

Role of the cell asymmetry apparatus and ribosome-associated chaperones in the destabilization of a *Saccharomyces cerevisiae* prion by heat shock

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SUPPLEMENTAL MATERIALS

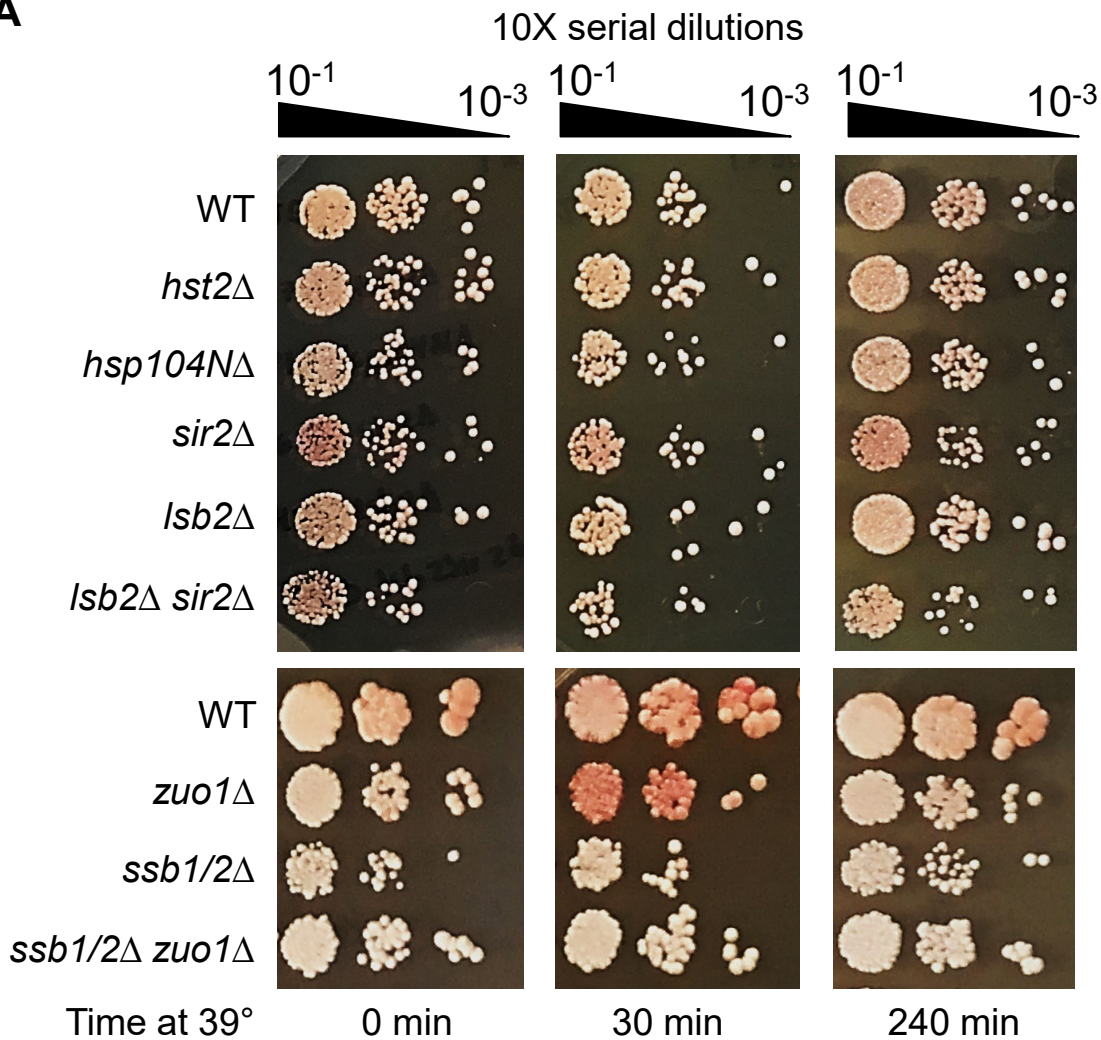
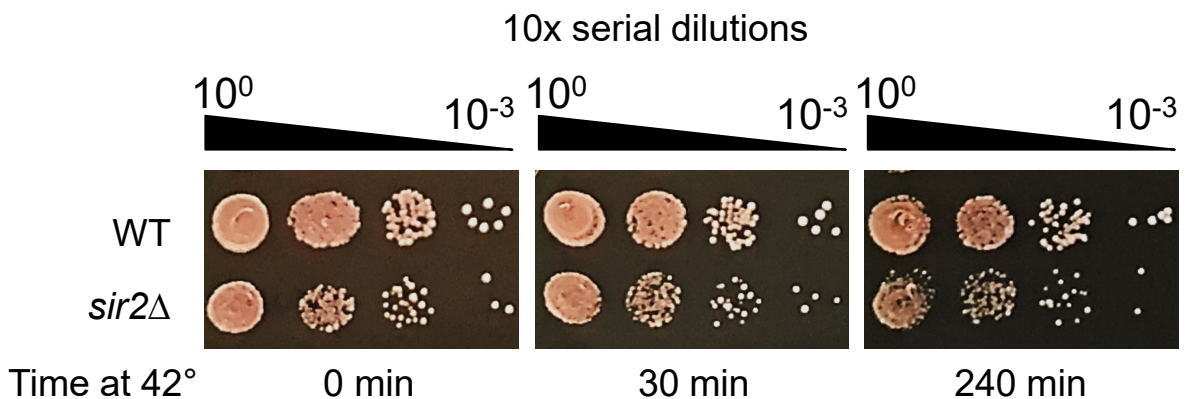
A**B**

Figure S1. Viability of wild-type and deletion after heat shock. Exponential cultures grown at 25° were sampled and heat shocked either at 39° (A) or 42° (B) as described in Materials and methods. Samples were taken at each time point, and serial decimal dilutions were spotted (5 μ L per plate) onto YPD plates, from 1:10 dilution (A) or from undiluted culture (B). Images of plates are taken after for 4-6 (A) or 2 (B) days of incubation at 25°.

