Supporting Information

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SI Materials and Methods

Strains and Genotyping. The hsp31Δ strain was a kind gift from Marek Skoneczny (Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw) and hsp32Δ was obtained by sporulation of the BY4743 strain heterozygous for the HSP32 deletion (Thermo Scientific Open Biosystems). The hsp33Δ and hsp34Δ strains (in the BY4742 background) were generated by PCR-based gene disruption as previously described (1). Because the flanking regions of these genes are identical, the same pair of primers was used to amplify the MX cassettes and delete the genes. The primers used are listed in Table S6. The strategy for gene-deletion verification consisted in performing the following PCR reactions: (*i*) amplification of HSP32 and HSP33: forward primer complementary to HSP32, HSP33, and HSP34 (HSP32/33/34F) together with a reverse primer that hybridizes with HSP32 and HSP33 3′ flanking regions (HSP32/33R); (*ii*) amplification of HSP34: forward primer HSP32/33/34 and a reverse primer specific to HSP34 3′ flanking region (HSP34R); (*iii*) amplification of the cassette integrated either at the HSP32 or HSP33 locus [forward primer complementary to the cassette (MX4F) and HSP32/33R]; (*iv*) amplification of the cassette integrated at the HSP34 locus (MX4F + HSP34R). To distinguish between HSP32 and HSP33, the 3′ flanking region was amplified and digested with BsgI, which has a unique restriction digestion site in the HSP32 flanking region. To further confirm the knockout strains, the flanking regions of the deleted genes were analyzed by DNA sequencing and real-time quantitative PCR (qRT-PCR). The knockout strains were crossed to generate a heterozygous diploid quadruple knockout. By sporulation and tetrad dissection of the diploid strain, WT and single knockout strains were selected (Lx strains). Experiments were performed on the original strains and repeated with Lx strains with no differences observed among the phenotypes analyzed. Microarrays were performed with RNA retrieved from Lx strains. The remaining knockout strains used: gis1Δ, sod1Δ, and yap1Δ were obtained from EUROSCARF (Table S5). W303 was used for the colocalization studies performed in Fig. 6 *C* and *D*.

Plasmids. HSP31, HSP32, HSP33, HSP34, and DCP1 ORFs were subcloned using Gateway Cloning technology. ORFs were moved from entry clones (HSP31–34 plasmids kindly provided by Tony Hazbun, Purdue University, West Lafayette, IN) into the expression vector pAG416GPD-ccdB-HA (HSP31-34) and pAG415GPD-ccdb-DsRed (DCP1) (Addgene). HSP31 and HSP32 were also cloned by the Gateway system into p416GPD-ccdb-GFP. The plasmid pRS316 GFP-Atg8 (2) was kindly given by Yoshinori Ohsumi (Tokyo Institute of Technology, Tokyo) and pRS315 HA-Atg13 by Daniel Klionsky (University of Michigan, Dearborn, MI) (3). Both genes are under control of their endogenous promoters. The plasmid BG1805 (encoding the Leu2-His6-HA-protease-3Csite-ZZ domain) was obtained from the ORF library (Thermo Scientific Open Biosystems); the protein of interest is expressed under control of the GAL1 promoter. pTA19 (kindly given by Uesono Yukifumi, University of Tokyo, Tokyo) was used to integrate GFP in the genome to fuse with Kog1 as described previously (4). Plasmids pRP1362 (Pab1-GFP, URA) (5), pRP1575 (Edc3-mcherry, TRP) (6), and pRP1662 (Pub1-m-cherry, TRP) (6) were kindly given by Roy Parker (University of Colorado, Boulder, CO).

Media and Growth Conditions. Yeast strains were grown on standard media: YPD [2%(wt/vol)], synthetic complete media containing all of the amino acids (SC) or lacking uracil (SC-URA) or leucine (SC-LEU). SC contains yeast nitrogen base (YNB), 2% (wt/vol) glucose, and a mixture of all of the amino acids or a URA or LEU dropout powders. For starvation experiments, cultures were grown until midlog phase in SC, SC-URA, or SC-LEU, washed once with water, and then resuspended either on carbon starvation media (SC-C), which is the respective synthetic complete media without any carbon source, or on nitrogen-starvation media (SC-N) that contains of 0.17% of YNB without amino acids and ammonium sulfate and glucose at 2% (wt/vol). Rapamycin (Sigma) was added to midlog phase cultures growing on SC-URA at a final concentration of 200 ng/mL. For *GAL1* induction analysis, cultures were grown in SC-URA with 2% (wt/vol) glucose until midlog phase, washed, and resuspended in SC-URA with 2% (wt/vol) galactose. Cultures were harvested for protein extraction at the indicated time points. Optical densities at wavelength 600 nm (OD_{600}) were measured on a microplate reader (Tecan Infinite M200TM).

