCOLAAIKIVPKKKAFVHCSNNGTVPNSYSSSMVTSNVSSPSIASREHSNHSQTNPYGIEREIVIMKLISHTNVMALFEVMENKSELYJVLEYV GSSGRVRLAKNIENCTLAAIKIVPKRTYNRRMRDQKMKTAGGVSSGTDSKDSSNREDPIKNGTDSALNPYGIEREIVIMKLISHPNVMCLLEVWENKSELYJVLEYV GSSGRVRLAKNMENCOLAAIKIVPKNKCLKGGTDNCSSNDNSTTITTNYSMENTSDKDIPINPYGIEREIVIMKLISHPNVMCLLEVWENKSELYJVLEYV GSSGRVRLAKNMETCOLAAIKIVPKKKCKNDPGSHSSKSTESDFSATMNTTTNTNRATTNGGTNANAGPSEVPANPYGIEREIVIMKLISHSNILCLYEVWENKSELY GSSGRVRLAKNMETCOLAAIKIVPKKKCKNDPGSRSSKSTESDFSATMNTTTNTNRATTNGGTNANAGPSEVPANPYGIEREIVIMKLISHSNILCLYEVWENKSELY GSSGRVRLAKNMETCOLAAIKIVPKKKCKNDPGSRSSKSESDASATMNTTTSTNRATTNATTNAGHSNVPANPYGIEREIVIMKLISHSNILCLYEVWENKSELY GSSGRVRLAKNMETCOLAAIKIVPKKKCKNDPGSRSSKSESDASATMNTTTSTNRATTNATTNAGHSNVPANPYGIEREIVIMKLISHSNILCLYEVWENKSELY GSSGRVRLAKNETCOLAAIKIVPKKKCNDPGSRSSKSESDASATMNTTSTNRATTNNTNAGHSNVPANPYGIEREIVIMKLISH SSGRVRLAKNETCOLAAIKIVPKKKKLPMKSSHSNSFFSASSNSNISTLATSPPMNNGSEKNOPNYGIEREIVIMKLISH SSGRVRLAKNMETCOLAAIKIVPKKKKLPMKSSHSNSFFGASSNSNSNISTLATSPPMNNGSEKNOPNYGIEREIVIMKLISH MUMALYEVWENKSELY SSSGRVRLAKNIETCOLAAIKIVPKNKKFNNQHRSKNTNSNNGDLFLSYSSIHNTTNTHNARNTDNTAIENSGGGNPYGIEREIVIMKLISHPNVMALYEVWENNSELYLEYV	(196) (208) (197) (224) (210) (218) (201) (217)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(197) (209) (198) (225) (211) (219) (202) (218)	DGGELFDYLVSKGKLPEREAIHYFKQIVEGVSYCHSFNICHRDLKPENLLLDKKNRRIKIADFGMAALELFNKLLKTSCGSPHYASPEIVMGRPYFGGPSDVWSCGIVLFALLTG DGGELFDYLVSKGKLSEPEAVHYFTQIIQGVSYCHSFNICHRDLKPENLLLDKKNKVIKIADFGMAALELFNKLLETSCGSPHYASPEIVMGKPYFGGPSDVWSCGIILFALLTG DGGELFDYLVSKGKLGEREAVHYFKQIVQGVSYCHSFNICHRDLKPENLLLDKKNKVIKIADFGMAALELSNKLLDTSCGSPHYASPEIVMGKPYFGGPSDVWSCGIILFALLTG DGGELFDYLVSKGKLGEREAVHYFKQIVQGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALEVSNKLLOTSCGSPHYASPEIVMGKPYFGGPSDVWSCGIILFALLTG DGGELFDYLVSKGKLGEREAVHYFKQIVQGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALEVSNKLLOTSCGSPHYASPEIVMGKPYFGGPSDVWSCGIILFALLTG DGGELFDYLVSKGKLGEREAVHYFKQIVQGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALEVSNKLLOTSCGSPHYASPEIVMGKSYFGGPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEKEAVHYFKQIIQGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALEVSNKLLOTSCGSPHYASPEIVMGKSYFGSPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEKEAVHYFKQIIGGSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALELSNKLLOTSCGSPHYASPEIVMGKSYFGSPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEKEAVHYFKQIIGGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALELSPNKLLOTSCGSPHYASPEIVMGKSYFGSPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEKEAVHYFKQIIGGVSYCHSFNICHRDLKPENLLLDKKNKSIKIADFGMAALELSPNKLLOTSCGSPHYASPEIVMGKSYFGSPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEKEAVHYFKQIIGGVSYCHSFNICHRDLKPENLLLDKKNKIKIKIADFGMAALELSPNKLLOTSCGSPHYASPEIVMGKSYFGSPSDVWSCGIILFALLTG