Roles of Hsf1, Sfl1 and Skn7 in mediating heat-shock protein genes and sustaining asexual cycle, stress tolerance and virulence in *Beauveria basssiana*

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Table S1. Paired primers used for the manipulation of *hsf1*, *sfl1* and *skn7* in *B. bassiana*.

Primer	Paired sequences (5′–3′)*	Purpose
Hsf1up-F/R	AAAAAGAATTCGCTGCGAGAGTCAAGAGAG / AAAAAAAGCTTGTGTCGGGTTGTGGGGTTAT	Cloning *hsf1* 5′-end (1045 bp)
Hsf1dn-F/R	AAAAACTCGAGAGGAGGAGAGCGTGGAGAAG / AAAAAACTAGTGGGGTTATGAACACCTCCAG	Cloning *hsf1* 3′-end (1072 bp)
Sfl1up-F/R	AAAAAGAATTCGGTAGATTGTAGCACAGACGGG / AAAAAGAGCTCACGAGGGTGGAAGTGGAATAAC	Cloning *sfl1* 5′-end (1583 bp)
Sfl1dn-F/R	AAAAATCTAGAGCTCGGTCCCAAAGAAAACA / AAAAAAGATCTCGAATCTGGCTCTCAGGTGTAT	Cloning *sfl1* 3′-end (1432 bp)
Skn7up-F/R	AAAAAGAATTCGGTAGCGACTGTGTTGTTGGC / AAAAACCCGGGGTTGACGACGGGGTGAAAAG	Cloning *skn7* 5′-end (2113 bp)
Skn7dn-F/R	AAAAAAGATCTATGCCGTAAGTTGCGTCGTC / AAAAAACTAGTCGTCGTAGGCTGTCTGCTGT	Cloning *skn7* 3′-end (2298 bp)
Hsf1fl-F/R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*GACCAGCGTCACGGCAAGAAT / *GGGGACAAGTTTGTACAAAAAAGCAGGCT*GGACTGAATGCGACAGGCGTT	Cloning full-length *hsf1* (5390 bp)
Sfl1fl-F/R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*GGGGAGAGAACAGATTTGGAC / *GGGGACAAGTTTGTACAAAAAAGCAGGCT*TCGTTGGACCTACTCCTTTTC	Cloning full-length *sfl1* (4336 bp)
Skn7fl-F/R	*GGGGACCACTTTGTACAAGAAAGCTGGGT*CTTAGCGATGTGAAGCAGGTT / *GGGGACAAGTTTGTACAAAAAAGCAGGCT*CAGAGCACCAACAAACAACAT	Cloning full-length *skn7* (5811 bp)
pHsf1-F/R	CAAGCACCTCACAACAACAAAG / CTCACGCTTGGCTTTGGCT	PCR detecting *hsf1*
pSfl1-F/R	CACTACACTTCGTCTTTACCGCCAG / TCGGGAGGCAGTTGAACAGGT	PCR detecting *sfl1*
pSkn7-F/R	TCGAACTCTTCTCTTCCGCG / TGGTCTCTTCCCACAGCATT	PCR detecting *skn7*
sbHsf1-F/R	TGTGACTCAAGGTGAAAAAAG / GAATATATTGTGGCAGTGGGT	Southern probe for *hsf1*
sbSfl1-F/R	GTAGGTAGGTATCACTTGCC / GCACTGGAGAGAGAAAAAAT	Southern probe for *sfl1*
sbSkn7-F/R	TTCTCCTCCTCATTCCGTCTT / GACACCGAAGGAGGTTGACAG	Southern probe for *skn7*
qHsf1-F/R	GTCCGATGAACCAGTCTT/AAAGGAGTCCAGGGATCT	qRT-PCR detecting *hsf1*
qSfl1-F/R	ATCCAGCATCTAATATCA/TAAAGTATTGGGAGAGAA	qRT-PCR detecting *sfl1*
qSkn7-F/R	GAACAATGCCAGTGAATT / CGACAACAACAAAGGTAT	qRT-PCR detecting *skn7*
qActin-F/R	TCTCTACGGCAACATTGTC / TGCTGGAAGGTGGATAGG	qRT-PCR detecting γ-actin gene