Microarray Analysis. WT, hsp31Δ, hsp32Δ, and hsp33Δ were harvested when cultures reached diauxic-shift (DS). RNA was obtained as described above for qRT-PCR analyses. Three independent RNA samples of each strain were used. RNA integrity was determined on the Agilent 2100 bioanalyzer. cDNA was synthetized from total RNA and amplified as fluorescent cRNA (cyanine 3-labeled CTP) using Agilent's Low Input Quick Amp Labeling Kit (one-color label) following the manufacturer's instructions. Labeled cRNA was purified and hybridized to Agilent yeast gene expression microarrays (containing 6,256 60-mer probes). Scanning was done on GenePix 4200A and normalization was performed in ArrayTrackTM using mean median scaling without background subtraction. WT expression profiles were compared individually to each knockout strain profile. Statistical significance was obtained using Welch's *t* test in ArrayTrack.

Immunoprecipitation and Mass Spectrometry. WT BY4741 cells expressing either GFP, Hsp31-GFP, or Hsp32-GFP were harvested at DS. The pellet was resuspended with immunoprecipitation buffer (50 mM Tris·HCl, pH 7.5, 0.5 mM, EDTA, 150 mM NaCl, 0.05% Tergitol solution Type Nonidet P-40). For immunoprecipitation, GFP Trap-A beads (Chromotek) were used, following manufacturer instructions. Samples were run on an SDS-polyacrylamide and were sent for mass spectrometry analysis at the proteomics facility at University Medical Center Göttingen, Germany.

Zymolyase Assay. Cells were collected by centrifugation and resuspended on Tris·HCl (10 mM) containing β-mercaptoethanol (40 mM) and 50 μg/mL of zymolyase-20T (Seikagaku). OD_{600} was measured every 10 min.

Cell Cycle Analysis. Cells in early stationary phase (SP) were fixed and stained with propidium iodide as previously described (7). Briefly, cells were pelleted and resuspended by vortexing in cold 70% (vol/vol) ethanol. After fixation, cells were washed and resuspended on sodium citrate pH 7, containing RNase A (Sigma) to a final concentration of 0.1 mg/mL. After 2 h incubation at 37 °C, cells were stained with propidium iodide (final concentration 4 μg/mL) and analyzed by flow cytometry on a BD LSR Fortessa and detected on the 575/26 nm channel. DNA content was quantified on FlowJo v9.6.2.

Survival Assays. Sensitivity to oxidative stress or thermotolerance was evaluated by spotting assays. Fresh colonies were grown in liquid YPD overnight, serially diluted, normalized to an OD_{600} of 0.1, and spotted on solid SC media containing hydrogen peroxide at different concentrations (1.6 mM and 2.3 mM). For heat shock, cultures were normalized by the number of cells and incubated at 50 °C at the indicated time points. For chronological lifespan (CLS) determination, cultures were grown on SC media containing 2% glucose, diluted back to synchronize their growth, and allowed to grow until cultures ceased dividing (beginning of SP). This time point was considered as day 0. Survival was measured by counting CFUs or by spotting assays (in the case of Fig. S1*F*) with 100% survival determined by the number of CFU or spot growth at day 0. CFU or spotting assays were performed by taking an aliquot of each culture at the indicated time points, serial diluting and plating or spotting the dilutions on YPD plates.

qRT-PCR. For oxidative stress treatments, liquid cultures of cells in midlog phase were treated with hydrogen peroxide (0.5 mM and 2 mM) for 1 h. Starvation was performed as described above for 1 h. After treatment, cells were harvested, pelleted, and stored at −80 °C until needed. RNA was extracted using Qiagen RNeasy Midi kits following the manufacturer's instructions. Genomic DNA contamination was removed from RNA using Turbo DNase (Ambion) according to the manufacturer's protocol. Next, 500 ng of total RNA was used as a template to synthesize cDNA with the Qiagen QuantiTect Reverse Transcription kit. To ensure that RNA had no genomic DNA contamination, a control reaction was included in which no reverse transcription was carried out. Primers used for qRT-PCR are listed in Table S6. Reactions were carried out using a LightCycler Real-Time PCR System (Roche) with the Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific). Primers were analyzed for specificity and efficiency. PCR efficiencies ranged from 0.80 to 1.0. Specificity was assessed by melting curve profile analysis; for *HSP32* and *HSP33* primers, because of their high homology, specificity was ensured by the absence of amplification when cDNA of the respective knockout strain was used as template. The crossing thresholds (CT) were calculated by the second derivative method using the LightCycler Relative Quantification Software. The relative quantification was corrected for PCR efficiency. TAF10 and UBC6 were used as housekeeping genes to normalize the cDNA input between samples. Three independent cDNA samples were analyzed. Statistical significance was determined using Student *t* test.

