Makhnevych et al., http://www.jcb.org/cgi/content/full/jcb.201111105/DC1

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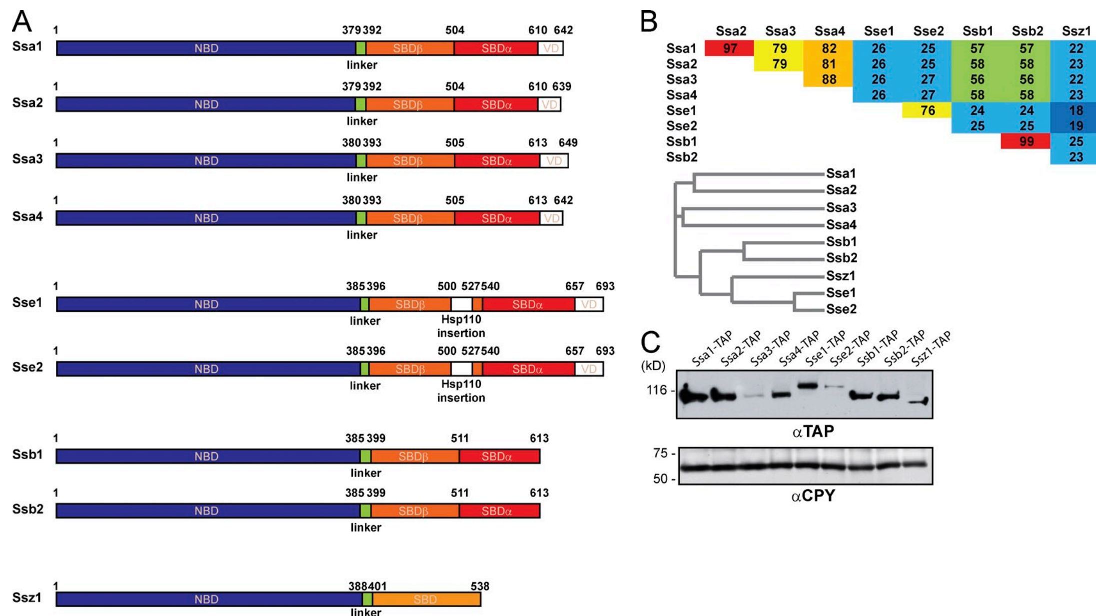
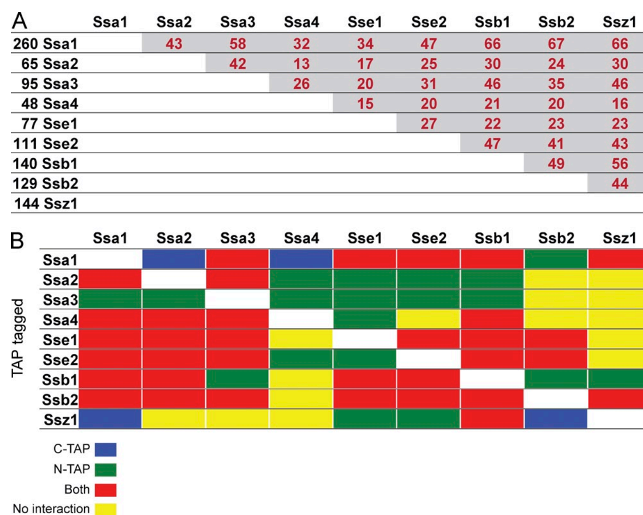


	Ssa2	Ssa3	Ssa4	Sse1	Sse2	Ssb1	Ssb2	Ssz1
Ssa1	97	79	82	26	25	57	57	22
Ssa2		79	81	26	25	58	58	23
Ssa3			88	26	27	56	56	22
Ssa4				26	27	58	58	23
Sse1					76	24	24	18
Sse2						25	25	19
Ssb1							99	25
Ssb2								23

Figure S1. **The cytoplasmic yeast Hsp70s/Hsp110s.** (A) Domain arrangement of the nine Hsp70/Hsp110 chaperones. (B) Pairwise sequence similarity between the chaperones. Protein sequences were compared using ClustalW (default settings). The percentage of identities are shown, computed by taking the number of identical residues between each of the two sequences after alignment and dividing by the length of the larger protein. (C) Log-phase cells grown at 30°C in YPD media expressing endogenously C-terminally TAP-tagged Hsp70s/Hsp110s were analyzed by Western blot using antibodies directed against the TAP tag and carboxypeptidase Y (CPY). Molecular mass markers are shown on the left of the gels.