DGGELFDYLVSKGKLSEREAVHYFKQIIGSPSDVWSCGIILFALLTG	(311) (323) (312) (339) (325) (333) (316) (332)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(312) (324) (313) (340) (326) (334) (317) (333)	HLPFNDDNIKKLILKVQSGKYQMESNLSSEARDLISKILVIDPEKRITTQEIIKHPLIKKVDDLPVNKVLRKMRK-DNMARGKSNSDIHLLNNV-SPSIVTLHSKGEIDESILR HLPFNDDNIKKLLLKVQSGRFQLPPYLTNDAKDLITRILVTNPEKRLTINEIINHPIKKVRNPPAHIRKLNLLGRGKSNSDIHVLEYNTPATVCLNSEEDIDQSILK HLPFNDDNIKKLLKVQSGRFRLPRNISLEAQDLIAKILVVNPRQRIKISDIIKHPLITKYRQFFKVKFNQVP-NPSATNINSEHMLTPNNLKLASRADIDESILR HLPFNDDNIKKLLKVQSGRFRLPRNISLEAQDLIAKILVVNPFRIATEEIINHPLITKYRQFFKVKFNQVP-NPSATNINSEHMLTPNNLKLASRADIDESILR HLPFNDDNIKKLLLKVQSGRFRLPRNISLEAQDLISKILVVNPFRIATEEIINHPLITKYDKFVKYRSSNPVALSQGKSNSDIHVLDASNSNIVDLRSREDIDDSIVS HLPFNDDNIKKLLLKVQSGRFHPQNLSPEAKDLISKILVVNPFRITTDRINHPLITKYDKFVKVKTSAN-MALSQGKSNSDIHVLEHASKIVKLSSNNIDASILK HLPFNDDNIKKLLKVQSGRFMPQNISLEADDLISKILVVNPFRITTDRINHPLIKYDNFVKVKTSAN-MALSQGKSNSDIHVLEHSPQFISLTSRNDIDASILK HLPFNDDSIKKLLKVQSGRFMPQNISLEADDLISRILVVDPSKRITTDRINHPIKYBDAGKNMVSGKSNSDIHVLEHSPQFISLTSRNDIDASILK HLPFNDDNIKKLLKVQSGRFMPQNISLEADDLISRILVVDPSKRITTDRINHPIKYBD	(423) (431) (417) (452) (437) (435) (425) (427)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(424) (432) (418) (453) (438) (436) (426) (426) (448)	SLOILWHGVSRELITAKLLQKPMSEEKLFYSLLLQYKQRHSISLSSSSENKKSATESSVNEPRIEYASKTANNTGLRSENNDVKTLH(8)TSTVNQNNAITGVNTEI SLOILWHGTPRORLVERLLVEGATEEKLFYSLLFOYKLKHSKPISATKLQKKIEDVVLENESTKTESKDSSNEPGIDNNNDTSDFTQDGNSSEMVNELHT NLQILWHGTSREIIIAKLLQYPMSAEKIFYSLLSYKEKHS(16)TDEIQQPQLAPLQPFSLEPTLAPEVVQQSLPAQDEEDEEELTKSQTTNEEDSSQLNESQDT NLQILWHGASRCLIVAKLLOPRMSEEKLFYSLLSYKEKHS(16)TDEIQQPQLAPLQPPSLEPTLAPEVVQQSLPAQDEEDEEELTKSQTTNEEDSSQLNESQDT NLQILWHGASRCLIVAKLLOPRMSEEKLFYSLLNYKEKHS(16)PPQSVQTVKSIQPIHSAESFDRAQEVQTTLPTE(23)EDEHEDEVBEDELSTDDANDEEAANNDIQDT NLQILWHGSRCLIIAKLLQTPMSEEKLFYSLLWQYKQRHTIQPIKQKEQTSLPVPKMISSLSHTPLVAKLQHAKDTSKIVIINEQDETEVEEGDKENKEN NLQILWHGTSRCLIISKLLYSLLWYKQXNIPLIQHTNHEASASNSPVSQSNSATDISIPFV(5)QFNKINPVVTIDTPVEN	(535) (531) (502) (570) (580) (537) (506) (553)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(536) (532) (503) (571) (581) (538) (507) (554)	