*Underlined regions denote the sites of restriction enzyme for the deletion of each target gene (*Eco*RI/*Hin*dIII and *Xho*I/*Spe*I for *hsf1*, *Eco*RI/*Sac*I and *Xba*I/*Bgl*II for *sfl1*, and *Eco*RI/*Xma*I and *Bgl*II/*Spe*I for *skn7*) via homogenous recombination of 5′ and 3′ fragments separated by *bar* marker or the gateway exchange fragments for complementation of each target gene.

Table S2. Paired primers used for assessing transcript levels of 46 genes in the mutants of *hsf1*, *sfl1* and *skn7* versus *B. bassiana* wild-type via qRT-PCR.

Gene ID	**Gene**	**Annotation**	**Sequences (5'-3') of paired primers**
Involved in growth and asexual development			
19887954	*fluG*	developmental protein	CCTCCCTAGTTTGGTCGCTTTCTC / CGCTGTCGGTAATCTGCTCCTC
19885980	*flbA*	developmental regulator	CCAATCCACTCGCCGCTCTC / CGGAGGAAAGAGAATCGGTAGAGG
19890000	*flbB*	bZIP transcription factor	GCACTGACACGCCGACAAGAGC / CCGCCGCCGAAGCCTGTTG
19886193	*flbC*	C_2H_2 conidiation transcription factor	TCCATCTCCAACTTGCTGGGTCTC / GGCGGCGTAGGCGGAAGG
19890271	*flbD*	MYB conidiophore development protein	CGGCAAGCGATGGGCAGAGATTG / ACGAGCAAGGTGACGGTAGAGGTG
19889611	*hyd1*	hydrophobin 1	TTCTCAGCGATCTTGATCTT / GCACTTGTTGTCGATTGG
19886027	*hyd2*	hydrophobin 2	CATGGTGGAAAGGATCTG / ATCTTGGTCGTCTTCTCG
Involved in Na^+ efflux			
19889202	*Ena1*	P-type Na^+/K^+ ATPase 1	TTCTCACCGACGATAACT / TATTCTCAGCCAGGACAT
19892062	*Ena2*	P-type Na^+/K^+ ATPase 2	TAATACCAGGCAAGAAGT / TTAGGACGACAATGAGAG
19886191	*Ena3*	P-type Na^+/K^+ ATPase 3	GAATCTCAGCAGACTCAT / AATCACGAATCTCTTCCT
19887969	*Ena4*	P-type Na^+/K^+ ATPase 4	CGATGATGACAGCAAGTT / CGATAGAGCACCACAGAT
19890673	*Ena5*	P-type Na^+/K^+ ATPase 5	CAAGGATACAAGCGGTAA / CGACGAAGTAATGAGGTT
HSP-coding genes			
19890898	*hsp20*	heat shock protein 20	TCAGGAGATGAGAAGAAC / GCTGTGATGATGTTTGTT
19891700	*hsp30a*	heat shock protein 30	TCTTCCCTTGGTCGTGAT/GTATTGGTCGCTGCTCTC
19885069	*hsp30b*	heat shock protein 30	ATGGCTTCCTTCTTTCCC/AGTATGTGTCAAACTCGTC