A

	Ssa1	Ssa2	Ssa3	Ssa4	Sse1	Sse2	Ssb1	Ssb2	Ssz1
260 Ssa1		43	58	32	34	47	66	67	66
65 Ssa2			42	13	17	25	30	24	30
95 Ssa3				26	20	31	46	35	46
48 Ssa4					15	20	21	20	16
77 Sse1						27	22	23	23
111 Sse2							47	41	43
140 Ssb1								49	56
129 Ssb2									44
144 Ssz1									

Figure S2. **The Hsp70–Hsp110 chaperone–chaperone interaction network.** (A) The table gives the total number of hits for each chaperone, listed on the vertical axis, and the number of hits that overlap between two chaperones. Data are based on experiments completed once. (B) TAP tag–based interaction between chaperones. The TAP-tagged chaperones are listed on the vertical axis. The different colors indicate whether the interaction was detected when the chaperone was N- or C-TAP tagged. For example, C-TAP–tagged Ssa1 pulled down Ssa4, whereas N-TAP–tagged Ssa1 pulled down Ssb2. Data are based on experiments completed once.

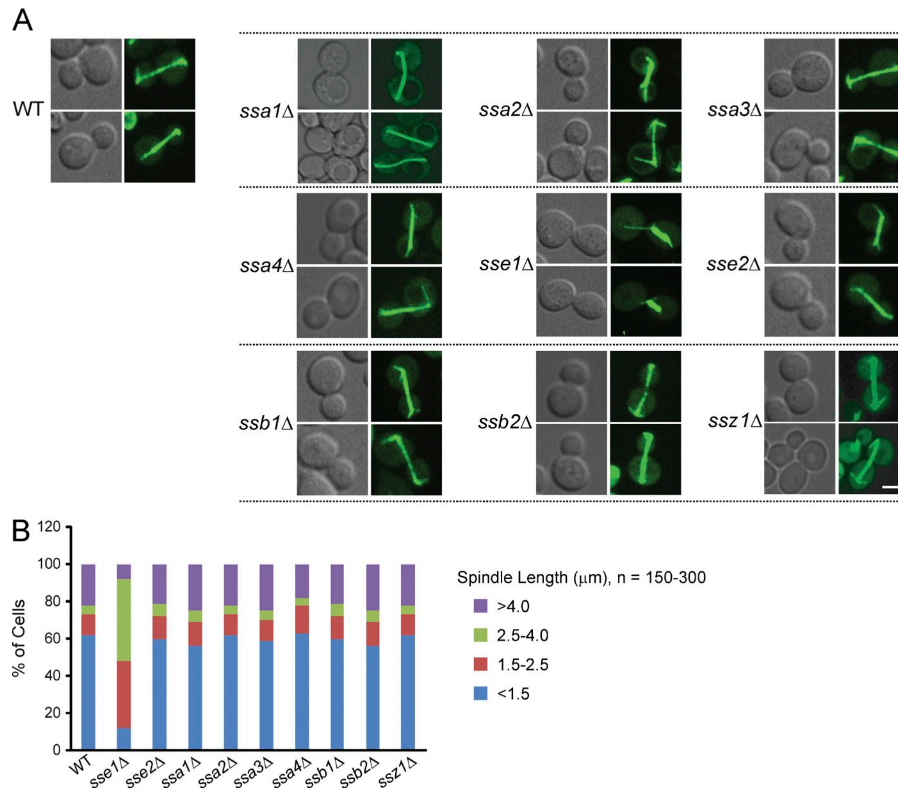


Figure S3. **Spindle morphology in different chaperone knockout strains.** (A) The morphology of the spindle in large budded cells grown in synthetic defined–URA media at 30°C was checked in WT and different knockout strains expressing GFP-Tub1 from the plasmid. Bar, 5 µm. (B) Bar graph showing the distribution of spindle lengths observed in WT and chaperone-deleted cells. The results are based on measuring the spindle length of 150–300 cells. The data shown are from a single representative experiment out of three repeats.