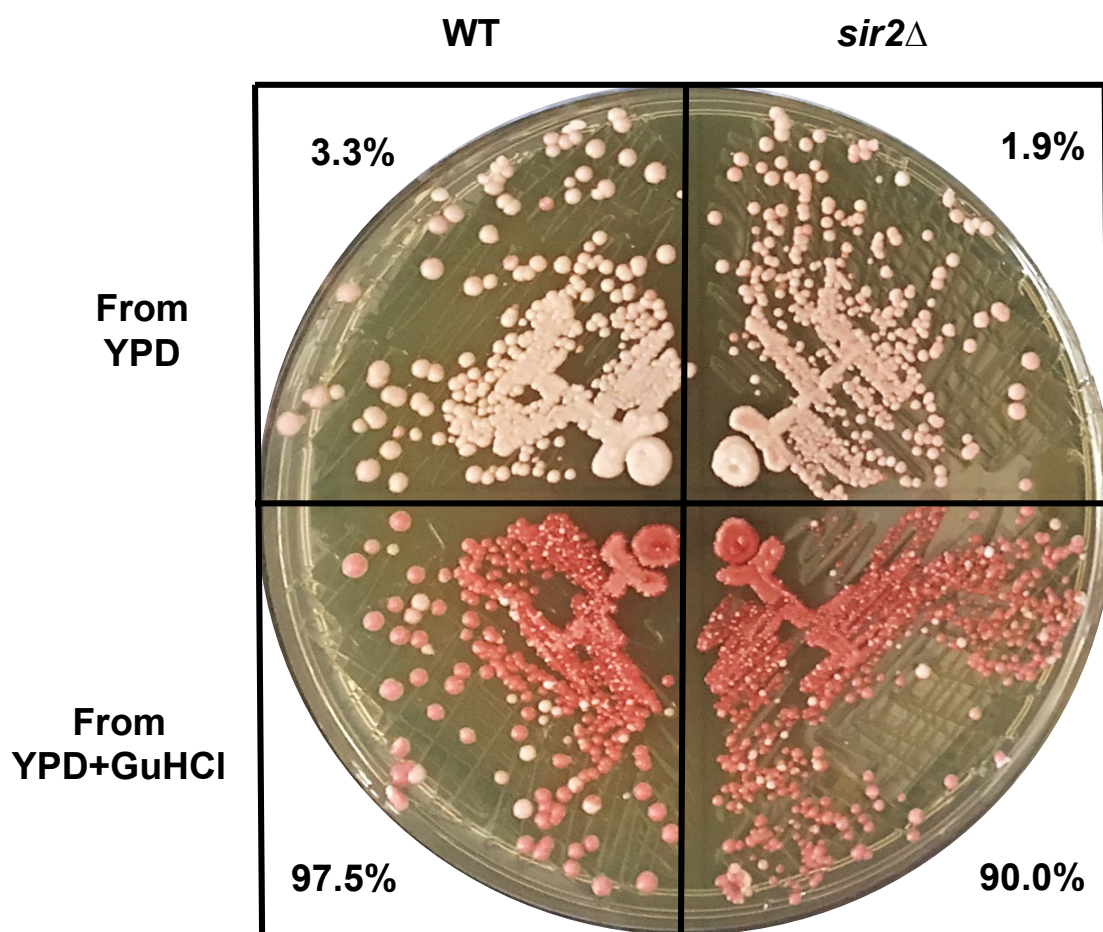


Figure S2. Curing of $[PSI^+]$ by growth in the presence of 5 mM guanidine hydrochloride (GuHCl) in wild-type (WT) and *sir2*Δ strains. Cultures were passaged three times on either YPD or YPD with 5 mM GuHCl plates, and then spots streaked out for single colonies on a fresh YPD plate. Percentage of red ($[psi^-]$) colonies for each culture/treatment is shown in the same quadrant.