19886748	*hsp40a*	heat shock protein 40	GAGATTGACCTCTATGAG / CTCCTTGAATTTGACCTC
19891452	*hsp40b*	heat shock protein 40	AGAAGACTACTACAAGATCC / TTCCTTGGCAGTATCATC
19889942	*hsp40c*	heat shock protein 40	ATTTCACACGACGAACAAG / TGCGAGTCCATAGTAAGC
19888317	*hsp40d*	heat shock protein 40	CAGGTACTTTCCGACAAG / CGAAAATAGACGAGAAGAAC
19885590	*hsp40e*	heat shock protein 40	GACAAGAACAAGGACGAT / ACCTCTCAGCAGAAATTC
19883188	*hsp40f*	heat shock protein 40	AATCGGTGCTCTCAAGTT / TGTCGGAAAGAATTTCGTAAG
19888479	*hsp60a*	heat shock protein 60	TTTACATCTTCACTACGA/CCTTCTCTAATCAGTTCT
19892463	*hsp70a*	heat shock protein 70	GTGGTAAAGGCATGATTT / GAGTCAGAAGTTGGAAAG
19889799	*hsp70b*	heat shock protein 70	GGACCTATGGCTCAACAG / AATGCGACACCAGAAAAG
19891640	*hsp70c*	heat shock protein 70	TTGGCTCAGTTGAATGTG / GCAAGTCTGGAAAGCAATA
19892721	*hsp70d*	heat shock protein 70	TTCTTGTAGCCGTTGATT / GGTATTATCTCCTCCTTCAC
19884009	*hsp70e*	heat shock protein 70	AGCAGAAGCAATGTCAAT/TGGCTGATGAAGAAGTTG
19886620	*hsp70f*	heat shock protein 70	AGCCGTCTTCTTCTTCTC/GCCTTGATGTACTCTGTG
19883269	*hsp70g*	heat shock protein 70	CATGGTTCTCCTCAAGAT / CTGGTTGTCGTTGAAGTA
19888598	*hsp70h*	heat shock protein 70	TGCTGGTGGTATGATGAC / TCGGAGAAGGTAGAGAAGA
19886917	*hsp70i*	heat shock protein 70	ATTATGGCACTGTCATTG / TGATACGGTTTCCTTGAT
19885360	*hsp70j*	heat shock protein 70	ACATCAAGGTCACTAAGC / CTTCTCTTCGTCTTCGTC
19889859	*hsp70k*	heat shock protein 70	TCAACATTCACGGCATTC / CTTCTTGTCAGTGTCCAT
19884520	*hsp70l*	heat shock protein 70	TCTGTCCTTAACGATACC / TCACGAAGAGCAGTAATC
19883953	*hsp70m*	heat shock protein 70	ACTCTACTTCAATATGGC / TAGGTCTACTTCCTCAAG
19886284	*hsp70n*	heat shock protein 70	CATTGTTGTCTCTATTGACT / TCTCCTTGATCTCCACTA
19883808	*hsp70o*	heat shock protein 70	TACAATCTGACTGAAGAACATATC / ATCGTTGAGCCTGACATT
19889528	*hsp90*	heat shock protein 90	TTCTCTCCCTCATCATCAAC/CTTGTAGCGGACCTTGTC
19885295	*hsp104*	heat shock protein 104	CTTTCGCCACAATTTCAC/ACACCGATGTAGGTATCTT
19884587	*hsp78*	heat shock protein 78	ATACAGACCTGCCGCTAT/TATGTTCGTGTGACCATTG