Stress Granule/P-Body Analysis. Cycloheximide was added 2 min before heat shock at 100 μg/mL. Treated and nontreated cells were washed twice and resuspended in fresh SC media lacking the appropriate amino acids. These cells were incubated at 46 °C for 30 °C and imaged immediately after heat shock. For glucose starvation, cells were washed twice and left with SC-C for 1 h. Glucose was readded to starved cells at 2% and imaged after 30 min. The percentage of cells with foci was scored with ImageJ software (http://rsbweb.nih.gov/ij/index.html). Brightness and contrast were adjusted with Adobe Photoshop when required.

Microarray Validation. Technical validation of the microarrays was performed by relative quantification of the expression of 11 genes by qRT-PCR, using as a template cDNA obtained from the RNA used for the microarrays. The qRT-PCR primers are listed in Table S6. Gene ontology was analyzed using the online DAVID Functional Annotation Tool (8) (http://david.abcc.ncifcrf.gov).

Immunoblotting, Immunoprecipitation, and Kinase Assays. At the indicated time points, the volume of cells equivalent to OD_{600} = 1.5 was collected and precipitated with TCA. Protein extracts were separated by SDS/PAGE, transferred to nitrocellulose membranes (Bio-Rad), followed by immunoblotting with α-GFP (University of California, Davis/National Institutes of Health NeuroMab Facility), α-HA (Santa Cruz Biotechnology), or α-Pgk1 (Invitrogen) antibodies. For HA-Atg13 immunoprecipitation, the volume corresponding to an OD_{600} = 7 was harvested and lysed as described in ref. 9. For log-phase samples, 10 optical densities were collected. The resulting protein extract was incubated with α-HA (Roche) followed by incubation with Protein G agarose beads (Invitrogen) with rotatory agitation at 4 °C. Beads were washed, resuspended on 2× loading buffer [200 mM Tris·HCl, 8% (wt/vol) SDS, 40% (vol/vol) glycerol, 0.4% bromophenol blue, 6% β-mercaptoethanol], boiled, and loaded on an SDS-polyacrylamide gel. After protein separation, the protein extracts were transferred to nitrocellulose membranes and blotted for phospho-(Ser/Thr) PKA substrate antibody (Cell Signaling). After incubation with the secondary antibody and exposure to X-ray film, the membrane was washed with a stripping solution (25 nM glycine hydrochloride, SDS 1%, pH 2) and incubated with α-HA (Santa Cruz Biotechnology). For phosphatase treatment, HA-Atg13 was immunoprecipitated and incubated with 200 units of λ phosphatase (New England Biolabs) for 1 h at 30 °C.

Measurement of Reactive Oxygen Species. To quantify reactive oxygen species (ROS), cells were incubated with 5 μM dihydroethidium (DHE) for 10 min and washed three times with PBS, or incubated with dihydrorhodamine 123 (DHR 123) at 15 μM for 90 min and washed three times with PBS, followed by staining with propidium iodide (500 μg/mL) to exclude dead cells. Positive cells were counted by flow cytometry.

Microscopy. Yeast cells were imaged on a Zeiss Axiovert 200M microscope using a 100× objective. Images were further processed using ImageJ software (http://rsbweb.nih.gov/ij/index.html). For colocalization studies, cells were imaged on a Zeiss LSM 710 microscope using a 63× 1.4 NA objective.

1. Wach A (1996) PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *S. cerevisiae*. *Yeast* 12(3):259–265.
2. Suzuki K, et al. (2001) The pre-autophagosomal structure organized by concerted functions of APG genes is essential for autophagosome formation. *EMBO J* 20(21): 5971–5981.
3. Cheong H, Nair U, Geng J, Klionsky DJ (2008) The Atg1 kinase complex is involved in the regulation of protein recruitment to initiate sequestering vesicle formation for nonspecific autophagy in *Saccharomyces cerevisiae*. *Mol Biol Cell* 19(2):668–681.
4. Araki T, Uesono Y, Oguchi T, Toh-E A (2005) LAS24/KOG1, a component of the TOR complex 1 (TORC1), is needed for resistance to local anesthetic tetracaine and normal distribution of actin cytoskeleton in yeast. *Genes Genet Syst* 80(5):325–343.
5. Brengues M, Parker R (2007) Accumulation of polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-bodies in *Saccharomyces cerevisiae*. *Mol Biol Cell* 18(7):2592–2602.
6. Buchan JR, Muhlrad D, Parker R (2008) P bodies promote stress granule assembly in *Saccharomyces cerevisiae*. *J Cell Biol* 183(3):441–455.
7. Costello G, Rodgers L, Beach D (1986) Fission yeast enters the stationary phase G0 state from either mitotic G1 or G2. *Curr Genet* 11:119–125.
8. Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44–57.
9. Miller-Fleming L, Cheong H, Antas P, Klionsky DJ (2014) Detection of *Saccharomyces cerevisiae* Atg13 by western blot. *Autophagy* 10(3):10.

