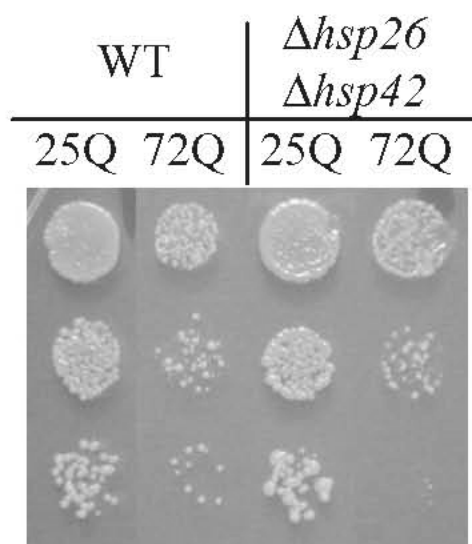
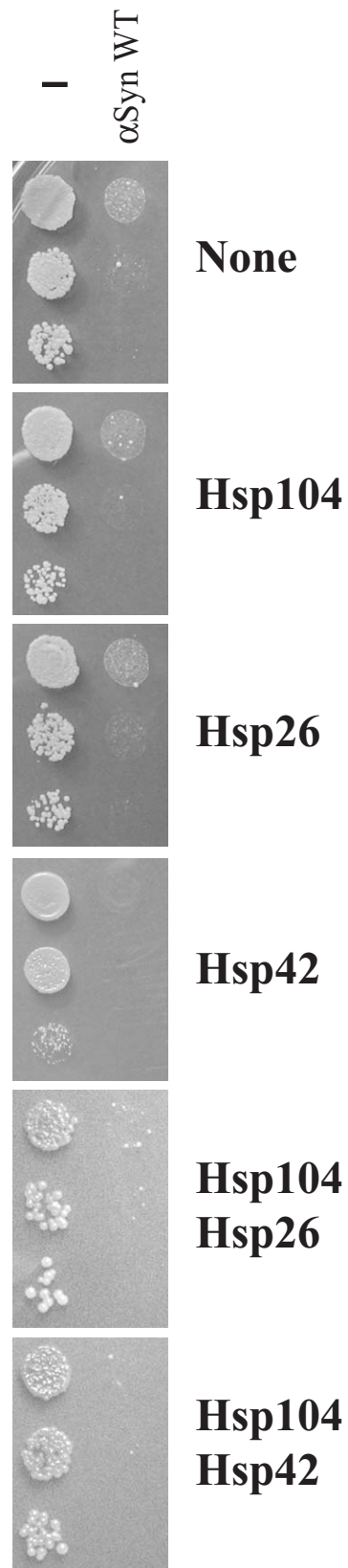


SUPPLEMENTARY MATERIAL

Supplementary Figure 1: Effect of sHsp deletion on the polyglutamine-induced toxicity in yeast. Plasmids expressing 25Q or 72Q were transformed into cells that were deleted for both Hsp26 and Hsp42.



Supplementary Figure 2: Effect of over expression of sHsps and Hsp104 on α -Synuclein-induced toxicity in yeast. Over-expression of wild-type α -synuclein causes toxicity to yeast cells (27).



Supplementary Table 1

Strains used in this study

Strain Name	Description	Genotype	Reference
W303-1A	Wild-type	<i>MATa, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, ade2-1</i>	(35)
SL304a	$\Delta hsp104$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp104::LEU2</i>	(36)
SL327	$\Delta hsp26$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp26::LEU2</i>	(8)
AGC23	$\Delta hsp26\Delta hsp104$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp104::LEU2, hsp26::KanMX</i>	This study
AGC12	$\Delta hsp42$	<i>MATa, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, ade2-1, hsp42::HygB^R</i>	This study
AGC13	$\Delta hsp42\Delta hsp104$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp104::LEU2, hsp42::HygB^R</i>	This study
AGC14	$\Delta hsp26\Delta hsp42$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp26::LEU2, hsp42::HygB^R</i>	This study
AGC15	$\Delta hsp26\Delta hsp42\Delta hsp104$	<i>MATa, leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 lys2D can1-100 hsp26::LEU2 hsp104::KanMX, hsp42::HygB^R</i>	This study