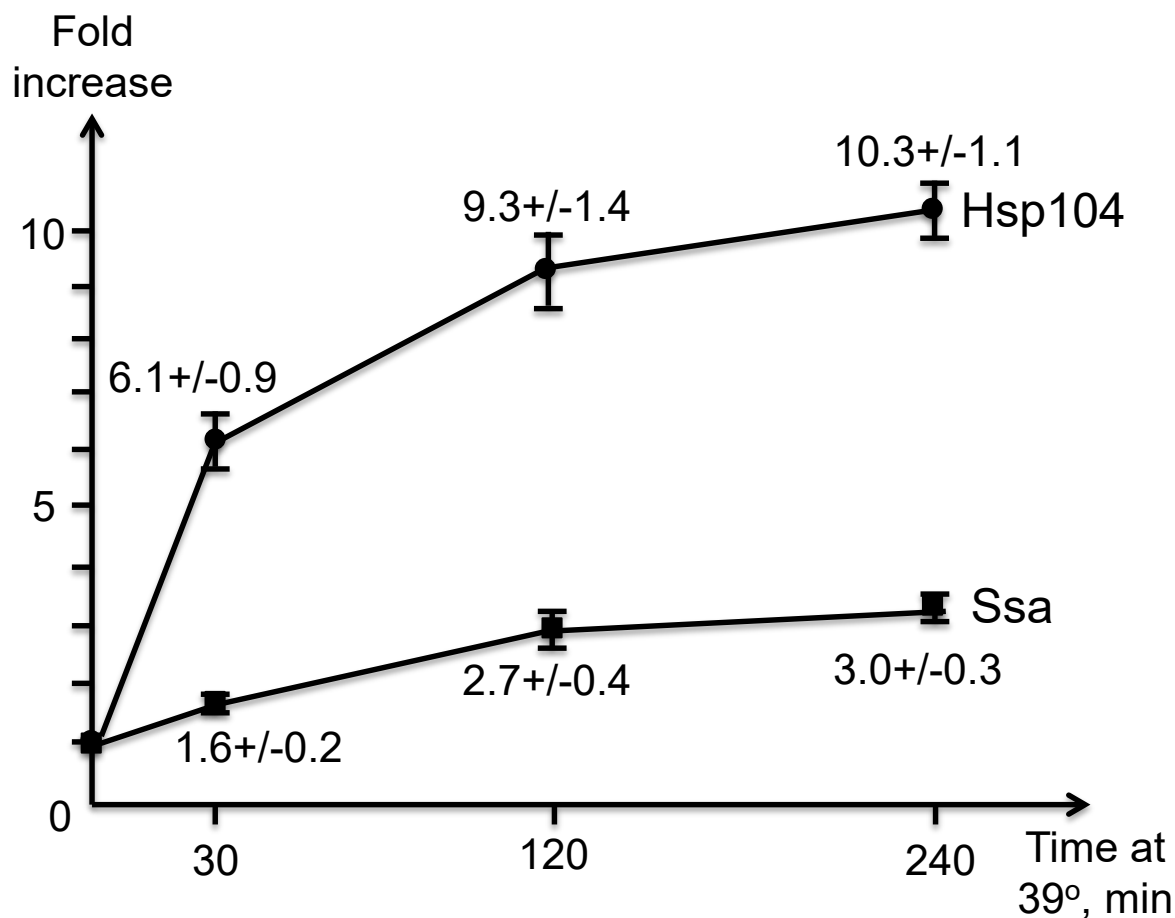


Figure S3. Hsp induction in the wild type strain during heat shock at 39°. Levels of the Hsp104 and Ssa proteins were determined by Western blotting, followed by reaction to respective antibodies, densitometry and normalization by loading control (Ade2 or Coomassie stained proteins). Fold increase relative to the level of respective protein in the exponential culture before heat shock is shown. Results are averaged from 8 to 12 repeats per each time point. Bars represent standard errors.

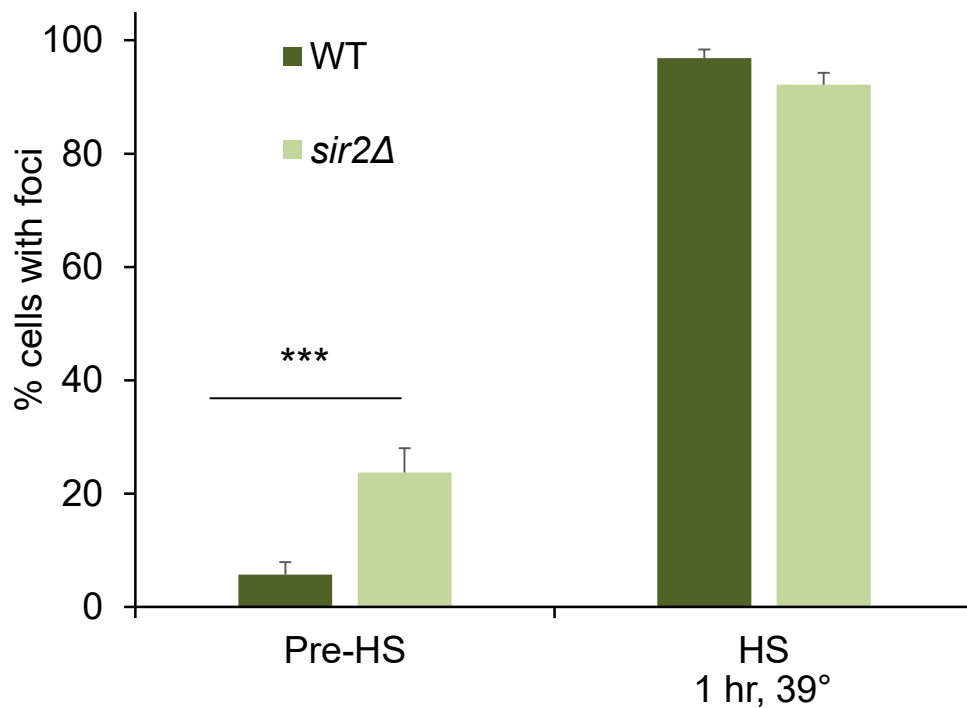


Figure S4. Accumulation of cells with aggregates in wild-type (WT) and *sir2Δ* strains at 39°. Cells were grown at 25°, sampled before heat shock (Pre-HS) and after 1 hr at 39° (HS), and analyzed by fluorescence microscopy.

