Supplemental Materials

Molecular Biology of the Cell

Garcia et al.

Table S1. Strains and Plasmids

Yeast strains	**Source**
BY4741 *MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Open Biosystems
BY4741 *sse1Δ::kanMX*	(Abrams *et al.*, 2014)
BY4741 *SSE1::LEU2*	this study
BY4741 *SSE1nbd::LEU2*	this study
BY4741 *SSE1sbd:LEU2*	this study
BY4741 *SSE1::LEU2 sse2Δ:: kanMX*	this study
BY4741 *SSE1nbd::LEU2 sse2Δ:: kanMX*	this study
BY4741 *SSE1sbd::LEU2 sse2Δ:: kanMX*	this study
W303 *MATa ura3-52 trp1 leu2-3,112 his3-11,15 ade2-1 can1-100*	(Rothstein, 1991)
W303 *sse1Δ::kanMX sse2Δ:: LEU2*	(Trott *et al.*, 2005)
BY4741 pRH2081(P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *sse1Δ::kanMX* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1::LEU2* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1nbd::LEU2* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1sbd::LEU2* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1::LEU2 sse2Δ* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1nbd::LEU2 sse2Δ* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study
BY4741 *SSE1sbd::LEU2 sse2Δ* pRH2081 (P_{TDH3}-*CPY*‡*-GFP, ADE2 URA3*)	this study

Plasmids	**Source**
pSSA3-lacZ, *URA3*-based expression plasmid, *GPD* promoter	(Liu and Thiele, 1997)
pCH-Flag-RatGR, *HIS3*-based expression plasmid	(Liu *et al.*, 1999)
pYRP-G2, 2μ,*URA3*-based expression GRE-lacZ reporter	(Liu *et al.*, 1999)
p413TEF, *HIS3*-based expression plasmid, *TEF* promoter	(Mumberg *et al.*, 1995)
p413TEF-FLAG-SSE1, *HIS3*-based expression plasmid, *TEF* promoter	(Abrams *et al.*, 2014)
p413TEF-FLAG-$SSE1_{nbd}$, *HIS3*-based expression plasmid, *TEF* promoter	(Shaner *et al.*, 2004)
p413TEF-FLAG-$SSE1_{sbd}$, *HIS3*-based expression plasmid, *TEF* promoter	this study

