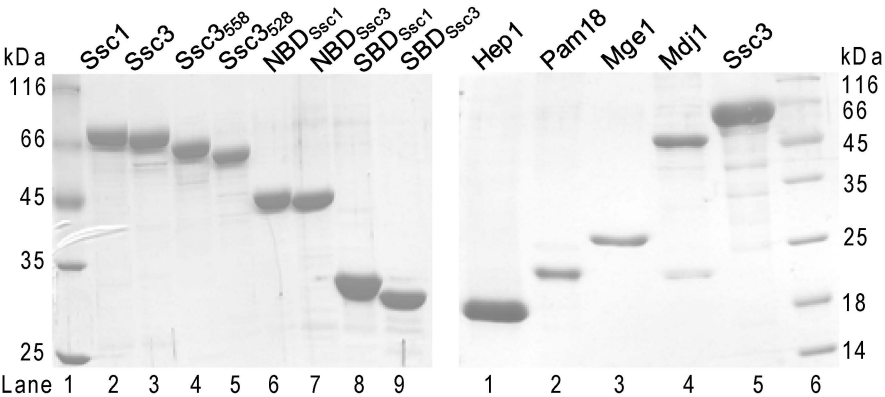


Supplemental Fig. S1



Supplemental Fig. S1:

SDS-PAGE analysis of purified Ssc1, Ssc3 and their variants. *A*, 5 μ g of different proteins are separated on 12.5% SDS-PAGE: molecular weight marker (*lane 1*), Ssc1 (*lane 2*), Ssc3 (*lane 3*), Ssc3₅₅₈ (*lane 4*), Ssc3₅₂₈ (*lane 5*), NBD_{Ssc1} (*lane 6*), NBD_{Ssc3} (*lane 7*), SBD_{Ssc1} (*lane 8*), SBD_{Ssc3} (*lane 9*). *B*, SDS-PAGE analysis of recombinantly expressed and purified Hep1 (*lane 1*), Pam18 (*lane 2*), Mge1 (*lane 3*), Mdj1 (*lane 4*), Ssc3 (*lane 5*) and molecular weight marker (*lane 6*).

Supplemental Fig. S2

A

Ssc1 **MLAAKNILNRSSLSSSFRIATRLQSTKVGQSVIGIDLGTNSAVAIMEGKVPKIIENAEG** 60
 Ssc3 **MLPSWKAFKAHNI---LRILTRFQSTKIPDAVIGIDLGTNSAVAIMEGKVPRIIENAEG** 57

Ssc1 **SRTTPSVVAFKEGERLVGIPAKRQAVVNPENTLFATKRLIGRRFEDAQVDIKQVFPYK** 120
 Ssc3 **SRTTPSVVAFKDGGERLVGEPAKRQSVINSENTLFATKRLIGRRFEDAQVDINQVFPFK** 117

Ssc1 **IVKHSNGDAWVEARGQTYSPAQIGGFVLNKMKETAEAYLIGKPVKNNAVVTVPAYFNDSQRQ** 180
 Ssc3 **IVKHSNGDAWVEARNRTYSPAQIGGFILNKMKETAEAYLAKSVKNNAVVTVPAYFNDAQRQ** 177

Ssc1 **ATKDAGQIVGLNVLRVVNEPTAAALAYGLEKSDSKVVAVFDLGGGTFDISILDIDNGVFE** 240
 Ssc3 **ATKDAGQIIGLNVLRVVNEPTAAALAYGLDKSEPKVIAVFDLGGGTFDISILDIDNGIFE** 237

Ssc1 **VKSTNGDTHLGGEDFDIYLLREIVSRFKTETGIDLENDRMAIQRIREAEEKAKIELSSTV** 300
 Ssc3 **VKSTNGDTHLGGEDFDIYLLQEIISHFKKETGIDLSNDRMAVQRIREAAEKAKIELSSTL** 297

Ssc1 **STEINLPFITADASGPKHINMKFSRAQFFLTAPLVKRTVDPVKKALKDAGLSTSDISEV** 360
 Ssc3 **STEINLPFITADAAGPKHIRMPFSRVQLENI TAPLIDRTVDPVKKALKDARITASDISDV** 357

Ssc1 **LLVGGMSRMPKVVEITVKSIFGKDPKAVNPDEAVAI GAAVQGAVLGSEVTDVLLLDV** 417
 Ssc3 **LLVGGMSRMPKVADITVKKLFGKDASKAVNPDEAVALGAAIQAAVLGSEVTDVLLLDV** 414