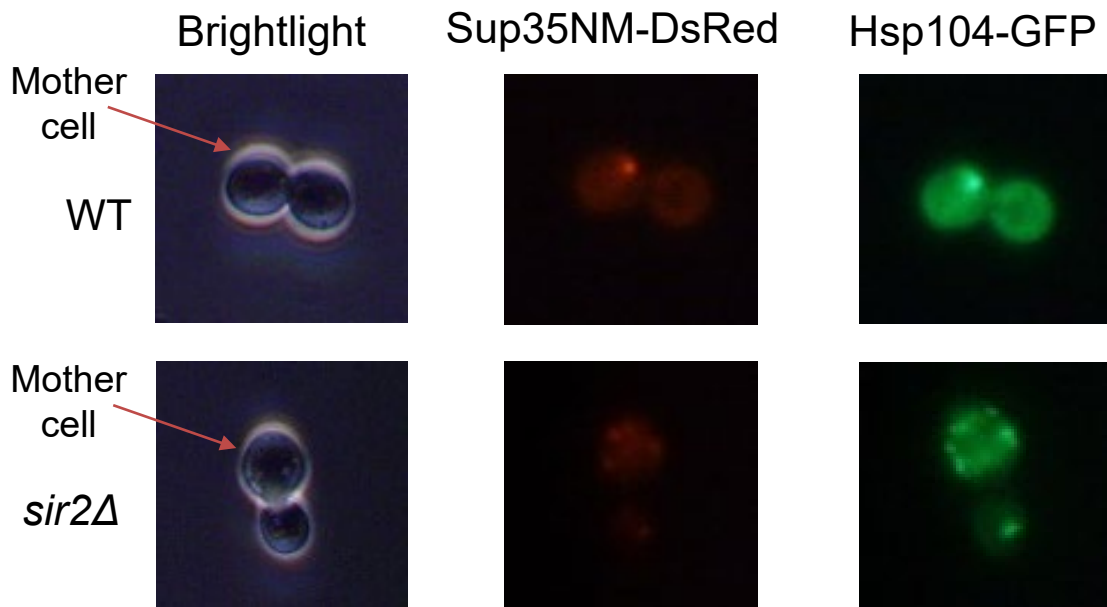


Figure S5. Differences in mother-specific aggregate retention between wild-type and *sir2Δ* cells. Cultures were heat shocked for 1hr at 42° and recovered for 4 hrs at 25°. Examples of cell pairs containing aggregates in mother cell (WT strain) or in both mother and daughter cells (*sir2Δ* strain) are shown.

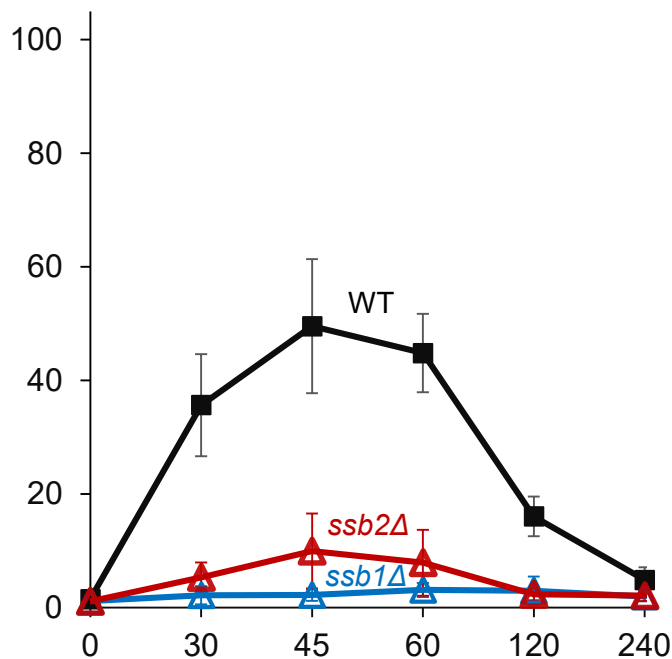


Figure S6. Comparison of $[PSI^+]$ destabilization by 39°C heat shock in the wild-type (WT) strain and single *ssb1Δ* or *ssb2Δ* deletion strains. Heat shock was performed at 39° as described previously. Differences are significant in all cases where bars corresponding to standard deviations do not overlap. WT data are the same as on Fig. 5B, as experiments were run in parallel. See Table S13 for colony numbers and Table S14 for p values.