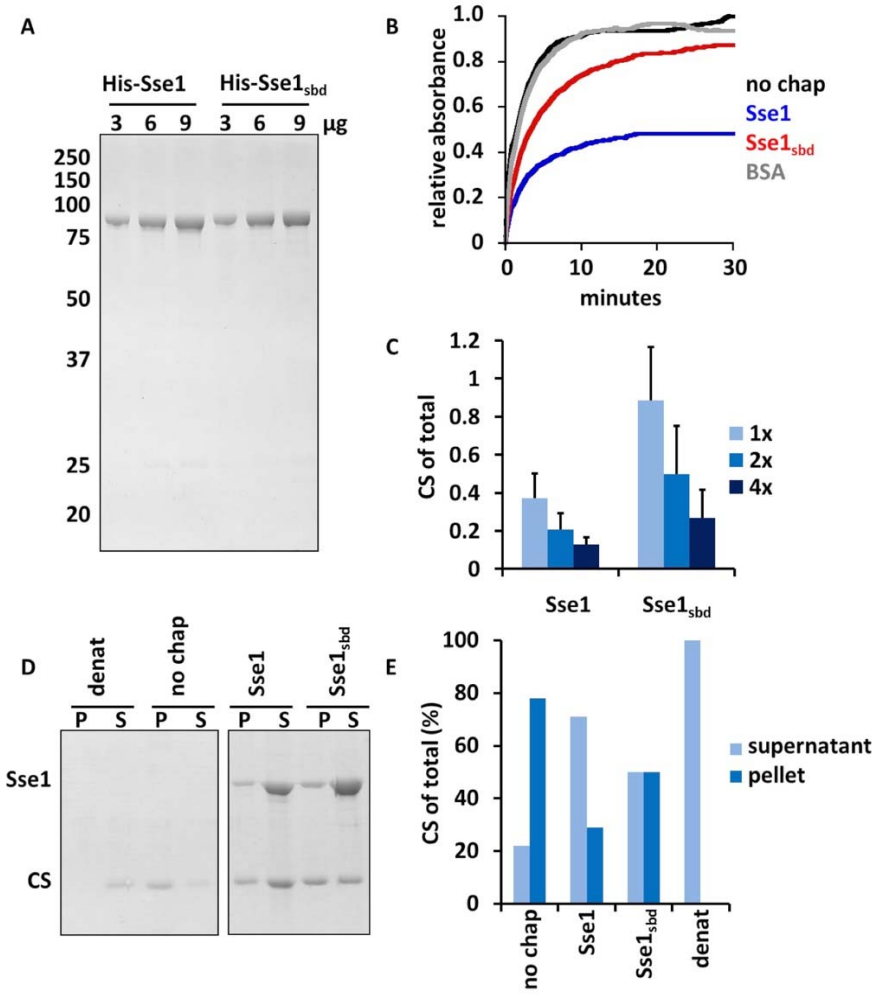

Figure S1. The novel substrate binding domain mutant $Sse1_{sbd}$ impairs holdase activity with denatured citrate synthase. A) 6xHis-tagged Sse1 protein purifications used in experiments that required recombinant proteins. B) Substrate aggregation experiments conducted using chemically denatured CS (200 nM) diluted into refolding buffer without chaperone, with Sse1 (200 nM), with $Sse1_{sbd}$ (200 nM), or with BSA (400 nM) as a non-chaperone control. C) Analysis of holdase experiments using denatured CS with varying ratios of chaperone using the endpoint amount of aggregation detected. D) Analysis of CS aggregation in the absence of chaperone in refolding or denaturing buffer or in the presence of different chaperones after 30 min in a holdase assay via differential centrifugation. Samples were analyzed via SDS-PAGE and Coommassie stain. E) Semi-quantitative analysis of experiments conducted in (D).

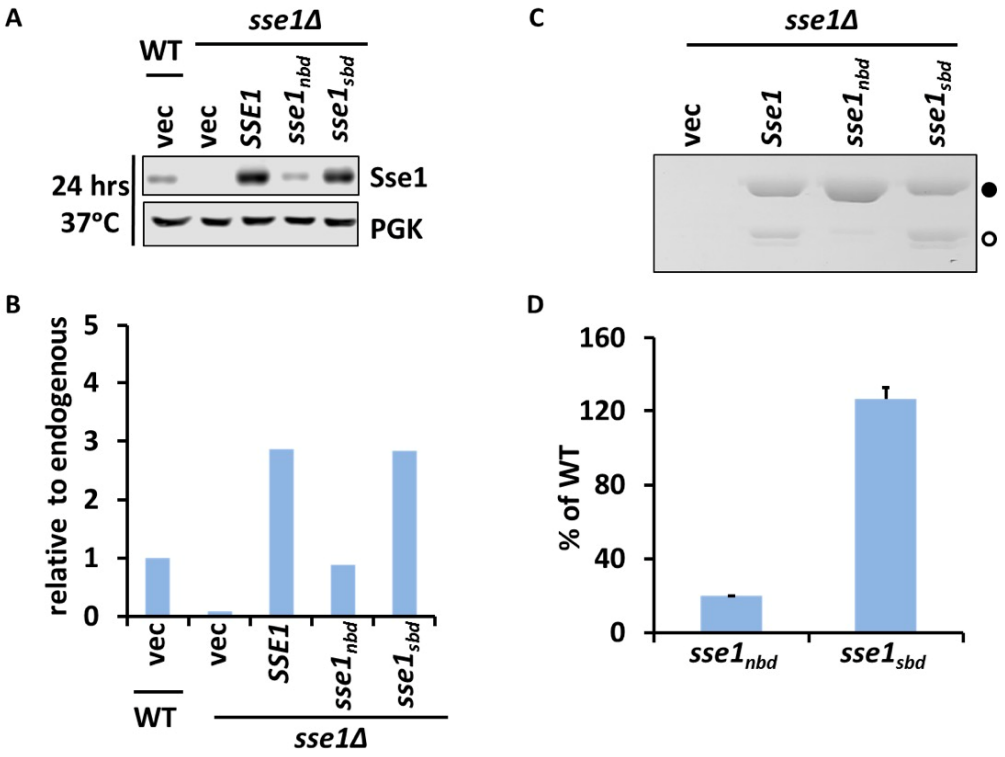

Figure S2. Sse1$_{sbd}$ is stable when expressed in vivo. A) Protein lysates from cells expressing Sse1 alleles from plasmids were cultured at 37°C for 24 h and analyzed by immunoblot. B) Semi-quantitative analysis of the immunoblot in (A). C)Co-immunoprecipitation experiments using FLAG-tagged Sse1 variants were performed at 30°C to assess interactions with endogenous Ssa and Ssb proteins. Samples were analyzed via Coommassie stain using SDS-PAGE. Sse1; closed circle, Ssa/Ssb; open circle. D) Quantitation of (C).