NAPVLAQKSQFSINTLSQPESDKAEAEAVTLPEAIPIFNASSIRIERNSYTSISSRSR-SLRLSNSRLSLSASTER(7)MPLPQL-(7)SLSRRAIHASPETKSIHK SAPMLEQKSQFSQHSLKSKETVPISDLPPLPEVIPTFAASSIKAERRSKSNLSSYLKRPKLENARSRTSLFNSSNTSITSS(15)SPSNITLTSLVSSRSLHN GPDLAENESNGIPPDSEIIIARVKQKSQFGLSABADSILDNSVKVDIPTVQPAAIFPASSSKVFSRSGSRLQMKNSASKSKSLSN SAPKLPQKSQFSASSIDQHS(12)SGVPSVPANSLPEAMPIFTAPSSRTFKNSGPLSIPSKK-SLKHSASKTSLNHSASKKSLHHSASRKSLVHASPETTSLHN SAPKLQQKSQFSIPSIKQES(12)SGVPSVPANSLPEAMPIFTAPSSRTFKNSGFLSUSSRTFKNSGSTLFLQSRR-SLKKSASKSSLSNSTSRRSLHHSSGRLQMKNSASKQ-PSPETTSLHN MTPKLLQKPQFSIPSIKQES(12)SGVPSLPSNSLPEAMPIFTAPSSRTFKNSGSTLFLQSRR-SLKKSASKSSLSNSTSRRSLHHSSKRTLRVSASKOLLK HAPKLDQKSQFSIPALVRQESATDVPSLPEAVPIFTAPSSRSFSTTSLISKKLMSTSTSKKSISKSASKRTLRVSASKOLLK HAPKLDQKSQFSIPALVRQESATDVPSLPEAVSIFPASSSRSFKSSTSLMSKK-SIKVSHSRTSLNKNVPSVSKSSLHK DAPKILQKAQFSLHSLPKSG-SESEKDMQFDKNLPEALP(5)IPPVSSKVFKKSSTISISRSISSISSISSTS	(649) (646) (589) (682) (691) (627) (594) (650)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(650) (647) (590) (683) (692) (628) (595) (651)	SLSRKNIAATVAARRIIONSASKRSLYSLOSISKRSLNINDLUVPDDPLPSKKPASENVNKSEPHSLESDSDEEILCOQILFGNALDRI BBBDNEKERDTQRQ SASKKSLKNL-Q-KRIIONSESKRSLYSOTSISKRSLNINDVLIGEPSNSAELPPLPKLDSNNEBEMLCOKILINTSALDNI BBBDNEKERDTQRQ SASKKSLKNL-Q-KRIIONSESKRSLYSOTSISKRSLNINDVLIGEPSNS	(754) (742) (688) (783) (793) (733) (703) (760)
SC_HS11 Cg_HS11 Kn_HS11 Zr_HS11 Zb_HS11 Nc_HS11 Ka_HS11 Vp_HS11	(755) (743) (689) (784) (794) (734) (704) (761)	RQNDTKSSADTFTISGVSTNKENEGPEYPTKIEKNQFNMSYKPSENMSGLSSFPIFEKENTLSSSYLEEQKPKRAALSDITNSFNKMNKQEGMRIEKKIQREQLQKKNDRPSPLK NDVHNEDTIIRKEMHDFEINVNNHQPEFKRQPSDFSVNGPDTS-IFSIEPMPEGLGISKKPVRVQPPTFNFIIDEDKVDDSIKVKQVSGTGPSNNVL-KDIS-NQINNGKGI SHVSDGRNISVEDESSVILPRSDKRHSSKSAVMGVQRP-AFDFFTEPIAELEETAAESNHSNLPLNDITNSLAESRAPRSNLSLKQK-PKLRGMFVASSGGI SQRTLTKVDASQRSPHSSDSKNK-TPAAKTIPPAFELNTPESEEEQQLRASAHKNLLAEENNRYPFKDITNNPTLAPSDSKPSKSKRDVSQKSDLSEVLN-SNQFGGSTFSSNDV SEKTITKADVSPSPEKSKDD-ASSSADHPSTFFFSEEHSGIAPRSLASKKATPDVPRSPFRDITNVPTSSGFKTFKLKRDASQKSNLSEVLK-PNQAATPTYASINI SPTNLIQSDETVIKNSDFPTEQTTTTPSNVFMNASSVSMDTSMERENNYPDMKPMFALSGPALNENSPLASILNRNRNPTKQETTTRVPLKDVTNRPEFNEMYKIDKSNF KIINNENSSSVGKFSKESLKGINNVTTTPNSTFNDSFNSTNLNSFDENLLETNSNSVRRTVQPLSLLDPSRCNALKDITNFNTQSVRVEGKTVKKSDHNGSYENKRIDSSRLK SVHLMESENVSEHTIKATIEKFEESSLEKNDITERKDSVVFDEIKNSFVLEAARTIDEANTIEESEVREVEKKRSPLQDLTNNYHAIKNSKQEKEAIMVKPNLITL	(869) (850) (788) (896) (899) (843) (816) (866)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(870) (851) (789) (897) (900) (844) (817) (867)	