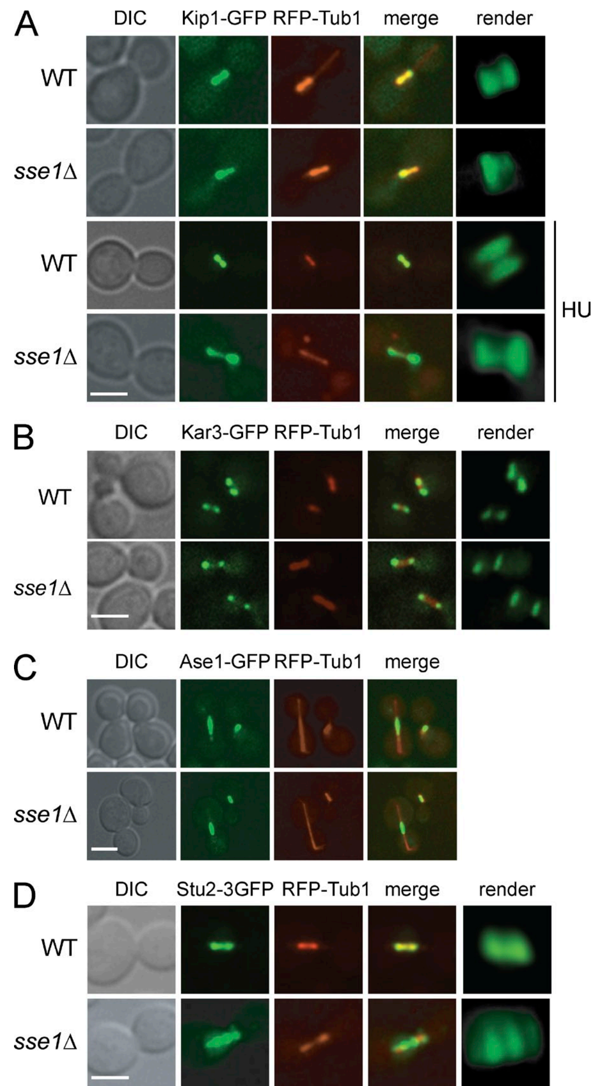


Figure S4. **The effect of *SSE1* deletion on Kip1, Kar3, Ase1, and Stu2 localization.** (A–D) Kip1-GFP (A), Kar3-GFP (B), Ase1-GFP (C), or Stu2-3GFP (D) WT and *sse1Δ* cells also expressing RFP-Tub1 were synchronized in S phase with HU for 2.5 h at 26°C and then imaged using fluorescence confocal microscopy. DIC, differential interference contrast. Bars, 5 µm.

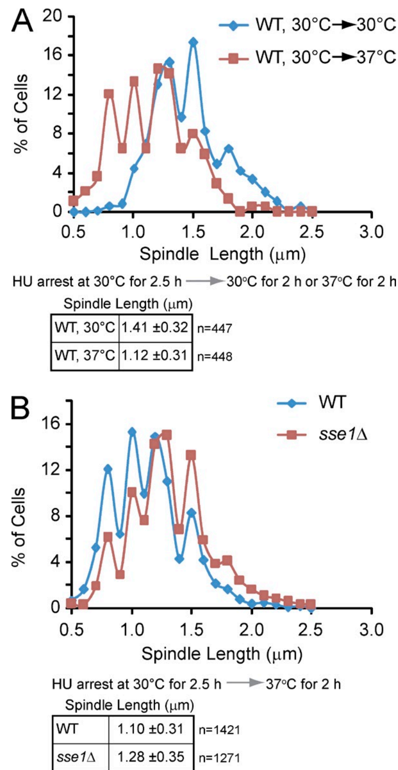
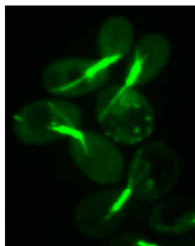


Figure S5. **The effect of heat shock on spindle length.** (A) Logarithmically growing WT cells expressing endogenously Spc42-RFP were synchronized in S phase using 100 mM HU for 1.5 h at 30°C. Half of the culture was then transferred to a new flask, and the temperature was shifted to 30°C (control) or 37°C for 2 h. Spindle length was measured using Spc42-RFP fluorescence. The data shown are from a single representative experiment out of three repeats. (B) Spindle length measurement in HU-arrested WT and *sse1Δ* cells at 37°C was performed as described in A. The data shown are from a single representative experiment out of three repeats.



Video 1. **Time-lapse microscopy of the spindle.** Spindles were visualized in *sse1Δ* cells using plasmid-borne GFP-Tub1 and examined by time-lapse spinning-disk confocal microscopy. Images were captured using a spinning-disk confocal system (WaveFX) with an ultra-cooled 512 back-tinned EM charge-coupled device camera. Images were captured at room temperature after loading the cells on gelatin pads. Stacks of 11 optical sections spaced 0.3 μm apart or 5 optical planes spaced 0.2 μm apart were captured every 1 min for 40 min. GFP was excited using a 488-nm laser, and its emission was collected using a 505-nm long-pass filter. The magnification used was 63×.