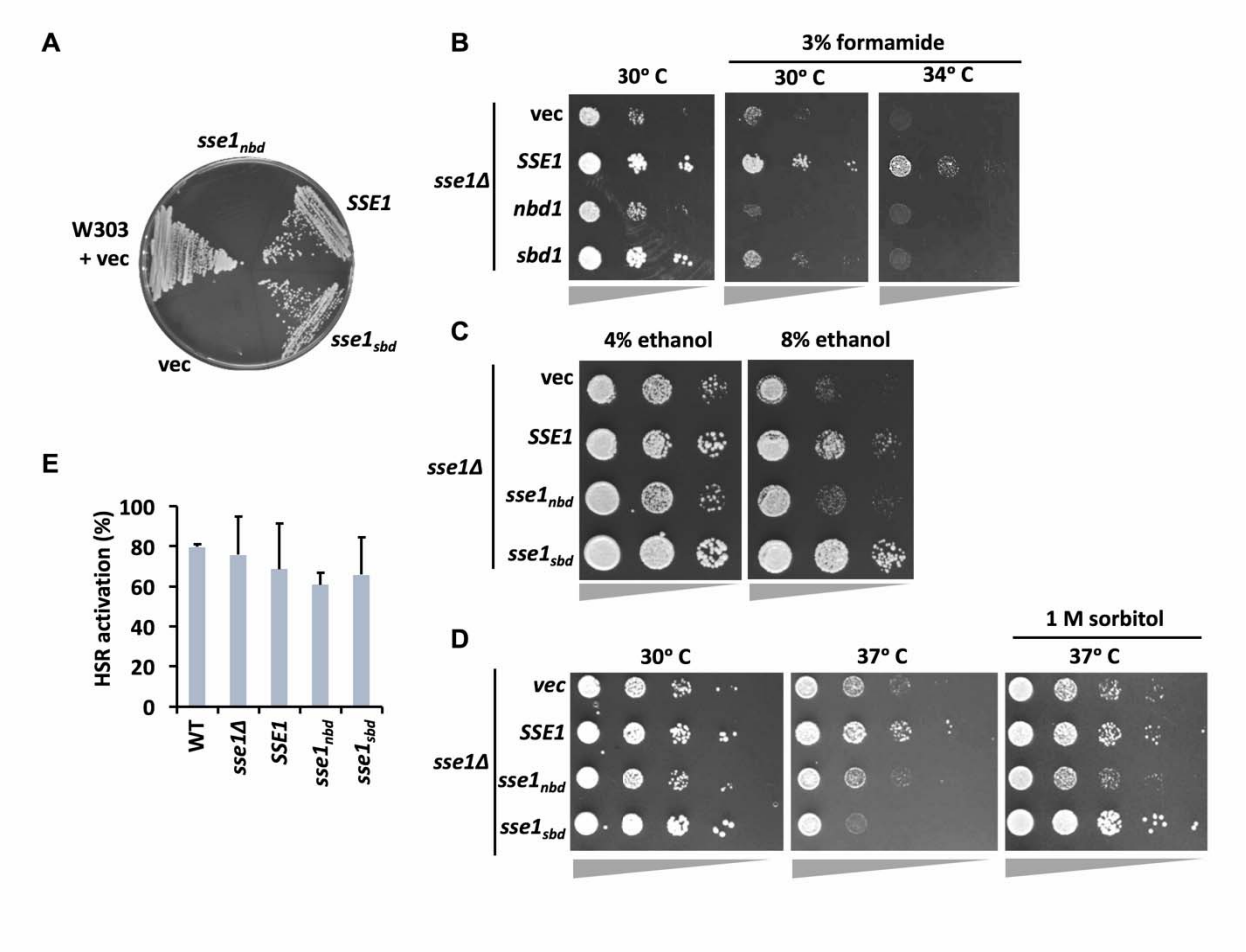

Figure S3. $Sse1_{sbd}$ substrate binding domain function is required under some stress conditions.A) Complementation of *sse1Δsse2Δ* with plasmid-expressed *SSE1* alleles at 30°C on plates containing 5-fluoroorotic acid (5-FOA). B) Serial dilution plating of *sse1Δ* cells complemented with the indicated plasmid-based alleles cultured on the indicated concentrations of formamide at 30˚C. C)Serial dilution plating of *sse1Δ* cells complemented with the indicated plasmid-based alleles cultured on the indicated concentrations of ethanol at 30˚C. D) Serial dilution plating of *sse1Δ* cells complemented with the indicated plasmid-based alleles cultured at 30°C or 37°C and in the presence of 1 M sorbitol. E) β-galactosidase activity assays from cells expressing the indicated *SSE1* alleles integrated at the endogenous locus and expressing the HSF reporter *pSSA3HSE-lacZ*. Cultures were split and incubated at 30˚C in SC-URA or the same media supplemented with 1 M sorbitol for 6 h, and activity from the sorbitol-treated cultures expressed as percent of non-treated cultures.