B

Ssc1 **DVLLLDVTPLSLGIETLGGVFTRLIPRNTIPTPKKSQIFSTAAAGQTSVEIRVFQGEREL** 60
 Ssc3 **DVLLLDVTPLSLGIETLGGVFTKLI PRNSTIPNKKSQIFSTAAAGQTSVEKVFQGEREL** 60

Ssc1 **VRDNKLIIGNFTLAGIPPAPKGVVQIEVTFDIDADGIINVSARDKATNKDSSITVAGSSGL** 120
 Ssc3 **VKDNKLIIGNFTLAGIPPAPKGTTPQIEVTFDIDANGIINVSARDKDLASHKSSITVAGASGL** 120

Ssc1 **SENEIEQMVNDAEKFKSQDEARKQAIETANKADQLANDTENS LKEFEGKVDKAEAKVVD** 180
 Ssc3 **SDTEIDRMVNEAERYKNQDRARRNAIETANKADQLANDTENS IKEFEGKLDKTDTSQRLKD** 180

Ssc1 **QITSLKELVARVQGGEEVNAEELKTKTEELQTSMMKLFQLYKNDNNNNNNNGNNAESG** 240
 Ssc3 **QISSRELWRSQAGDEVNDDVGTKIDNLRITSSMKLFQLYKN-SDNPETKNGR-----** 234

Ssc1 **ETKQ** 244
 Ssc3 **ENK-** 237

Supplemental Fig. S2:

Sequence comparison of Ssc1 and Ssc3. *A*, Sequence alignment of nucleotide-binding domain (NBD); and *B*, substrate-binding domain (SBD) of Ssc1 and Ssc3 using ClustalW2 tool. Identical amino acids are shaded by gray boxes. Secondary structure elements including β strands ($\beta 1$ - $\beta 8$) and α helices (αA - αE) are indicated by modelling the structure of Ssc1 using Pymol software and structure of DnaK as a template (2KHO1).

Supplemental Fig. S3

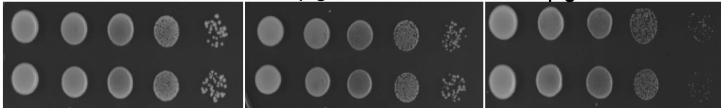
Control

10 $\mu\text{g}/\text{ml}$ CFW

25 $\mu\text{g}/\text{ml}$ CFW

WT

ssc3 Δ



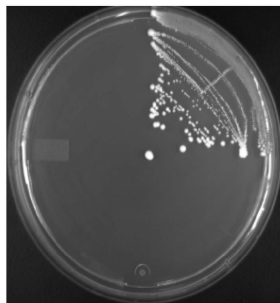
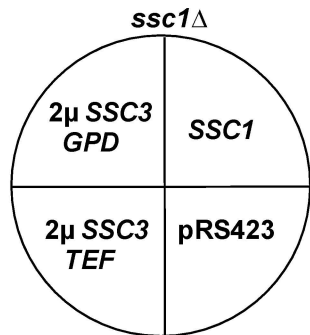
Ten fold serial dilutions

Supplemental Fig. S3:

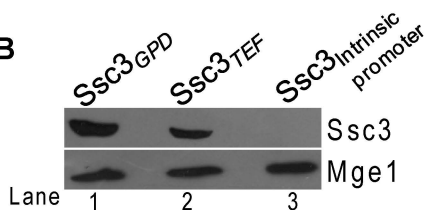
Calcofluor white (CFW) hypersensitivity test of *ssc3Δ* strain. CFW hypersensitivity of *ssc3Δ* strain was checked by drop test on YPD plates containing 10 μ g/ml (*middle panel*) and 25 μ g/ml (*right panel*) of CFW solution. Parent strain BY4741 *MATa* (*his3Δ1 leu2Δ met15Δ ura3Δ*) was used as a control.