PIQHQELRVNSLPNDQ-GKPSLSLDFRNISCEVNSKVESLLQGLKFKKEPASHWTHERGSLFMSEHVEDEKPVKASDVSIESSYVPLTTVATSS ENSKLHPTSNNNYKN-IRNVTAPECNFTSKTFSLDFRNISCEPKESLFHSLLNNKSKNAEENTQFESKS-KQ-(5)KTAHSEFEKERLNAHRDADRSNDQN HDTKKNPTNSRPSKSSLKLSSAPSGNVALTKFSLDFRNIFSCETAPSVVTKLLSTKQRNFTASNAIQNRISEETT-KE(11)DGAMLHRLLATADLHRQTTRSTTES LRNNSKSEFKPSLRVTNDVRSASENQSKPVQPSYSLDFRNATCEVNFVVASLLKRAKTKNIRDARKSGDWSVISGSH(43)DTRSSLWQPSLRDSHVESDAEHNTSGT MRNGSRQNNTQSRSRHPAELRSTSEGRPKQQGYSLDFRNATCEVNFVVASLLKRAKTKNIRDARKSGDWSVISGSH(46)KTNSSYWQPSLRGSQTDSEMEQNSTNN SKNAEGLQQKKNSIFGDVILRITSEPPV5DKSHSLDFRNATCENGVUESMLKMLKSKPASNKKLKDVENVFSSTSGFN(46)KTNSSSDYNKFDVMTSNSD KFSVEEMLFKKGVTNFQNIRISSAPSGNNHQSLDFRNFFCENGVUESMLKMLKSKFASNKKLKDVENVFSSTSGSINSKLNSSDSNKSDXSSDYNKFPKMTLRTTTN AKFPEETEKKEVPVKKEYNIRAASTKIETVSRPSLDFRNFTNETSDRVESLLKNI(5)KKALSDKAWFESRIKVTSSTKE(28)DRMSMAESRLISFDHISNVASKRFSL	(963) (952) (902) (1047) (1051) (948) (926) (1005)

Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(964) (953) (903) (1048) (1052) (949) (927) (1006)	RDPSVIAESSTIQKPMLSLPSSFLNTSMTEKNISQILADDGDDKHLSVPQNQSRSVAMSHPLRKQSAKIS-LTPRSNLNANLSVKRNQGSPGSYLSNDLDGISD VINETSVIAQSSTIHES-PLLSIPSTLNNTSMTEKNIVDMLQENKLDDINETVAKESSAP-LKKKSTKLS-LAPHSNLVSDLNKKTSGINTLASVDRSSB NEFPDTSDVNVLADSSIIKE-PLLDMPSTLAHDSMTESDISHFFVDGGENANTSNVNPP-LEQSTPQRKGLKKTNTCDRSINTDGYYSD NEFPDTSDVNVLADSSIIQKP-NDVTRQPSLLSHTGTEKNISELIHKEGNANNIANRSHEENPSNVMKKSTELN-LAPRSTLTAALDPKDRSMTNSFMSNMSD TGFFETSDVNVLADSSIIQKS-TISRQPSILSSTGTERNINEQLQRNDDQIITRDQSNPTDHSNVLKKHSTELT-LAPRSTLTAALDPKDRSMTNSFMSNMSD SASIETGRDESIAHSSIIQKS-TISRQPSILSSTGTERNINGQLGRNDQQITTRDQSNPTDHSNVLKKHSTELT-LAPRSTLTAALGPKDRSMTNSFFSNMSD SITRTDRSVLAQSSIIHN-PLLSLPSGLLNSSMTEKNINQFITDES-DGAELLPESKTLSRLRSSTLRRTQSRTK-LSEILVQENDGGHKHISSTSNADSDFNE-FSD ASIETGRDESIAHSSTLQK-PLVSLPSAMLNQSTTERDISKFLEDDSQEDSMMYPTKSSQCFNFSSAKLASVKKIPSIMRLDLPTNQDLMEEYGSSFISRDLDDISD STNTDMSNPSVLAHSSTIKNYGSLLEMPKSFKTTSTFKDISEFLVSDESVNFLELSKSGTGNIKKSPSLKKDISMYSALSIPKLEAKAAFNLNASHGDLSSVRSLSASDRDVSD	(1066) (1050) (989) (1150) (1048) (1048) (1033) (1120)
		HsI7-binding region (1144-1200)	
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(1067) (1051) (990) (1151) (1049) (1050) (1034) (1121)	MTFAMEIPTNTFT QAIQLMNNDT(11)SSFTKEKVIKSAAYISKEKEPDNSDT(8)PNTYDEKAINIFED SAJDLPTNTNIREIVILSGNNGSSEKNIFMLSNDNNETELFHTDENKTSIKSAVNIFEDPPSDSTSLQTSSESDSRASVHRKAVSIDTMATTNVLTPAT MSLTEIPTKTFT BAVRISFASPNGRPEIEHQTDEGPDNEMTQLSGKDALPQNANLHVNIFEDASDSTSLQTSSESDSNTHKRAVSIDTLNSTNVLTPT MSYVMEMPSRTYEQAIEVSNNSSADDITDLPVNSDREFGQSSSQGQHTTATFEDNNVNIFEDAPVDSDSSDTAS-E-DSQONVRRKAVSIDTLHTTNVLTPAT