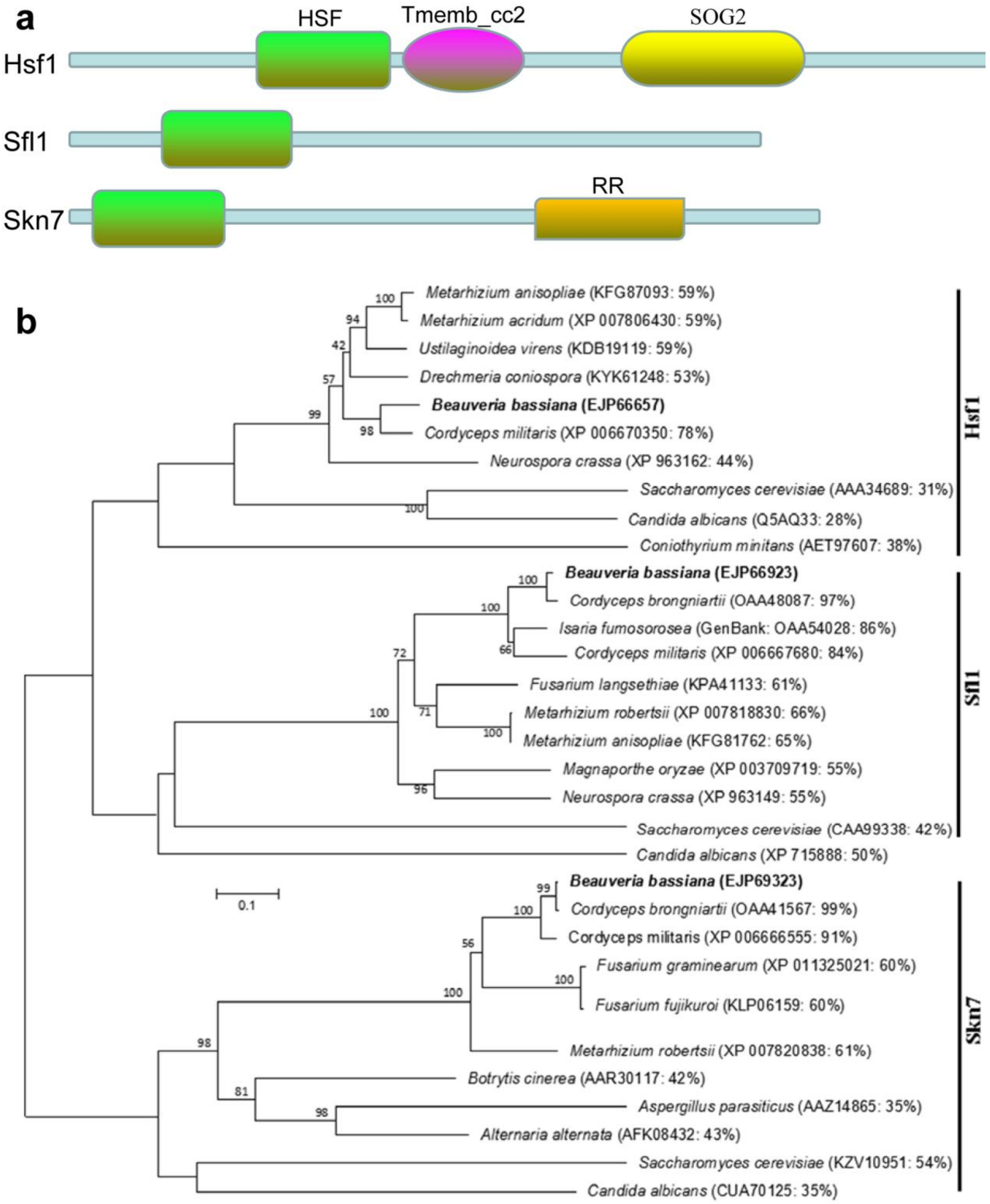

Fig. S1. Structural and phylogenetic features of three HSF-containing genes found in the genome database of *B. bassiana*. (**a**) Conserved domains predicted from three HSFs via sequence alignment and online blast analysis at http://blast.ncbi.nlm.nih.gov/blast.cgi. (**b**) Phylogenetic tree for three HSFs of *B. bassiana* and other fungi based on a neighbor-joining method in MEGA7 at http://www.megasoftware.net. The NCBI accession code of a given protein and its sequence identity to that of *B. bassiana* are given in the parentheses following each fungal name. Scale bar: branch length proportional to genetic distance.