Supplemental Fig. S4

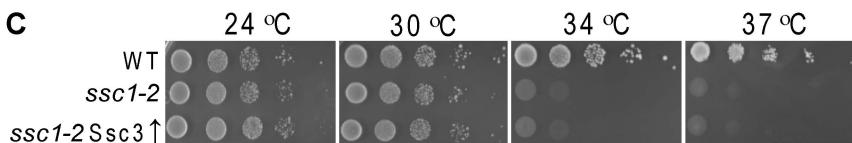
A



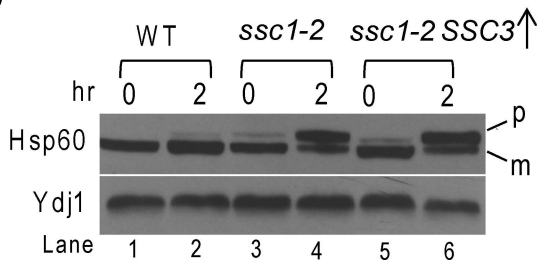
B



C



D

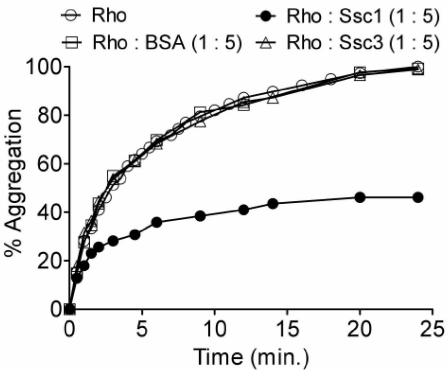


Supplemental Fig. S4:

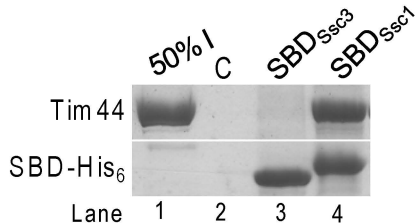
Growth complementation analysis of *ssc1Δ* cells upon Ssc3 overexpression. *A*, Haploid *ssc1Δ* strain expressing wild type copy of *SSC1* gene from a *URA* plasmid was transformed with either *SSC1* on a *CEN* plasmid or *SSC3* from a high copy pRS423 plasmid driven by *TEF* or *GPD* promoter. The cells were streaked on media containing 5-fluoroorotic acid and grown at 30 °C for 3 days. The empty pRS423 plasmid was used as a vector control. *B*, Total lysates prepared from cells overexpressing HA-tagged Ssc3 under different promoters as indicated were resolved by SDS-PAGE, electroblotted, and immunodecorated with anti-HA antibodies. Mge1 was used as a loading control. *C*, 10-fold serial dilutions of an equivalent number of *ssc1Δ* cells expressing either wt Ssc1 from its own promoter (WT), or *ssc1-2*, or *ssc1-2* overexpressing wild type Ssc3 from *GPD* promoter (*ssc1-2* Ssc3[↑]) were spotted on selective medium and incubated at indicated temperatures for 3 days. *D*, Wild type (WT), *ssc1-2* and *ssc1-2* Ssc3[↑] yeast strains were grown at permissive temperatures in minimal media to early log phase. The culture was shifted to 37 °C for 2 h to induce the temperature sensitive phenotype. Subsequently, the whole cell lysates were resolved by SDS-PAGE followed by immunoblotting using Hsp60-specific antibodies; (p), precursor form of Hsp60; (m), mature form of Hsp60.

Supplemental Fig. S5

A



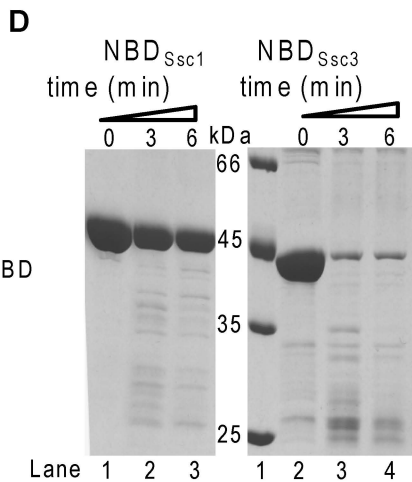
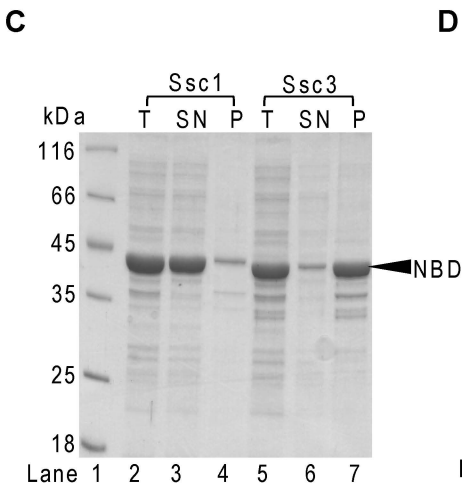
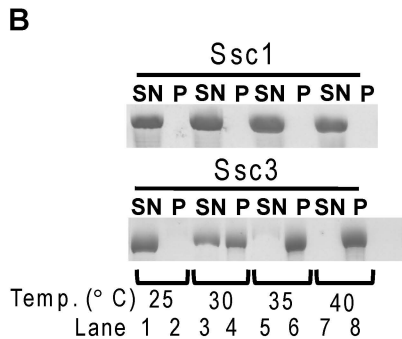
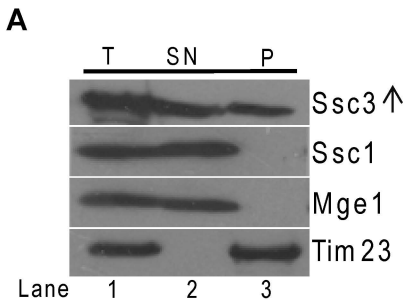
B