MSDVMEMPTNTYVKAVEVSNNSSAEHISDFQMESDRDVDPSSSQEQRTTATFEDNNINIFEDAPVDSDSSIDTS-TDSQONVRRKAVSIDTLHTTNVLTPAT LSFAMDIQPRIFKPQAVQITNNG	(1190) (1153) (1092) (1252) (1256) (1143) (1145) (1216)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(1191) (1154) (1093) (1253) (1257) (1144) (1146) (1217)	NVRVSLYWNNNSSGIPRETTEBILSKLRLSPENPSN-THMQKRFSSTRGS-RDSNALGISQSLQSMEKDLEBDQDGH-TSQADILESSMSYSKRRPSEESVNPKQRV DVRVSLYGNNVSNS-NT-MPRETTEBLISRFKLTPEKP-Q-HQVQKRFSSLTQTGRNTDSIALSQSMISMFKDAEBNGNNKSVKVLDQVEERNASKLTPTSPKNRV NVRVSLYNKYNSPK-KK-LHRETTEBLISKFQLPDSTSKQ-RTVQKRFSNVSNK-RISDAMNLSQSMVSMERDLDEDVSSNDVSQAEMLIG(11)QLAVTEEAVVPQVPEEKKRV NVRVSLYNNNNHT-ESPLKRETTEBIISKFKLSPEKSSQ-PLVQKRFSALAAN-RNSDIHSTMTMFKDLBBEQDAEDISQWKPSES(11)VTMLFDNEEEMPQLEHQITP NVRVSLYMNNNANS-ESPLQRETTEBIISKFKLSPEKSSQ-PLVQKRFSALAAN-RNSDMHSTMTIFKDLEBEQDAEDISQWKPSES(11)VTMLFDNEEEMPQLEHQITP NVRVSLYMNNNANS-ESPLQRETTEBIISKFKLSPEKSSQ-PLVQKRFSALAAN-PNSDMHSTMTIFKDLEBEQDAEDISQWKPSES(11)VTMLFDNEELPQLEHQITP NVRVSLYMNNANS-SSPLQRETTEBIISKFKLSPEKSSQ-PLVQKRFSALAAN-PNSDMHSTMTIFKDLEBEQDAEDISQWKPSES(11)VTMLFDNEELPQLEHQITP NVRVSLYMNNNANS-SSPLQRETTEBIISKFKLSPEKSSQ-PLVQKRFSALAAN-PNSDMHSTMTIFKDLEBEDGEGSSSEQINDE-(9)VTMLFDNDELPQLKQTNSA DVRVSLYVNNQQTS-ANDLPRETTEBIISKFKLSPEKPTSIEKRFSDLLKPSNISTISEGAASMFKDLBEESSIQSNVLPIAKGAQRSKSVYTNNKDPENRV DVRVSLYVNNNLNS-NLILPRETTEBLISKFKLTPEKTTNEYVQKRYSMVPVN-DNENSLALSHSVISMEKDLEBEMDQTPTKENVQSTMKIELHTNDDNVTKPNRV	(1294) (1255) (1210) (1367) (1368) (1245) (1248) (1322)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(1295) (1256) (1211) (1368) (1369) (1246) (1249) (1323)	TMLFDEEEEESKKVGGGKIKEEHTKLDNKISEESSQLVLPVVEKKENANNTENNYSKIPKPSTIKVTKDTAMESNTQTHTKKPILKSVQNVEVEEAPSS TMLFDDYEAQQNITSD-QSNKSSKVIEIPNDSPIKVKVQKASTIFEKEIISAAEADENDHLRIPYE-SKSKKEIAQVHQQEQKEQQVRKPTESQEKAVQK TMLFDDYEEKIASE-IS-RVATIQEHSGEHEVEPKVSAEPVPLVTKEVQQKPPQRVKPVKPTQPFLTEQKQFTQPAETTKPKIVNIKKIKS- TPEVPQKNENRSVPDTKSTRASEEKSMKSIDBGLQKA(10)KQLKEPPHAPPQPKSKVKSGSPLPAKSKKSKPMKEKSQTATAASTATPSAPPNVSNSAAATP AQKQDNVQREEDQSQEGINPEKTKQSEPKYQEKKDCNEKQE(14)SQMHERAMQINKSLKRKKR(17)NAAPPAPVPATKPPPPSAKQLAKTLTESTPVSNPPVSNPPVSNP- TMLFDEEQLNEESALEVVKEXKATKLPTVTKDTKKESTKFENRPSSYS TLLFDDDE	(1393) (1353) (1299) (1478) (1504) (1329) (1324) (1422)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(1394) (1354) (1300) (1485) (1517) (1330) (1325) (1423)	KKNMFYKDFQNFSSHNNATKASKNHVTNISBDDAHMLTINBFNKNSIDYQLKNDDHKFGRKVVEYDCKFYKGNBKBKIKITSTPNASTVIDVKKRSKHSNT