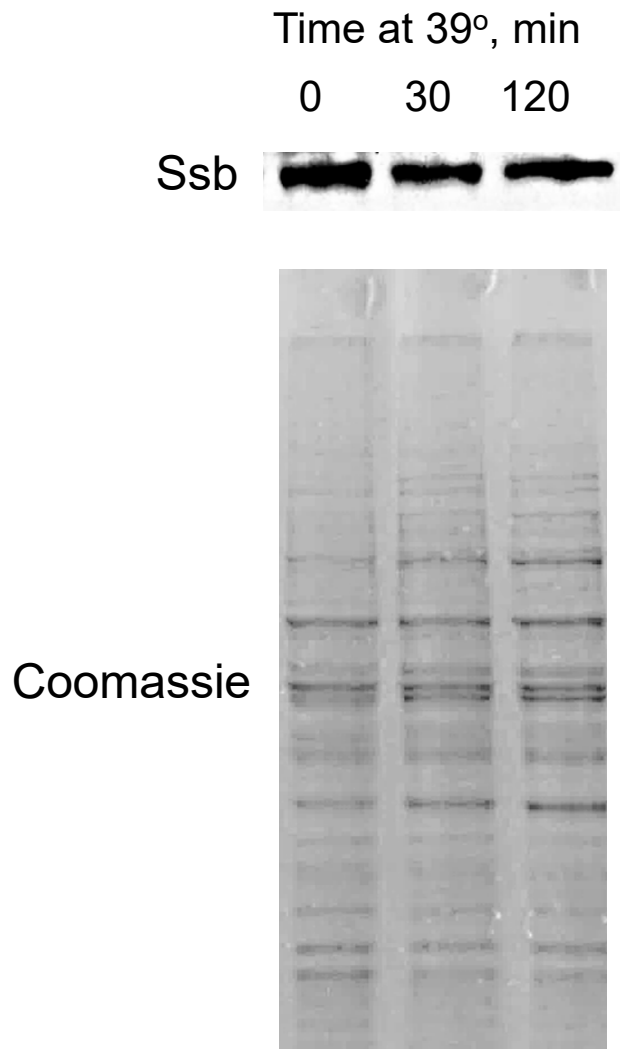


Figure S7. Ssb protein levels are not increased during mild heat shock. An example of *zuo1Δ* strain is shown. Coomassie stained gel is included as a loading control. Similar results were observed for the wild-type strain.

Table S1. Viability of yeast strains during incubation at 39°.

Strain	Cfu*/ml at start ($\pm$ SD**)	Fold change after incubation at 39° $\pm$ SD	
		30 min	240 min
WT	$(1.15 \pm 0.015) \times 10^7$	1.35 ± 0.53	1.37 ± 0.60
<i>hsp104-ΔN</i>	$(1.04 \pm 0.45) \times 10^7$	1.24 ± 0.63	1.30 ± 0.39
<i>sir2Δ</i>	$(1.45 \pm 0.545) \times 10^7$	1.13 ± 0.32	1.22 ± 0.24
<i>ssb1/2Δ</i>	$(1.24 \pm 0.29) \times 10^7$	0.96 ± 0.25	1.06 ± 0.16

*cfu - colony-forming units

**SD - standard deviation

Table S2. Effects of 39°C heat shock on wild-type and *hsp104-ΔN* strains (numbers of colonies for Fig. 1A).

Strain	Number of cultures analyzed	Incubation at 39°C, min	Number of colonies				
			Red ([<i>psi</i> '])	Mosaic ([<i>PSI</i> ']/[<i>psi</i> '])	% red and mosaic $\pm$ SD	White/pink ([<i>PSI</i> '])	Total
WT	3	0	1	0	0.2 ± 0.4	533	534
		30	10	149	32.8 ± 0.7	326	485
		60	11	121	28.1 ± 3.0	342	474
		120	15	4	4.2 ± 2.6	425	444
		240	13	0	3.4 ± 0.6	373	386
<i>hsp104-ΔN</i>	3	0	0	0	0.0	466	466
		30	0	4	0.8 ± 0.5	552	556
		60	0	4	0.9 ± 0.8	419	423
		120	1	0	0.3 ± 0.5	336	337
		240	0	0	0.0	349	349

Table S3. Effects of 39° heat shock on wild-type, *sir2Δ*, *hst2Δ*, *lsb2Δ* and *lsb2Δ sir2Δ* strains (numbers of colonies for Fig. 1C).

Strain	Number of cultures analyzed	Incubation at 39°, min	Number of colonies				
			Red ([<i>psr</i> '])	Mosaic ([<i>PSI</i> ']/[<i>psr</i> '])	% red and mosaic $\pm$ SD	White/pink ([<i>PSI</i> '])	Total
WT	8	0	13	8	2.0 $\pm$ 1.0	985	1006
		30	43	286	31.5 $\pm$ 3.4	708	1037
		45	38	288	37.9 $\pm$ 5.0	532	858
		60	27	306	29.1 $\pm$ 3.7	803	1136
		120	22	128	14.2 $\pm$ 3.1	908	1058
		240	21	44	5.9 $\pm$ 2.2	1032	1097
<i>sir2Δ</i>	8	0	8	2	1.0 $\pm$ 1.0	1002	1012
		30	11	19	2.4 $\pm$ 2.9	1123	1153
		45	12	29	3.7 $\pm$ 4.9	891	932
		60	9	25	3.4 $\pm$ 3.0	978	1012
		120	8	16	2.4 $\pm$ 3.8	951	975
		240	9	12	2.1 $\pm$ 1.8	1041	1062
<i>hst2Δ</i>	6	0	3	8	1.5 $\pm$ 1.3	712	723
		30	11	131	17.6 $\pm$ 2.9	675	817
		45	12	143	19.4 $\pm$ 3.1	649	804
		60	7	121	16.0 $\pm$ 3.0	657	785
		120	11	38	7.0 $\pm$ 3.3	654	703
		240	10	21	3.8 $\pm$ 0.7	775	806
<i>lsb2Δ</i>	3	0	3	1	1.0 $\pm$ 1.2	396	400
		30	18	131	45.8 $\pm$ 6.0	177	326
		45	25	201	55.5 $\pm$ 1.6	181	407
		60	31	204	66.8 $\pm$ 5.0	119	354
		120	10	112	32.3 $\pm$ 8.3	248	370
		240	9	38	11.9 $\pm$ 3.9	334	381
<i>lsb2Δ sir2Δ</i>	5	0	0	2	0.4 $\pm$ 0.9	504	506
		30	2	11	2.2 $\pm$ 1.0	554	567
		45	2	24	5.1 $\pm$ 2.5	487	513
		60	5	19	5.1 $\pm$ 6.4	554	578
		120	10	28	5.6 $\pm$ 7.4	697	735
		240	5	15	3.0 $\pm$ 2.1	600	620

Table S4. Statistical comparisons by *t*-test for the experiments shown on Fig. 1C.