Table S1. **Strains used in this study**

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2 Δ0 lys2 Δ0 ura3 Δ0 met15 Δ0*	Brachmann et al., 1998
BY4743	*MATa/α his3 Δ1/his3 Δ1 leu2 Δ0/leu2 Δ0 LYS2/lys2 Δ0 met15 Δ0/MET15 ura3 Δ0/ura3 Δ0*	Brachmann et al., 1998
TM028	*MATa TAP-SSA1*	This study
TM029	*MATa TAP-SSA2*	This study
TM030	*MATa TAP-SSA3*	This study
TM031	*MATa TAP-SSA4*	This study
TM032	*MATa TAP-SSE1*	This study
TM033	*MATa TAP-SSE2*	This study
TM034	*MATa TAP-SSB1*	This study
TM035	*MATa TAP-SSB2*	This study
TM036	*MATa TAP-SSZ1*	This study
TM037	*MATa ssa1::SSA1-TAP::HIS3*	Ghaemmaghami et al., 2003
TM038	*MATa ssa2::SSA2-TAP::HIS3*	Ghaemmaghami et al., 2003
TM039	*MATa ssa3::SSA3-TAP::HIS3*	Ghaemmaghami et al., 2003
TM040	*MATa ssa4:: SSA4-TAP::HIS3*	Ghaemmaghami et al., 2003
TM041	*MATa sse1::SSE1-TAP::HIS3*	Ghaemmaghami et al., 2003
TM042	*MATa sse2::SSE2-TAP::HIS3*	Ghaemmaghami et al., 2003
TM043	*MATa ssb1::SSB1-TAP::HIS3*	Ghaemmaghami et al., 2003
TM044	*MATa ssb1::SSB2-TAP::HIS3*	Ghaemmaghami et al., 2003
TM045	*MATa ssz1::SSZ1-TAP::HIS3*	Ghaemmaghami et al., 2003
TM003	*MATa SSE1::NATR MAD2::KANR*	This study
TM141	*MATa SPC42-RFP::KANR SSE1::NATR pGFP-TUB1::URA3*	This study
TM003	*MATα SSE1::NATR can1 Δ::STE2pr-Sp_his5 lyp1 Δ*	Costanzo et al., 2010
TM004	*MATa SSE1::KANR*	Winzeler et al., 1999
TM136	*MATa SSE1::NATR CIN8::KANR pGFP-TUB1*	This study
TM137	*MATa SSE1::NATR KAR3::KANR*	This study
TM117	*MATa CIN8-GFP::HIS3 pRFP-TUB1*	This study
TM118	*MATa CIN8-GFP::HIS3 SSE1::NATR pRFP-TUB1*	This study
TM119	*MATa KIP1-GFP::HIS3 pRFP-TUB1*	This study
TM120	*MATa KIP1-GFP::HIS3 SSE1::NATR pRFP-TUB1*	This study
TM146	*MATa NDC80-GFP::HIS3 SPC42::KANR*	This study
TM148	*MATa NDC80-GFP::HIS3 SPC42::KANR SSE1::NATR*	This study
TM162	*MATa ndc80-1 CIN8-GFP::HIS3 SPC42-RFP::KANR*	This study
TM163	*MATa ndc80-1 CIN8-GFP::HIS3 SPC42-RFP::KANR SSE1::NATR*	This study
TM151	*MATa SSA1::KANR SSA2::NATR pGFP-TUB1*	This study
TM156	*MATa ssa1::KANR sse1::NATR*	This study
TM195	*MATa ura3-52::mCherry-TUB1::URA3 cin8 ΔNLS-3xmyEGFP::hphNT6*	Roostalu et al., 2011
TM200	*MATa SSE1::NATR ura3-52::mCherry-TUB1::URA3 cin8 ΔNLS-3xmyEGFP::hphNT6*	This study
TM300	*MATa ndc80-1::NATR ura3-52::mCherry-TUB1::URA3 cin8ΔNLS-3xmyEGFP::hphNT6*	This study
TM128	*MATa ASE1-GFP::HIS3 pRFP-TUB1*	This study
TM129	*MATa ASE1-GFP::HIS3 SSE1::NATR pRFP-TUB1*	This study
TM130	*MATa KAR3-GFP pRFP-TUB1*	This study
TM131	*MATa KAR3-GFP SSE1::NATR pRFP-TUB1*	This study
TM139	*MATa PDS1-18myc::LEU2*	Shirayama et al., 1998
TM141	*MATa PDS1-18myc::LEU2 SSE1::NATR*	This study
TM202	*MATa stu2-3GFP::HIS3MX6 pRFP-TUB1*	Wolyniak et al., 2006
TM203	*MATa stu2-3GFP::HIS3MX6 SSE1::NATR pRFP-TUB1*	This study
TM204	*MATa promURA3::tetR::GFP::LEU2 CENIV::tetOX448::URA3 TUB1-mCherry::URA3*	Liu et al., 2008
TM205	*MATa promURA3::tetR::GFP::LEU2 CENIV::tetOX448::URA3 TUB1-mCherry:: URA3 SSE1::KANR*	This study

Table S2 is provided as an Excel file and shows interactions identified in this study.

Table S3 is provided as an Excel file and shows interactions previously reported in published literature.

Table S4 is provided as an Excel file and shows hits obtained using both N- and C-TAP–tagged chaperones.

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