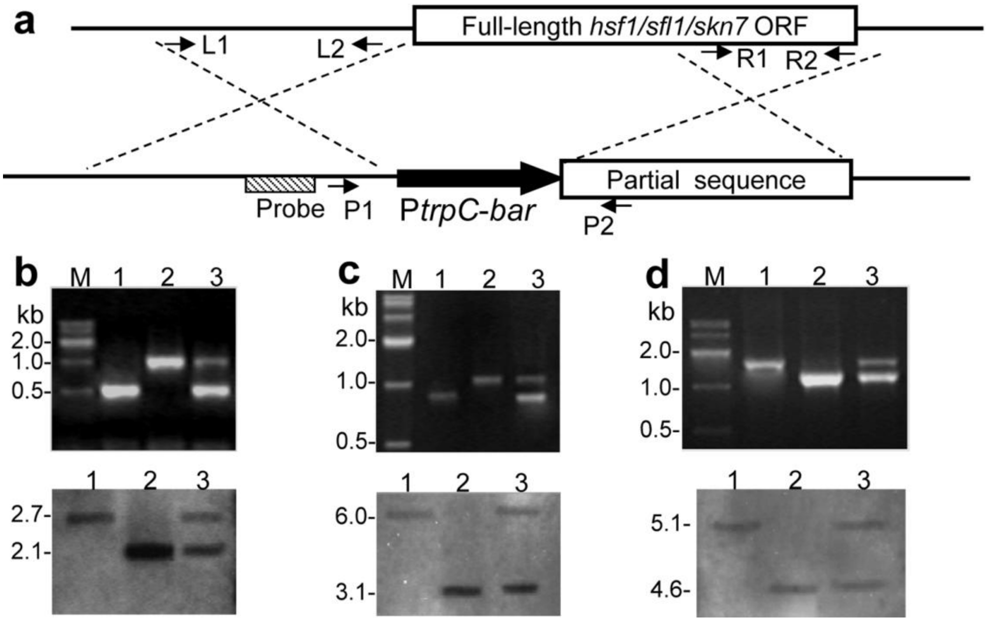

Fig. S2. Construction and identification of *B. bassiana hsf1*, *sfl1* and *skn7* mutants. (**a**) Schematic diagram for the deletion strategy of each target gene. (**b**–**d**) The *hsf1*, *sfl1* and *skn7* mutants identified via PCR (*upper panel*) and Southern blot hybridization (*lower panel*), respectively. *Lane 1*: wild-type strain. *Lane 2*: deletion mutant. *Lane 3*: complemented mutant. Genomic DNAs used for Southern blotting of *hsf1*, *sfl1* and *skn7* were digested with *Xho*I/*Xho*I, *Xba*I/*Xba*I and *Bst*XI/*Eco*RI, respectively.

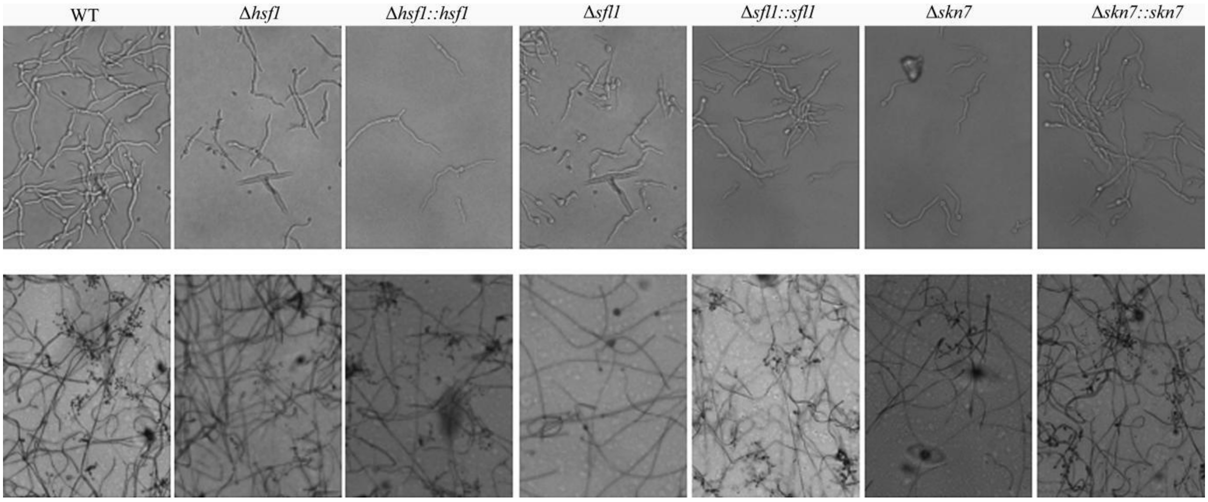

Fig. S3. Images of germ tubes and early hyphae after conidia were cultivated on GM plates for 16 h (up) and conidiophores and conidia after conidia were cultivated on SDAY plates for 3 days (down), visualized at 400× magnification.