Compared strains	<i>p</i> value at incubation time (min at 39°)*					
	0	30	45	60	120	240
WT and <i>hst2Δ</i>	0.39257	<0.00001	0.00001	0.00001	0.00140	0.04464
WT and <i>sir2Δ</i>	0.05287	<0.00001	<0.00001	<0.00001	0.00001	0.00195
WT and <i>lsb2Δ sir2Δ</i>	0.01138	<0.00001	<0.00001	<0.00001	0.01298	0.03711
WT and <i>lsb2Δ</i>	0.18042	0.00065	0.00041	<0.00001	0.00035	0.00902
<i>sir2Δ</i> and <i>hst2Δ</i>	0.42602	<0.00001	0.00002	<0.00001	0.03368	0.05127
<i>lsb2Δ sir2Δ</i> and <i>lsb2Δ</i>	0.41340	<0.00001	<0.00001	0.00001	0.00320	0.00509
<i>lsb2Δ sir2Δ</i> and <i>sir2Δ</i>	0.33135	0.88703	0.56017	0.51873	0.31863	0.42620

*Statistically significant values are in red.

Table S5. Effects of 42° heat shock on wild-type and *sir2Δ* strains (numbers of colonies for Fig. 1D).

Strain	Number of cultures analyzed	Incubation at 42°, min	Number of colonies				
			Red ([<i>psi</i>])	Mosaic ([<i>PSI</i> ⁺]/[<i>psi</i>])	% Red and mosaic ± SD	White/pink ([<i>PSI</i> ⁺])	Total
WT	4	0	2	4	1.5±1.3	411	417
		30	178	327	76.8±5.9	143	648
		45	313	313	92.4±5.2	45	671
		60	337	279	94.2±2.3	39	655
		120	291	253	96.9±2.1	17	561
		240	175	383	93.3±4.8	44	602
<i>sir2Δ</i>	4	0	8	3	2.1±1.0	598	609
		30	15	42	8.3±4.5	568	625
		45	46	126	26.2±6.6	490	662
		60	79	186	41.8±7.6	360	625
		120	46	191	43.9±5.5	312	549
		240	67	192	38.1±10.1	412	671

Table S6. $[PSI^+]$ loss following induction of P_{GAL} -HSP104 in wild-type and *sir2Δ* strains (numbers for Fig. 1E).

Strain	Number of cultures analyzed	Incubation in galactose (hrs)	Number of colonies			
			Ade ⁻ ($[psi^-]$)	% $\pm$ SD	Ade ⁺ ($[PSI^+]$)	Total
WT	3	0	14	4.0 $\pm$ 3.0	350	364
		12	349	84.9 $\pm$ 8.6	60	409
		24	474	95.8 $\pm$ 4.6	21	495
<i>sir2Δ</i>	3	0	2	0.5 $\pm$ 0.5	464	466
		12	103	26.7 $\pm$ 9.9	289	392
		24	363	68.5 $\pm$ 8.7	171	534

Table S7. Comparison of normalized Hsp levels in wild-type and deletion strains.

Strain	Protein	Mean ratio of the protein levels in the deletion versus wild-type strain after incubation at 39° (with standardized error)			
		0 min	30 min	120 min	240 min
<i>sir2Δ</i>	Hsp104	0.97 $\pm$ 0.32	0.98 $\pm$ 0.11	1.19 $\pm$ 0.13	1.50 $\pm$ 0.61
	Ssa	1.13 $\pm$ 0.26	1.01 $\pm$ 0.15	0.96 $\pm$ 0.13	0.93 $\pm$ 0.14
<i>ssb1/2Δ</i>	Hsp104	0.95 $\pm$ 0.05	1.04 $\pm$ 0.10	1.06 $\pm$ 0.21	0.84 $\pm$ 0.09
	Ssa	1.10 $\pm$ 0.24	1.38 $\pm$ 0.12	1.19 $\pm$ 0.19	0.95 $\pm$ 0.13
<i>zuo1Δ</i>	Hsp104	1.28 $\pm$ 0.41	1.19 $\pm$ 0.24	1.20 $\pm$ 0.31	0.94 $\pm$ 0.14
	Ssa	0.87 $\pm$ 0.15	0.96 $\pm$ 0.24	0.89 $\pm$ 0.15	0.92 $\pm$ 0.13

Proteins were detected by specific antibodies. Band intensities were measured to densitometry and normalized by loading control (Ade2 or Coomassie). Ratios between the normalized protein levels in the deletion and control wild-type strains were always determined for the pairs run on one and the same gel. Mean ratios and errors were calculated for each time point, based on the results for 3 to 7 independent cultures per each time point.

Table S8. Effects of 39° heat shock on wild-type strain in the absence and presence of nicotinamide (NAM) (numbers of colonies for Fig. 3A).

Strain	Number of cultures analyzed	Incubation at 39°, min	Number of colonies				
			Red ([<i>psi</i> ⁻])	Mosaic ([<i>PSI</i> ⁺]/[<i>psi</i> ⁻])	% red and mosaic $\pm$ SD	White/pink ([<i>PSI</i> ⁺])	Total
WT	6	0	13	2	1.8 $\pm$ 1.4	776	791
		30	36	190	29.7 $\pm$ 3.0	541	767
		45	38	282	45.7 $\pm$ 10.6	393	713
		60	42	309	44.6 $\pm$ 11.2	445	796
		120	19	176	20.3 $\pm$ 4.4	793	988
		240	12	51	7.0 $\pm$ 4.5	844	907
WT+NAM	5	0	7	9	2.5 $\pm$ 1.6	674	690
		30	35	160	29.8 $\pm$ 9.3	468	663
		45	120	316	57.5 $\pm$ 18.4	282	718
		60	138	378	65.1 $\pm$ 17.2	253	769
		120	63	270	39.6 $\pm$ 13.6	522	855
		240	12	48	9.4 $\pm$ 5.3	560	620

Table S9. Effects of 39° heat shock on *sir2Δ* strain in the absence and presence of nicotinamide (NAM) (numbers of colonies for Fig. 3C).