Supplementary Table 2

Primers used for creating the various gene-deletion mutant strains

Name	Primer Sequences (5' to 3')
FdelHsp26	CAGGTATCCAAAAAAGCAAACAAACAACTAAACAAATTAACATG TCAGCTGAAGCTTCGTACGC
RdelHsp26	AATGGTCCTCGCGAGAGGGACAACACTATAGAGCCAGGTCACITTA GCATAGGCCACTAGTGGATCTG
V1Hsp26	TCTGCGCACATCAATCATT
V2Hsp26	ATGGGAACAGGGACAAGTCA
V3Hsp26	ACGCAAATGGTGTTTTGACA
V4Hsp26	GGCGCTACGTATTTCTGCAT
FdelHsp42	TTGTCCATATCCCACACAAATTAAGATCATACCAAGCCGAAGCAAT GAGTCGTACGCTGCAGGTTCGAC
RdelHsp42	TATAAATATAAATGTATGTATGTGTGTATAAACAGATACGATATTC AATTATCGATGAATTCGAGCTCG
V1Hsp42	CACTAGCTTCACCTAAAAGCA
V2Hsp42	CCCTCTCTGGCCAGTTTG
V3Hsp42	CCCAACCCTACGGTAGAA
V4Hsp42	TTTGGTTTGGGAGCGGCC
FdelHsp104	CAAAGAAAAAAGAAATCAACTACACGTACCATAAAATATACAGAA TATCAGCTGAAGCTTCGTACGC
RdelHsp104	CTGATTCTTGTTTCGAAAGTTTTTAAAAATCACACTATATTAATTAG CATAGGCCACTAGTGGATCTG
V1Hsp104	TAGCTCAGCCGGAACCTAAA
V2Hsp104	AAGGACTTTCCTCCAAAGCAT
V3Hsp104	TTTGGTCATGGGTGCTGTTA
V4Hsp104	TCATCGCTGTTCGAATTTTCT
KanB	GGATGTATGGGCTAAATG
KanC	CCTCGACATCATCTGCCC

Supplementary Table 3
Plasmids used in this study

Name	Gene	Promoter	Replication	Markers	Source
pGPD _{LuxAB}	Bacterial luciferase fusion protein	GPD	Cen/ARS	His ⁺	(13)
pGALScHsp104	Hsp104	GAL1/10	Cen/ars	His ⁺	(37)
pUGPDHsp26	Hsp26	GPD	2 μ	Ura ⁺	Vogel J & SLL unpublished
pHGPDHsp42	Hsp42	GPD	Cen/ars	His ⁺	This study

Supplementary Table 4

Identification of Hsp26 by MALDI-TOF

Peptides identified

m/z submitted	MH+ matched	Delta ppm	Start	End	Peptide sequence
814.5	814.4787	26.1797	24	31	LLGEGGLR
842.5	842.4736	31.3460	38	45	QLANTPAK
1274.6	1274.6017	-1.3381	118	127	DIDIEYHQNK
1330.7	1330.6967	2.5035	38	50	QLANTPAKDSTGK
1363.6	1363.6494	-36.2146	203	214	KIEVSSQESWGN
1402.7	1402.6967	2.3750	117	127	KDIDIEYHQNK
1461.8	1461.7953	3.2015	177	190	ADYANGVLTTLVTPK
1729.9	1729.9012	-0.7178	161	176	VITLPDYPGVDADNIK
1805.9	1805.8935	3.6154	51	66	EVARPNNYAGALYDPR
1958.0	1958.0082	-4.2013	128	145	NQILVSGEIPSTLNEESK
2041.0	2041.0242	-11.8620	90	107	SVAVPVDILDHDNNYELK
2201.1	2201.1301	-13.6894	128	147	NQILVSGEIPSTLNEESKDK
2690.2	2690.2051	-1.8914	67	89	DETLDDWFDNDLSLFPSTGFGFPR
2698.2	2698.1850	5.5514	1	23	SFNSPFFDFFDNINNEVDAFNR
2719.5	2719.4671	12.1138	90	114	SVAVPVDILDHDNNYELKVVVPGVK

Sequence of Hsp26 showing the peptides identified (colored red). Vertical lines show trypsin cleavage sites.

SFNSPFFDFFDNINNEVDAFNR|LLGEGGLR|GYAPRR|QLANTPAK|DSTGK|EVARPNNYAGALYDPR|DETLDDWFDNDLSLFPSTGFGFPR|SVAVPVDILDHDNNYELK|VVVPGVK|SK|K|DIDIEYHQNK|NQILVSGEIPSTLNEESK|DK|VKVKESSSGKFKR|VITLPDYPGVDADNIK|ADYANGVLTTLVTPK|LKPQKDGKNHVK|KIEVSSQESWGN

Protein coverage: 180/214 = 84.1% by amino acid count

Supplementary Table 5

Structural properties of Hsp26:FFL complexes. Details of the experiment are provided in the legend to Figure 6. MW is molecular weight predicted assuming that the particles are spheres. %PD indicates polydispersity.

Sample	Temperature	Radius (nm)	Diameter (nm)	MW (kDa)	% PD
0.42 μ M Hsp26 oligomer	25°C	8.7	17.4	532	31.0
	45°C/10' \rightarrow 25°C	10.1	20.2	758	44.2
0.42 μ M Hsp26 oligomer + 0.1 μ M FFL	25°C	8.7	17.4	538	31.9
	45°C/10' \rightarrow 25°C	16.7	33.4	2427	68.2
0.42 μ M Hsp26 oligomer + 1 μ M FFL	25°C	9.1	18.2	585	28.8
	45°C/10' \rightarrow 25°C	33.2	66.4	12228	35.8