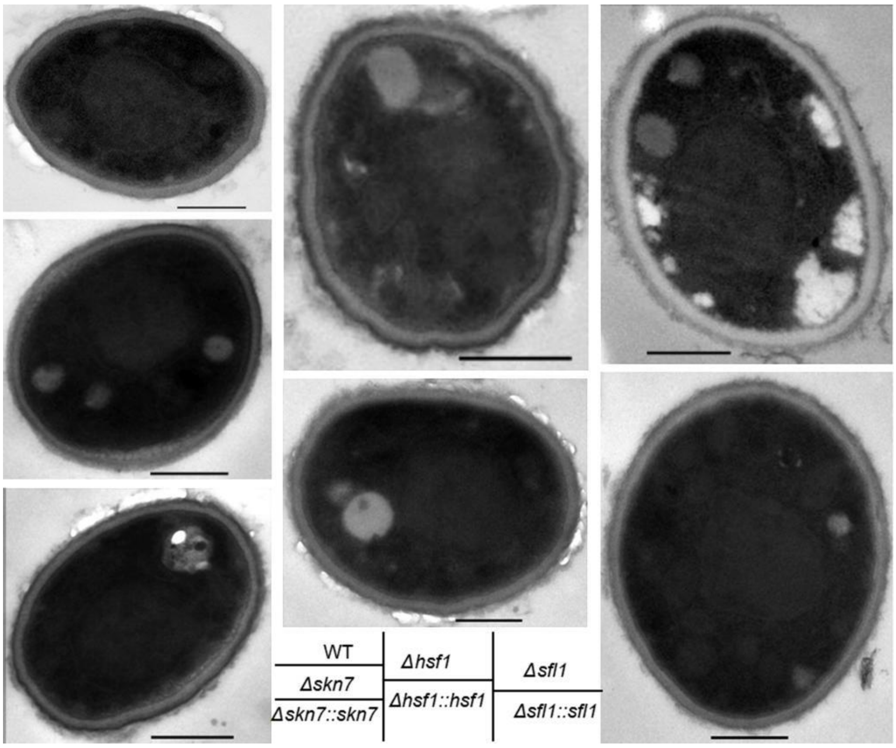

Fig. S4. Images for the ultrathin sections of conidia observed under transmission electron microscopy (TEM). Briefly, aerial conidia were washed three times in PBS (pH 7.0) and fixed overnight at 4 °C with 2% paraformaldehyde and 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After post-fixed in 1% OsO_4, conidia were dehydrated in a graded ethanol series, embedded in Spurr resin and stained with 2% uranyl acetate and Reynold's lead solution. The samples were examined under an H-7650 transmission electron microscope (Hitachi). Scale: 0.5 µm.

Table S3. Relative transcript levels of HSPs gene in the 3-d-old SDAY cultures of *hsf1*, *sfl1* and *skn7* mutants versus WT grown at 25°C or then shocked at 40°C for 1 h.