TTKPSGFSKDIHGFKTTGGSGHTKLIREHSTKMTFEEVHMITVKEFGNNGIDYQLKNDDKKGDREKVEYDCKFVKGNBEBKIKITSTPNASTVIDVKKGKSINSK AKR-NMFTKDFSGLKSHSLPVNLSQEHVTIDESDDVHIITLSEFSKTNISFKNTIDRKSTKSKVEYDCKFVKGNBKBGIKIVGEHTCAGRIMTIDVKKGKSTDQ (6)-PRKQNMFTKDFGGFKSQ-KSDLGRLQQDHFTKISFDDAHLLTHBEDKNAVEYHLKSDDHKLHDEKVEYDCKFPNG-FABKIKITTT-VLGQKNSTULDVKKKGRTSS (12)PRKQNMFSKDFGGLKSQRKSDTGKLQQDHFTKISFDDAHLLTHBEDKNAVEYHLKSDDHKLHDEKVEYDCKFPNG-FABKIKITS-SVFPGVSTVIDVKKKGRATSP KQNMFSKDFGGLKSQRKSDTGKLQQDHFTKISFDDAHLLTHBEDKNAVEYHLKGDEHKLHDEKVEYDCKFPNG-FABKIKITS-SVFPGVSTVIDVKKKGRATSP KQNMFSKDFGGLKSQRKSDTGKLQQDHFTKISFDDAHLLTHBEDKNAVEYHLKGDEHKLHDEKVEYDCKFPNG-FABKIKITSS-VFPGVSTVIDVKKKGRATSP KQNMFSKDFGGLKSQRKSDTGKLQDHFTKISFDDAHLLTHBEDKNAVEYHLKGDEHKLHDEKVEYDCKFPNG-FABKIKITSS-VFPGVSTVIDVKKKGRATSP KQNMFSKDFGGFGFHS-HVKLVQDHTSKVFDDVHLILSFGKGGIDVQLKKDRKGTERVEYDCNFVKGNFKKKIKITFNGNSTIVDVKKKGRATSP KQNMFSKDFGGFGHH-S-HVKLVQDHTSKVFDDVHLLKLNBFGKNGIDYHJRNDDRKNGTERVEYDCKFVQSNFRKIKITFNGNSTIVDVKKKSKSN- KNNMFSRDFGFKVN-DNISKKLVRDHVTNISFDLVNSLTLKEFSKYNIEVKLKNNHRTSARENVEYDCKFVGNFKSKIKIKEGIKESCONIVGLKKKSKLSGA	(1495) (1460) (1405) (1590) (1623) (1436) (1421) (1525)
Sc_Hsl1 Cg_Hsl1 Kn_Hsl1 Zr_Hsl1 Zb_Hsl1 Nc_Hsl1 Ka_Hsl1 Vp_Hsl1	(1496) (1461) (1406) (1591) (1624) (1437) (1422) (1526)	SSNKABEKENDDVERVIRNAGRS.(1518)ETTDLSORDVANIENLIKSAERNIESNPK.(1489)MSEDAELTENRKVADVLRDHEATSKYP.(1432)DVKGABOMENONVANVIREAEMGYNV.(1616)ESRSABOAENLSVAKVLRDAEHNV.(1647)HSEASEKTENNDITRILREMEATRA.(1461)-KNNEEVRENDITRILREMEATRA.(1461)HDT-AEEKSDQDVDKLIRDTERRIALHN.(1552)	

FIGURE S2. Alignment of HsI1 orthologs from various yeast species. CLUSTAL-W (Thompson et al., 1994) was used to align Hsl1 proteins (residue numbers in parentheses) from: Sc, Saccharomyces cerevisiae; Cg, Candida glabrata; Kn, Kazachstania naganishii; Zr, Zygosaccharomyces rouxii; Zb, Zygosaccharomyces bailii; Nc, Naumovozyma castellii; Ka, Kazachstania africana; and Vp, Vanderwaltozyma polyspora. Location of various domains and sequence elements are indicated: Kinase domain (85-369) (Russell and Nurse, 1987); UBA domain (410-462) (Hofmann and Bucher, 1996; Mueller and Feigon, 2002); septin-binding domain (611-950) (this study); KEN box (775-781) and D-box (849-857) (Burton and Solomon, 2001); HsI7-binding sequence (1144-1200) (Shulewitz et al., 1999; Shulewitz, 2000; Crutchley et al., 2009), and the KA1 domain (1357-1518) (Moravcevic et al., 2010). White-on-black letters, residues invariant across all eight fungal species; blue letters, residues strongly conserved (found in 7 of the 8 species).