Strain	Number of cultures	Incubation at 39°C, min	Number of colonies				
			Red ([<i>psi</i> ⁻])	Mosaic ([<i>PSI</i> ⁺]/[<i>psi</i> ⁻])	Red and mosaic (%)	White/pink ([<i>PSI</i> ⁺])	Total
<i>sir2Δ</i>	6	0	13	7	2.2 $\pm$ 1.3	893	913
		30	8	28	4.3 $\pm$ 2.6	785	821
		45	16	66	9.1 $\pm$ 5.9	792	874
		60	20	50	7.1 $\pm$ 5.4	918	988
		120	8	26	4.4 $\pm$ 2.8	760	794
		240	8	5	1.6 $\pm$ 1.9	781	794
<i>sir2Δ</i> +NAM	6	0	4	3	0.9 $\pm$ 0.5	789	796
		30	40	118	18.6 $\pm$ 5.4	706	864
		45	126	302	40.7 $\pm$ 12.6	609	1037
		60	222	354	48.7 $\pm$ 11.9	560	1136
		120	123	288	35.2 $\pm$ 9.1	733	1144
		240	20	71	8.6 $\pm$ 4.8	880	971

Table S10. Numbers of cells with cytologically detectable aggregates (for Fig. 4A)

Protein	Strain	Treatment	Number of cells with foci	Total number of of cells with fluorescence	% cells with foci $\pm$ SE**
Hsp104-GFP	WT	None (pre-HS*)	20	197	9 $\pm$ 2.0
		HS at 42°, 1 hr	121	0	100 $\pm$ 0.3
Hsp104-GFP	<i>sir2</i> Δ	None (pre-HS)	57	22	20 $\pm$ 2.4
		HS at 42°, 1 hr	325	16	95 $\pm$ 0.8
Sup35NM-DsRed	WT	None (pre-HS)	13	128	6 $\pm$ 1.5
		HS at 42°, 1 hr	25	37	21 $\pm$ 3.6
Sup35NM-DsRed	<i>sir2</i> Δ	None (pre-HS)	27	222	10 $\pm$ 1.6
		HS at 42°, 1 hr	115	188	34 $\pm$ 2.6

Data from two independent experiments are summarized.

*HS – heat shock.

**SE – standardized error

Table S11. Distribution of the Hsp104-GFP aggregates between mother and daughter cells in pairs (numbers for Fig. 4D).

Strain	Conditions	Numbers of cell pairs with aggregates in				Total number of cell pairs with fluorescence	% cell pairs with aggregates in mother* ($\pm$ SE)
		Mother	Daughter	Both	None		
WT	Pre-HS	8	0	3	71	82	73 ($\pm$ 13)
	HS at 42°, 1hr	0	0	68	0	68	0 (+1)
	Post-HS, 120 min	21	0	118	2	141	15 ($\pm$ 3)
	Post-HS 240 min	18	0	9	2	29	67 ($\pm$ 9)
<i>sir2</i> Δ	Pre-HS	12	9	8	56	85	41 ($\pm$ 9)
	HS at 42°, 1hr	0	0	217	0	217	0 (+1)
	Post-HS 120 min	4	4	116	0	124	3 ($\pm$ 2)
	Post-HS 240 min	26	2	19	4	47	55 ($\pm$ 7)

Data from two independent experiments are summarized. Designations are as in Table S10.

*Relative to the total number of cell pairs with aggregates.

Table S12. Distribution of the Sup35NM-DsRed aggregates between mother and daughter cells in pairs (numbers for Fig. 4E).

Strain	Conditions	Numbers of cell pairs with aggregates in				Total number of cell pairs with fluorescence	% cell pairs with aggregates in mother* ($\pm$ SE)
		Mother	Daughter	Both	None		
WT	Pre-HS	4	0	6	57	67	40 ($\pm$ 15)
	HS at 42°, 1hr	16	2	11	17	46	55 ($\pm$ 9)
	Post-HS 240 min	17	0	1	5	23	94 ($\pm$ 6)
<i>sir2</i> Δ	Pre-HS	7	0	5	43	55	58 ($\pm$ 14)
	HS at 42°, 1hr	5	4	47	10	66	9 ($\pm$ 4)
	Post-HS 240 min	26	3	12	13	54	63 ($\pm$ 8)

Data from two independent experiments are summarized. Designations are as in Table S10.

*Relative to the total number of cell pairs with aggregates.

Table S13. Effects of 39° heat shock on wild-type, *ssb1/2* Δ , *zuo1* Δ , *ssz1* Δ and *ssb1/2* Δ *zuo1* Δ strains (numbers of colonies for Figs. 5B and S7).