HSP genes	Relative transcript levels (mean ± SD) in normal cultures*							Relative transcript levels (mean ± SD) in heat-shocked cultures*						
	WT	Δ*hsf1*	Δ*hsf1::hsf1*	Δ*sfl1*	Δ*sfl1::sfl1*	Δ*skn7*	Δ*skn7::skn7*	WT	Δ*hsf1*	Δ*hsf1::hsf1*	Δ*sfl1*	Δ*sfl1::sfl1*	Δ*skn7*	Δ*skn7::skn7*
hsp20	1.00±0.09	13.5±0.97	1.71±0.35	1.38±0.11	1.02±0.08	0.24±0.01	0.97±0.02	1.00±0.07	2.14±0.03	1.00±0.02	0.11±0.02	0.95±0.04	0.47±0.03	1.00±0.02
hsp30a	1.00±0.09	1.25±0.14	0.97±0.13	0.83±0.02	1.00±0.02	0.58±0.01	1.00±0.01	1.00±0.03	0.10±0.01	0.95±0.03	0.78±0.02	0.97±0.04	0.54±0.02	0.95±0.03
hsp30b	1.00±0.11	1.50±0.10	1.02±0.06	0.23±0.02	0.96±0.02	0.25±0.01	0.96±0.03	1.03±0.31	0.43±0.04	1.00±0.03	0.09±0.01	0.93±0.06	0.94±0.02	1.00±0.02
hsp40a	1.00±0.06	0.17±0.01	1.00±0.01	1.03±0.06	1.02±0.06	0.62±0.03	0.93±0.04	1.04±0.39	0.54±0.03	1.00±0.02	0.79±0.04	1.00±0.03	3.34±0.33	1.35±0.39
hsp40b	1.00±0.10	0.10±0.00	0.95±0.04	1.33±0.02	1.01±0.02	0.45±0.02	0.96±0.04	1.01±0.14	1.21±0.04	1.00±0.03	0.17±0.04	0.95±0.03	1.14±0.07	1.00±0.05
hsp40c	1.00±0.06	6.30±0.31	1.63±0.35	1.09±0.02	0.99±0.02	0.24±0.01	0.99±0.01	1.00±0.02	0.30±0.01	1.00±0.01	0.61±0.05	1.00±0.03	0.14±0.01	0.93±0.10
hsp40d	1.01±0.13	7.45±0.67	1.71±0.47	1.03±0.07	1.02±0.06	0.19±0.01	0.95±0.04	1.00±0.12	1.07±0.02	1.00±0.01	0.14±0.01	0.99±0.01	0.07±0.00	0.87±0.01
hsp40e	1.01±0.14	8.99±0.90	1.74±0.18	1.57±0.17	1.11±0.02	0.20±0.02	0.95±0.04	1.00±0.04	0.84±0.03	1.00±0.02	0.21±0.00	1.00±0.00	0.12±0.00	0.84±0.03
hsp40f	1.00±0.12	5.53±0.12	1.67±0.50	0.95±0.02	1.00±0.02	0.28±0.00	0.97±0.03	1.00±0.08	0.49±0.04	0.97±0.03	0.24±0.01	1.00±0.01	0.18±0.00	1.00±0.00
hsp60	1.00±0.10	35.9±11.7	0.97±0.05	0.68±0.14	1.00±0.11	0.42±0.10	0.96±0.21	1.00±0.12	2.13±0.22	1.14±0.10	0.50±0.09	0.93±0.01	1.07±0.25	1.01±0.17
hsp70a	1.00±0.09	0.77±0.05	1.00±0.03	1.68±0.11	0.98±0.11	0.77±0.05	1.10±0.18	1.00±0.06	0.06±0.00	0.96±0.04	0.07±0.00	1.00±0.00	2.19±0.02	1.00±0.01
hsp70b	1.00±0.05	0.11±0.01	0.95±0.07	0.29±0.02	0.94±0.04	2.60±0.08	1.02±0.07	1.00±0.06	2.43±0.07	1.00±0.05	0.14±0.01	0.85±0.04	0.06±0.01	0.87±0.02
hsp70c	1.00±0.03	15.7±1.10	1.96±0.37	2.28±0.13	0.98±0.12	0.17±0.01	0.90±0.00	1.00±0.03	1.42±0.03	1.00±0.02	0.09±0.01	0.87±0.02	0.06±0.01	0.83±0.01