Supplemental Fig. S5:

***In vitro* analysis of chaperone like activity of Ssc3 by rhodanese aggregation assay and Tim44 interaction.** *A*, Bovine rhodanese was first denatured in 6 M guanidine hydrochloride and subsequently diluted into the reaction mixture and absorbance changes at 320 nm were used to monitor aggregation at different time intervals at 25 °C. Aggregation of denatured rhodanese (0.46 μ M) in the presence of either 2.3 μ M of Ssc1 or Ssc3 was monitored for 25 min by measuring the changes in turbidity. Percent aggregation values were plotted against time. Under similar conditions, 0.46 μ M denatured rhodanese alone was used as an internal control (absence of chaperones) and in the presence of 4.6 μ M bovine serum albumin (*BSA*) as a negative control. *B*, 4 μ M of His-tagged SBDs of Ssc1 and Ssc3 were preincubated with 1 μ M of non-tagged Tim44 in buffer F (20 mM HEPES pH 7.5, 100 mM KCl, 0.2 % Triton and 10 mM imidazole) for binding at 20 °C for 15 min. The reactions were then incubated with 10 μ l of Ni-NTA resin (bed volume) for 30 minutes. The resin was washed 3 times with buffer F and bound proteins were analysed by SDS-PAGE followed by Coomassie dye staining. 50% of the input was used as loading control and Ni-NTA resin alone (absence of SBDs of Ssc1/Ssc3) used as negative control (*C*).

Supplemental Fig. S6

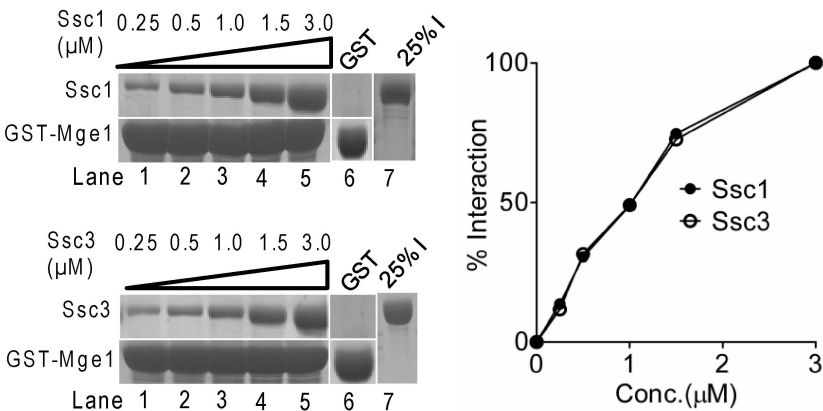


Supplemental Fig. S6:

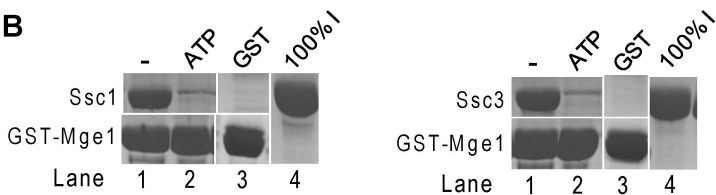
Analysis of alteration in stability and solubility of Ssc3 upon overexpression. *A*, Mitochondria from Ssc3 overexpressing yeast strains (under Ssc1 promoter) were isolated and subjected to fractionation using sonication by hypotonic swelling method. The sonicated mitochondrial samples were subjected to ultracentrifugation. The equivalent amounts of supernatant (S) and pellet (P), and unfractionated extract (T) were analyzed by SDS-PAGE and immunoblotted with yeast specific antibodies as indicated. *B*, Thermal stability of Ssc1 (*top panel*) and Ssc3 (*bottom panel*) were analyzed by incubating the proteins at indicated temperatures for 30 min in buffer A containing 5% glycerol. The samples were separated into a supernatant (SN) and pellet (P) fraction by centrifugation and analyzed by SDS-PAGE followed by Coomassie dye staining. *C*, The NBDs of Ssc1 and Ssc3 along with the linker region were coexpressed with Hep1 in *E. coli* cells. The cells were lysed as described above and analyzed for its solubility by SDS-PAGE. *D*, 5 μ g of NBDs of Ssc1 (*left panel*) and Ssc3 (*right panel*) were subjected to partial tryptic digestion as a function of time as described in Fig. 2. The samples were analyzed on SDS-PAGE and subjected to Coomassie dye staining.

Supplemental Fig. S7

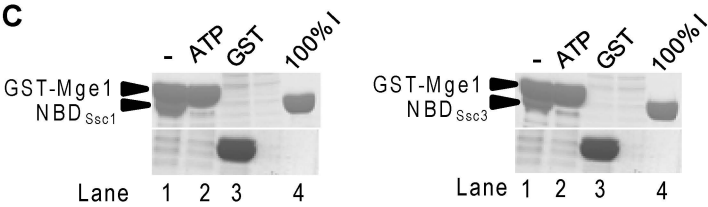
A



B



C

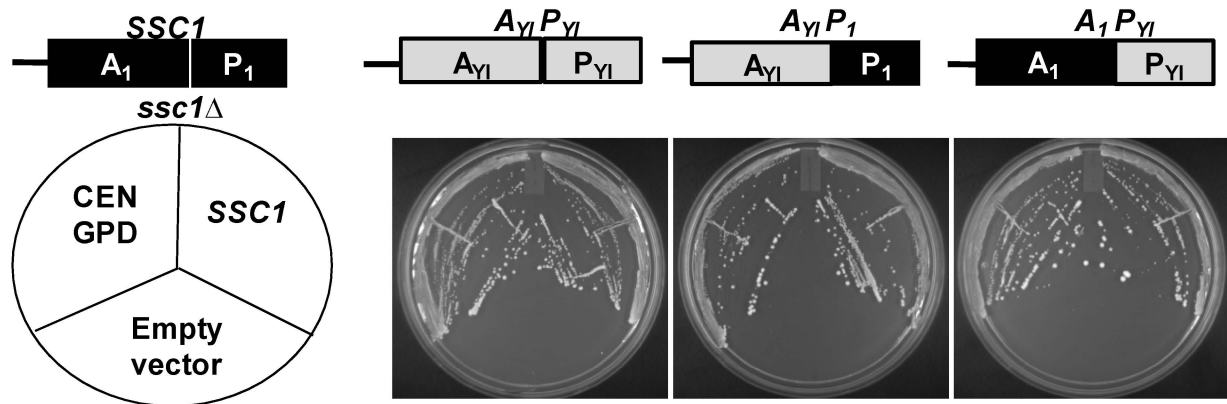


Supplemental Fig. S7:

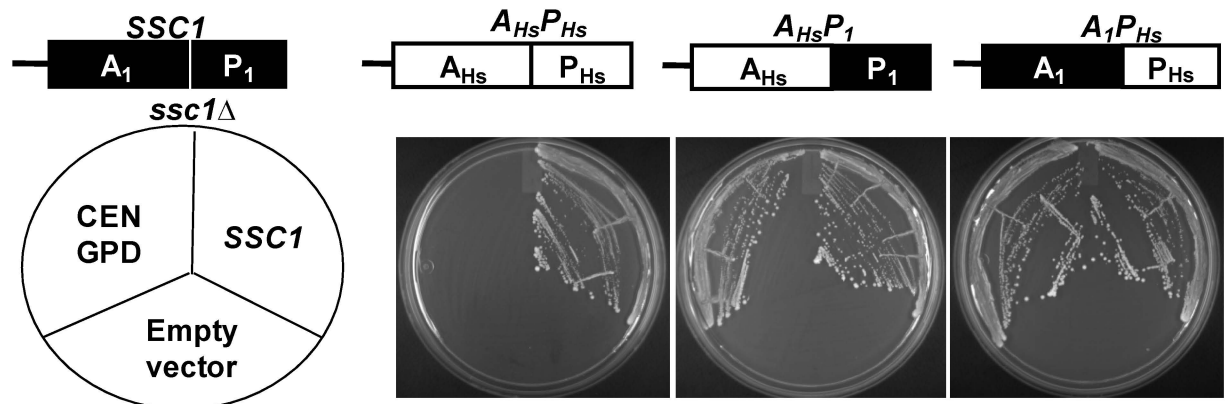
Nucleotide-dependent Mge1 interaction analysis of Ssc1, Ssc3 and their respective NBDs. *A*, Immobilized GST-Mge1 (1.5 μM) was incubated with increasing concentrations of full-length Ssc1 (*left top panel*) and Ssc3 (*left bottom panel*) as indicated in the absence of nucleotides. The bound proteins were analyzed by SDS-PAGE followed by Coomassie dye staining. GST alone was used as a negative control and 25% input (offered to the beads) was used as a loading control. The data was quantified by densitometric analysis using ImageQuant software (*right panel*). *B*, Immobilized GST-Mge1 (1.5 μM) was incubated with 2.5 μM of full-length Ssc1 (*left panel*) and Ssc3 (*right panel*) in the presence or absence of 250 μM of ATP. *C*, 1.5 μM of immobilized GST-Mge1 was incubated with 2.0 μM of NBD_{Ssc1} (*left panel*) and NBD_{Ssc3} (*right panel*) in the presence or absence of 250 μM of ATP. The bound proteins were analyzed by SDS-PAGE followed by Coomassie dye staining. GST alone was used as a negative control and 100% input was used as a loading control.

Supplemental Fig. S8

A



B



Supplemental Fig. S8:

5-FOA counterselection of *ssc1Δ* cells complemented with various mtHsp70 constructs. The *ssc1Δ* cells expressing wild type copy of *SSC1* gene from a *URA* plasmid was transformed with either *SSC1* on a *CEN* plasmid or *A*, *A_{YI}P_{YI}* (*left panel*), *A_{YI}P_I* (*middle panel*), *A_IP_{YI}* (*right panel*) from a *CEN* pRS413 plasmid driven by *GPD* promoter. *B*, *A_{Hs}P_{Hs}* (*left panel*), *A_{Hs}P_I* (*middle panel*), *A_IP_{Hs}* (*right panel*) from a 2μ pRS423 plasmid driven by *TEF* promoter. The cells were streaked on media containing 5-fluoroorotic acid and grown at 30 °C for 3 days. The empty vector was used as a negative control.

Supplemental TABLE S1:

Plasmids used in this study

Plasmid name	Expressed protein	Leader sequence	Vector	Host Organism used	Restriction sites
pRS314- <i>SSC1</i>	Ssc1	Ssc1	pRS314	<i>S.cerevisiae</i>	PstI/ BamHI
pRS313- <i>SSC3</i>	Ssc3	Ssc3	pRS313	<i>S.cerevisiae</i>	HindIII/BamHI
pRS423- <i>SSC3</i>	Ssc3	Ssc1	TEF/GPD pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS423- <i>A₁P₃</i>	<i>A₁P₃</i>	Ssc1	TEF/GPD pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS423- <i>A₃P₁</i>	<i>A₃P₁</i>	Ssc1	TEF/GPD pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS423- <i>A_{Hs} P_{Hs}</i>	<i>A_{Hs} P_{Hs}</i>	Ssc1	TEF pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS423- <i>A₁P_{Hs}</i>	<i>A₁P_{Hs}</i>	Ssc1	TEF pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS423- <i>A_{Hs}P₁</i>	<i>A_{Hs}P₁</i>	Ssc1	TEF pRS423	<i>S.cerevisiae</i>	BamHI/XhoI
pRS413- <i>A_{YI} P_{YI}</i>	<i>A_{YI}P_{YI}</i>	Ssc1	GPD pRS413	<i>S.cerevisiae</i>	BamHI/EcoRI
pRS413- <i>A₁P_{YI}</i>	<i>A₁P_{YI}</i>	Ssc1	GPD pRS413	<i>S.cerevisiae</i>	BamHI/EcoRI
pRS413- <i>A_{YI}P₁</i>	<i>A_{YI}P₁</i>	Ssc1	GPD pRS413	<i>S.cerevisiae</i>	BamHI/EcoRI
pRS314- <i>ssc1-2</i>	Ssc1-2	Ssc1	pRS314	<i>S.cerevisiae</i>	PstI/ BamHI
pRSFDuet-1-Ssc1	Ssc1(His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1-Ssc3	Ssc3(His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc3 ₅₅₈	Ssc3(1-558) (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc3 ₅₂₈	Ssc3 (1-528) (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pET-3a-SBD _{Ssc1}	SBD _{Ssc1} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-21d-SBD _{Ssc3}	SBD _{Ssc3} (His ₆)	-	pET-21d	<i>E. coli</i> BL21	NcoI/XhoI
pET-3a-SBD _{Ssc1} - (ABCD) _{Ssc3}	SBD _{Ssc1} - (ABCD) _{Ssc3} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-3a-SBD _{Ssc1} - (A) _{Ssc3}	SBD _{Ssc1} -(A) _{Ssc3} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-3a-SBD _{Ssc1} - (B) _{Ssc3}	SBD _{Ssc1} -(B) _{Ssc3} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-3a-SBD _{Ssc1} - (C) _{Ssc3}	SBD _{Ssc1} -(C) _{Ssc3} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-3a-SBD _{Ssc1} - (D) _{Ssc3}	SBD _{Ssc1} -(D) _{Ssc3} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pET-3a-SBD _{Ssc3} - (D) _{Ssc1}	SBD _{Ssc3} -(D) _{Ssc1} (His ₆)	-	pET-3a	<i>E. coli</i> BL21	NdeI/ BamHI
pRSFDuet-1-NBD _{Ssc1}	NBD _{Ssc1} (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1-NBD _{Ssc3}	NBD _{Ssc3} (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- V28IQ29PG30DS31A	Ssc1- V28IQ29PG30DS 31A(His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1-	Ssc1-	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI

I80EA86SV88IP90S	I80EA86SV88I P90S (His ₆)				
pRSFDuet-1- Ssc1- G135NQ136R	Ssc1- G135NQ136R(Hi s ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- G160AP162S	Ssc1- G160AP162S (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- S214P	Ssc1- S214P (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- V238I	Ssc1-V238I (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- S314A	Ssc1- S314A (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- N320RK322P	Ssc1- N320RK322P (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- A326VF328LT330NL 331I	Ssc1- A326VF328LT33 0NL331I (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- V336IK337D	Ssc1- V336IK337D (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- G351RL352IS353TT3 54A	Ssc1- G351RL352IS353 TT354A (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- S378K	Ssc1- S378K (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- P384A	Ssc1- P384A (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc1- I396LV400IG402A	Ssc1- I396LV400IG402 A (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1- Ssc3- A157GS159P	Ssc3- A157GS159P (His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pRSFDuet-1-Hep1	Hep1	-	pRSFDuet-1	<i>E. coli</i> BL21	BamHI/SalI
pRSFDuet-1-Mge1	Mge1(His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	BamHI/SalI
pRSFDuet-1-Mdj1	Mdj1(His ₆)	-	pRSFDuet-1	<i>E. coli</i> BL21	NdeI/XhoI
pET-3a-Pam18	Pam18(His ₆)	-	pET-3a	<i>E. coli</i> C41	NdeI/ BamHI
pET-20b-Tim44	Tim44 (TEV cleavable His ₆)	-	pET-20b	<i>E. coli</i> BL21	BamHI/XhoI

Supplemental TABLE S2:

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
PJ53-52C	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1</i>)	Voisine, C. et al. (41)
PJ53-52C <i>CEN SSC3</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS313-SSC3</i>)	This study
PJ53-52C 2μ <i>SSC3</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423-SSC3</i>)	This study
PJ53-52C <i>A₁P₃</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423- A₁P₃</i>)	This study
PJ53-52C <i>A₃P₁</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423- A₃P₁</i>)	This study
<i>A₁P₃</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS423- A₁P₃</i>)	This study
PJ53-52C <i>A_{Hs} P_{Hs}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423- A_{Hs} P_{Hs}</i>)	This study
PJ53-52C <i>A₁P_{Hs}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423- A₁P_{Hs}</i>)	This study
PJ53-52C <i>A_{Hs}P₁</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS423- A_{Hs}P₁</i>)	This study
<i>A₁P_{Hs}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS423- A₁P_{Hs}</i>)	This study
<i>A_{Hs}P₁</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS423- A_{Hs}P₁</i>)	This study
PJ53-52C <i>A_{YI} P_{YI}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS413- A_{YI} P_{YI}</i>)	This study
PJ53-52C <i>A₁P_{YI}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS413- A₁P_{YI}</i>)	This study
PJ53-52C <i>A_{YI}P₁</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS316-SSC1 pRS413- A_{YI}P₁</i>)	This study
<i>A_{YI} P_{YI}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS413- A_{YI} P_{YI}</i>)	This study
<i>A₁P_{YI}</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS413- A₁P_{YI}</i>)	This study
<i>A_{YI} P₁</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS413- A_{YI} P₁</i>)	This study
PJ53-52C <i>ssc1-2</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS314-ssc1-2</i>)	Voisine, C. et al. (41)
<i>ssc1-2</i> 2μ <i>SSC3</i>	(<i>trp1-1 ura3-1 leu2-3, 112 his3-11, 15 ade2-1 can1-100 GAL2+ met2-Δ1 lys2-Δ2 ssc1ΔClal::LEU2 pRS314-ssc1-2</i>)	This study

	pRS423- <i>Ssc3</i>)	
BY4741	<i>MATa his3Δ1 leu2Δ met15Δ ura3Δ</i>	<i>Open Biosystems</i>
BY4741 <i>ssc3Δ</i>	<i>MATa his3Δ1 leu2Δ met15Δ ura3Δ ssc3Δ::kanMX4</i>	<i>Open Biosystems</i>

Supplemental TABLE S3:**Kinetic parameters for F-P5 binding with SBD_{Ssc1}, SBD_{Ssc3} and chimeric proteins.**

Protein	K_d (μM)	R²
SBD _{Ssc1}	0.264 ± 0.041	0.98
SBD _{Ssc1} -(ABCD) _{Ssc3}	22.724 ± 1.008	0.97
SBD _{Ssc1} -(A) _{Ssc3}	0.293 ± 0.031	0.99
SBD _{Ssc1} -(B) _{Ssc3}	0.351 ± 0.033	0.99
SBD _{Ssc1} -(C) _{Ssc3}	0.285 ± 0.032	0.98
SBD _{Ssc1} -(D) _{Ssc3}	20.782 ± 0.983	0.97
SBD _{Ssc3}	26.897 ± 2.639	0.97
SBD _{Ssc3} -(D) _{Ssc1}	0.911 ± 0.087	0.99

Supplemental TABLE S4:**Aggregation of various mtHsp70 proteins at 37 °C in bacterial system.**

Protein	Aggregation at 37 °C
Ssc1	
WT	-
V28IQ29PG30DS31A	-
I80EA86SV88IP90S	*
G135NQ136R	-
G160AP162S	**
S214P	-
V238I	*
S314A	-
N320RK322P	-
A326VF328LT330NL331I	-
V336IK337D	-
G351RL352IS353TT354A	*
S378K	-
P384A	-
I396LV400IG402A	-
Ssc3	
WT	*****
A157GS159P	****

Subjective scoring is based on the amount of protein present in pellet (P) fraction upon solubility analysis at 37 °C in *E.coli*. Here (*****) has been assigned to Ssc3 owing to its maximum tendency to aggregate, while others are assigned relative to Ssc3 based on their distribution in supernatant and pellet fraction upon solubility analysis. (-) sign indicates no aggregation under similar conditions.

Supplemental TABLE S5:

Growth complementation of *ssc1Δ* strain (PJ53-52C) with various Hsp70 constructs at indicated temperatures.

Strain	Growth complementation at			
	24 °C	30 °C	34 °C	37 °C
WT PJ53-52C	+++	+++	+++	+++
A_3P_3	-	-	-	-
A_3P_1	-	-	-	-
A_1P_3	+++	+++	++	-
$A_{Yl}P_{Yl}$	++	++	+	-
$A_{Yl}P_1$	++	++	+	-
A_1P_{Yl}	+++	+++	+++	+++
$A_{Hs}P_{Hs}$	-	-	-	-
$A_{Hs}P_1$	+	+	+	-
A_1P_{Hs}	++	++	++	+

Subjective scoring is based on temperature sensitive phenotype mentioned in Fig.8 from strongest (+++) to weakest (+). Minus (-) indicates lethal phenotype at indicated temperature.