FIGURE S3. Quantification of GFP fluorescence at the plasma membrane using ImageJ. (A) A strain expressing Cdc10-mCherry (GFY-42), and harboring a plasmid expressing the KA1 domain of HsI1(1357-1518) fused to GFP (pGF-IVL524) as a plasma membrane (PM) marker, is used here as an example. First, for each cell, four randomly placed lines were drawn across the cell periphery (*left panel*, pink lines). The maximal / peak pixel intensity of each line represents the PM contribution. Second, four lines were randomly drawn within the cell body excluding the PM (*middle panel*, green lines). To calculate the PM-to-cytosolic ratio, the average maximum PM pixel intensity was divided by the mean cytosolic intensity. *Scale bar*, 2 μ M. (B) The pixel intensity profile of a single line scan across the same cell used in (A) indicating the portions corresponding to the PM peaks (pink) and the cytosolic region (green) described in (A). In the calculation of the PM-to-cytosolic ratio, no subtraction of background fluorescence (black) was included.



FIGURE S4. Bud neck recruitment of Hsl7 requires its interaction with a short segment of Hsl1 (residues 1144-1200) located between the septin-binding elements and the C-terminal membrane-binding KA1 domain. (A) Plasmids expressing GFP-tagged derivatives of full-length Hsl1 and the indicated set of internal deletions (pGF-IVL521, pGF-IVL667, and pGF-IVL775 through pGF-IVL778) were introduced into a strain (GFY-42) expressing Cdc10-mCherry from the endogenous CDC10 locus and examined by fluorescence microscopy (all images were scaled identically). Dotted white lines, cell periphery. None of these deletions prevents Hsl1 localization to the septin collar. Scale bar, 2 µM. (B) Cells lacking Hsl1 (hsl1A) or producing the same set of Hsl1 constructs as in (A), expressed from the endogenous HSL1 locus (and Cterminally tagged with the 3XHA epitope, instead of GFP, to permit confirmation of expression) in cells also expressing Cdc10-mCherry from the endogenous CDC10 locus (strains GFY-1561 through GFY-1566) were transformed with a vector expressing N-terminally tagged GFP-HsI7 (pGF-IVL650) and imaged by fluorescence microscopy as in (A). Scale bar, 2 µM. The Hsl1 constructs that lack any portion of the region 1144-1200 fail to recruit HsI7 to the bud neck prior to splitting of the septin collar and, instead, GFP-Hsl7 localizes as a single dot. It has been demonstrated before that this single dot is the spindle pole body; when Hsl1 is absent (after its APC-mediated destruction upon the onset of anaphase and until early G1, or in an $hs/1\Delta$ mutant or, as shown here, in Hsl1 mutants that cannot bind Hsl7), Hsl7 localizes to the spindle pole body and not the bud neck (Shulewitz et al., 1999; Shulewitz, 2000; Cid et al., 2001).



C-terminal GST-MBP-GFP fusions



FIGURE S5. A triple protein fusion localization assay demonstrates that residues 611-950 in Hsl1 contain a cryptic NLS and at least two separable elements sufficient to support bud neck recruitment. A plasmid (pGF-IVL672) expressing a GST-MBP-eGFP chimera and derivatives in which the indicated fragments of Hsl1 were fused to its N-terminus (pGF-IVL673, pGF-IVL701, pGF-IVL750, pGF-IVL753 through pGF-IVL756, and pGF-IVL821) were introduced into a strain expressing Cdc10-mCherry (GFY-42) and visualized by fluorescent microscopy (all images were scaled identically). Dotted white lines, cell periphery. Scale bar, 2 µM. The GST-MBPeGFP chimera is excluded from the nucleus (left column, upper panel), whereas an Hsl1(611-710)-GST-MBP-eGFP fusion exhibits efficient nuclear import (left column, middle panel). Mutation to Ala of the seven basic residues (Set 1) in an apparent classical bipartite NLS (635RRAIHASPSTKSI-HKSLSRK654) (Robbins et al., 1991) in the same fusion largely abrogates nuclear entry and reveals retention at the bud neck (left column, lower panel). Mutation to Ala of additional basic residues within a conserved region downstream (Set 1 & Set 2) prevents both nuclear import and bud neck localization (middle column, upper panel), suggesting that the conserved region (residues 663-688, see Fig. S2) contains residues important for septin binding. In agreement, mutation to Ala of only those conserved residues (Set 3) allows nuclear import (middle column, lower panel). An Hsl1(711-950)-GST-MBP-eGFP fusion shows robust retention at the bud neck (*middle column*, bottom panel), even when progressively pared down to ~100 residues (850-950) (middle column, top, middle and lower panel), which also contain a small conserved element (residues 877-910, see Fig. S2).