hsp70d	1.01±0.14	17.4±0.83	1.84±0.07	1.74±0.14	1.02±0.09	0.74±0.03	1.01±0.02	1.00±0.05	1.82±0.01	1.00±0.01	0.07±0.00	0.95±0.05	0.06±0.01	0.85±0.03
hsp70e	1.00±0.10	0.92±0.02	1.00±0.02	0.16±0.00	0.97±0.06	0.20±0.01	0.94±0.06	1.09±0.50	0.83±0.08	1.00±0.05	0.13±0.01	0.90±0.01	0.34±0.06	1.00±0.04
hsp70f	1.00±0.10	0.90±0.03	1.00±0.03	0.16±0.01	0.94±0.05	0.15±0.00	0.94±0.05	1.00±0.04	0.91±0.04	1.00±0.03	0.12±0.01	0.90±0.00	0.06±0.00	0.95±0.04
hsp70g	1.00±0.08	0.06±0.00	1.00±0.00	0.51±0.06	0.95±0.02	2.82±0.04	0.99±0.03	1.00±0.08	2.32±0.08	1.00±0.06	0.09±0.01	0.88±0.03	1.38±0.14	1.03±0.06
hsp70h	1.00±0.07	0.59±0.07	0.95±0.00	0.33±0.03	1.00±0.02	2.60±0.08	0.98±0.06	1.00±0.02	0.53±0.03	1.00±0.02	0.48±0.01	1.00±0.00	0.45±0.07	1.00±0.05
hsp70i	1.00±0.03	0.47±0.01	1.00±0.01	0.12±0.01	0.96±0.04	2.78±0.22	0.98±0.02	1.00±0.06	1.22±0.03	1.00±0.02	0.17±0.01	0.98±0.02	0.52±0.04	1.00±0.03
hsp70j	1.00±0.06	4.82±0.24	1.62±0.36	2.01±0.03	0.99±0.03	0.44±0.01	0.96±0.02	1.00±0.05	1.84±0.17	1.00±0.12	0.12±0.01	0.90±0.01	0.07±0.00	0.87±0.02
hsp70k	1.00±0.06	4.17±0.23	1.03±0.15	1.31±0.11	0.98±0.11	2.30±0.11	1.03±0.11	1.00±0.08	0.81±0.04	1.00±0.03	0.52±0.02	1.00±0.01	0.42±0.04	1.00±0.03
hsp70l	1.00±0.11	0.46±0.09	0.96±0.01	0.15±0.03	0.84±0.03	2.39±0.08	1.02±0.08	1.00±0.09	1.48±0.04	1.00±0.03	0.43±0.03	1.00±0.02	0.74±0.09	1.01±0.16
hsp70m	1.00±0.10	17.7±0.71	1.71±0.36	1.41±0.12	1.09±0.06	0.11±0.01	0.97±0.05	1.00±0.04	1.01±0.00	1.00±0.00	0.06±0.00	0.87±0.04	0.06±0.00	1.00±0.00
hsp70n	1.00±0.01	15.4±0.75	1.70±0.19	1.07±0.04	1.01±0.04	0.38±0.04	0.94±0.02	1.00±0.07	0.71±0.03	1.00±0.02	0.03±0.00	0.86±0.03	0.92±0.01	1.00±0.01
hsp70o	1.01±0.15	14.1±0.78	1.69±0.12	1.37±0.08	1.02±0.06	0.12±0.00	0.98±0.04	1.00±0.05	0.98±0.05	1.00±0.04	0.06±0.00	0.84±0.03	0.05±0.00	0.94±0.07
hsp90	1.00±0.02	0.75±0.02	1.01±0.02	0.33±0.01	0.99±0.02	0.54±0.01	1.00±0.00	1.00±0.07	0.23±0.02	1.00±0.01	0.48±0.04	0.97±0.03	1.06±0.04	1.00±0.02
hsp104	1.00±0.04	2.73±0.21	0.98±0.12	0.63±0.02	1.00±0.02	0.36±0.00	1.00±0.00	1.00±0.09	0.42±0.05	1.00±0.04	3.61±0.39	1.46±0.09	0.57±0.04	1.00±0.03
hsp78	1.00±0.00	0.93±0.00	1.00±0.00	0.15±0.00	0.90±0.00	0.20±0.01	0.80±0.01	1.00±0.08	0.66±0.01	1.00±0.01	0.63±0.03	0.97±0.03	3.71±0.86	1.44±0.13

* Red: HSP genes significantly upregulated by ≥ 2-fold. Green: HSP genes significantly downregulated by ≥ 50%.