Strain	Number of cultures analyzed	Incubation at 39°, min	Number of colonies				
			Red ([<i>psi</i> '])	Mosaic ([<i>PSI</i> ']/[<i>psi</i> '])	% red and mosaic $\pm$ SD	White/pink ([<i>PSI</i> '])	Total
WT	12	0	23	10	1.5 $\pm$ 1.1	1840	1873
		30	88	471	35.7 $\pm$ 9.0	933	1492
		45	75	509	49.6 $\pm$ 11.8	613	1197
		60	94	532	44.8 $\pm$ 6.9	774	1400
		120	28	217	16.1 $\pm$ 3.5	1267	1512
		240	18	60	4.9 $\pm$ 2.3	1521	1599
<i>ssb1/2</i> Δ	11	0	19	14	2.6 $\pm$ 2.1	1550	1583
		30	20	58	7.2 $\pm$ 4.9	1252	1330
		45	27	117	11.9 $\pm$ 4.5	1137	1281
		60	26	95	9.7 $\pm$ 2.7	1122	1243
		120	25	22	3.4 $\pm$ 2.1	1323	1370
		240	23	17	2.8 $\pm$ 2.7	1212	1252
<i>zuo1</i> Δ	8	0	150	13	9.7 $\pm$ 8.3	1828	1991
		30	1023	433	85.1 $\pm$ 14.3	219	1675
		45	1042	346	94.3 $\pm$ 7.2	82	1470
		60	869	383	88.8 $\pm$ 10.7	162	1414
		120	255	395	76.7 $\pm$ 19.2	148	798
		240	89	199	28.5 $\pm$ 19.4	606	894

Table S13 (continuation). Effects of 39° heat shock on wild-type, *ssb1/2Δ*, *zuo1Δ*, *ssz1Δ* and *ssb1/2Δ zuo1Δ* strains (numbers of colonies for Figs. 5B and S7).

Strain	Number of cultures analyzed	Incubation at 39°, min	Number of colonies				
			Red ([<i>psi</i> ⁻])	Mosaic ([<i>PSI</i> ⁺]/[<i>psi</i> ⁻])	% red and mosaic $\pm$ SD	White/pink ([<i>PSI</i> ⁺])	Total
<i>ssz1Δ</i>	3	0	10	1	2.0 $\pm$ 1.2	441	452
		30	281	128	90.1 $\pm$ 5.5	44	453
		45	456	135	91.2 $\pm$ 2.3	60	651
		60	522	143	90.6 $\pm$ 5.8	69	734
		120	116	245	78.0 $\pm$ 4.7	106	467
		240	22	63	21.4 $\pm$ 5.5	320	405
<i>ssb1/2Δ zuo1Δ</i>	7	0	0	5	1.0 $\pm$ 2.5	1742	1747
		30	14	166	13.5 $\pm$ 11.1	1266	1446
		45	10	217	24.2 $\pm$ 11.4	868	1095
		60	43	221	24.6 $\pm$ 16.8	705	969
		120	2	101	11.2 $\pm$ 5.5	694	797
		240	2	6	1.5 $\pm$ 2.1	614	622
<i>ssb1Δ</i>	4	0	3	3	1.2 $\pm$ 1.2	515	521
		30	2	11	2.2 $\pm$ 1.6	560	573
		45	1	10	2.2 $\pm$ 1.0	468	479
		60	3	17	3.1 $\pm$ 1.2	590	610
		120	6	10	3.0 $\pm$ 2.5	497	513
		240	4	7	2.0 $\pm$ 0.8	536	547
<i>ssb2Δ</i>	4	0	0	5	1.1 $\pm$ 2.2	468	473
		30	4	22	5.3 $\pm$ 2.7	461	487
		45	6	43	10.0 $\pm$ 6.6	454	503
		60	4	32	7.9 $\pm$ 5.8	458	494
		120	1	9	2.3 $\pm$ 1.0	429	439
		240	3	6	2.1 $\pm$ 1.0	424	433

Table S14. Statistical comparisons by *t*-test for the experiments shown on Figs. 5B and S6.

Compared strains	<i>p</i> value at incubation time (min at 39°)*					
	0	30	45	60	120	240
WT and <i>zuo1</i> Δ	0.00306	<0.00001	<0.00001	<0.00001	<0.00001	0.00051
WT and <i>ssb1</i> Δ	0.64251	<0.00001	<0.00001	<0.00001	0.00001	0.02760
WT and <i>ssb2</i> Δ	0.63781	0.00001	0.00002	<0.00001	<0.00001	0.03662
WT and <i>ssb1/2</i> Δ	0.13444	<0.00001	<0.00001	<0.00001	<0.00001	0.05805
WT and <i>ssz</i> Δ	0.45949	<0.00001	0.00005	<0.00001	<0.00001	<0.00001
WT and <i>ssb1/2</i> Δ <i>zuo1</i> Δ	0.57846	0.00019	0.00027	0.00170	0.03101	0.00433
<i>ssb1</i> Δ and <i>ssb1/2</i> Δ	0.24076	0.07209	0.00115	0.00051	0.72827	0.57312
<i>ssb2</i> Δ and <i>ssb1/2</i> Δ	0.25740	0.49207	0.53157	0.41086	0.34897	0.64900
<i>zuo1</i> Δ and <i>ssz</i> Δ	0.15879	0.58052	0.49785	0.78879	0.91227	0.56066
<i>ssb1/2</i> Δ and <i>zuo1</i> Δ	0.01363	<0.00001	<0.00001	<0.00001	<0.00001	0.00041
<i>ssb1</i> Δ and <i>ssb2</i> Δ	0.94726	0.09068	0.05940	0.15694	0.65100	0.82029
<i>ssb1/2</i> Δ and <i>ssb1/2</i> Δ <i>zuo1</i> Δ	0.17676	0.11170	0.00497	0.00957	0.00058	0.28012
<i>zuo1</i> Δ and <i>ssb1/2</i> Δ <i>zuo1</i> Δ	0.02018	<0.00001	<0.00001	<0.00001	<0.00001	0.00296

*Statistically significant values are in red.