FIGURE S6. Native Hsl1 likely does not normally undergo nucleocytoplasmic shuttling. We tested whether Hsl1 would accumulate in the nucleus in any strain lacking the function of one of the four known karyopherins that mediate nuclear export ("exportins") of various classes of proteins (Strom and Weis, 2001; Strahl et al., 2005). Although los1 Δ and msn5 Δ strains are viable, CSE1 and XPO1/CRM1 are essential genes; hence, we acquired, tested and confirmed the phenotypes of strains carrying either a cold-sensitive allele (cse1-1) of the former (Panel A, upper half) or a temperature-sensitive allele (xpo1-1) of the latter (Panel A, lower half). Two colonies of each of the indicated strains were streaked on rich medium (YPD) plates and incubated for either 4 days (25°, 30°, and 37°C) or 14 days (11°C). (B) Both the cse1-1 and xpo1-1 strains contained an ade2 mutation, which causes intracellular accumulation of a red pigment that increases the intrinsic background fluorescence of the cells and also moderately impairs growth even on plates containing adenine (Weisman et al., 1987). To eliminate the background fluorescence problem, the cse1-1 and xpo1-1 strains were streaked for single colonies and spontaneous white derivatives that do not accumulate the red pigment (because they have acquired a mutation in a gene for an enzyme that acts further upstream in the adenine pathway) (Ugolini and Bruschi, 1996) were picked for subsequent use. Each of the exportin-defective strains was transformed with a URA3-marked plasmid (pGF-IVL774) expressing HsI1-GFP and selected on SD-Ura (+Ade, where necessary) plates at 30°C. Exponentially-growing cultures of the los1A and msn5A cells expressing Hsl1-GFP were visualized by fluorescence microscopy at 30°C. Strains harboring the cse1-1 or xpo1-1 mutations were cultured overnight at 25°C, back diluted to an $A_{600 \text{ nm}} = -0.3$ for 5 h, and then shifted to the restrictive temperature (either 12°C or 37°C, respectively) for 6 h and then imaged. Dotted white lines, cell periphery. Scale bar, 2 µM. No nuclear accumulation was observed in any of the exportin-deficient cells. Thus, unlike other cell cycle regulators (Keaton et al., 2008), Hsl1 does not appear to undergo nucleocytoplasmic shuttling.



FIGURE S7. The KEN box and D box are not required for localization of the bud neckassociated fragment HsI1(611-950). The KEN box motif (K-E-N-X₃-E/D/N; residues 775-781) and the D box motif (R-X₂-L-X₄-N/D/E; residues 828-836) required for APC-mediated ubiquitinylation and subsequent degradation of Hsl1 were characterized previously (Burton and Solomon, 2001). Native Hsl1(611-950) alone (left column) or fused at its C-terminus to a Cterminal fragment containing the Hsl1 KA1 domain (right column), or derivatives containing the indicated mutational alterations of the key residues in the KEN box and/or D-box motifs (pGF-IVL536, pGF-IVL612, and pGF-IVL652 through pGF-IVL657) were expressed in a strain (GFY-42) co-expressing Cdc10-mCherry and imaged by fluorescence microscopy (all images were scaled identically), Dotted white lines, cell periphery. Scale bar, 2 µM. Regardless of any alteration of the KEN and/or D box, Hsl1(611-950) localizes weakly to the bud neck, but predominantly to the nucleus due to the action of its cryptic NLS (see Fig. S5). Likewise, regardless of any alteration of the KEN and/or D box, Hsl1(611-950; 1245-1518) localizes exclusively to the bud neck (because presence of the KA1 domain prevents nuclear entry, presumably by first retaining the protein at the PM prior to its association with the septin collar. Results shown in Fig. 2D eliminate the possibility that presence of a cryptic potential NES, L-X-I-X₃-L-X₂-M (residues 1245-1254), contributes to the behavior of Hsl1(611-950; 1245-1518).

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