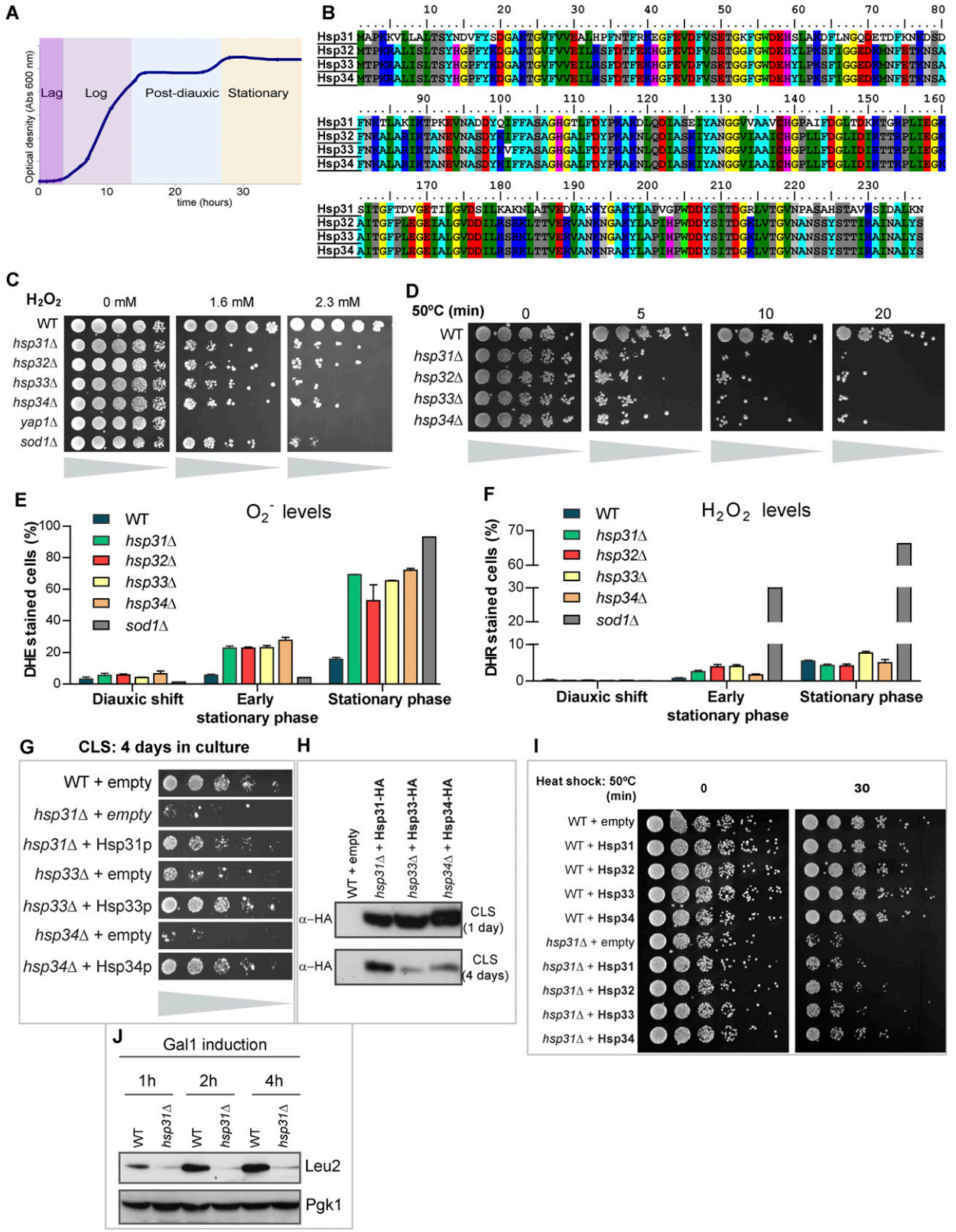

Fig. S1. *hsp31*Δ, *hsp32*Δ, *hsp33*Δ, and *hsp34*Δ strains are more sensitive to oxidative and heat stress, have reduced CLS, and accumulate higher levels of ROS. (*A*) Yeast growth in glucose-rich liquid culture. Yeast cells grown in glucose-rich media exhibit exponential division. Log phase: In this phase, cells obtain energy through fermentation and produce ethanol. When glucose becomes limiting (known as DS) cells enter postdiauxic phase, in which growth rate decreases. Cells readjust their metabolism to aerobic respiration and consequently exhibit higher levels of ROS. In parallel, cells up-regulate stress-response genes, induce autophagy, acquire thicker cell walls, accumulate trehalose, and exhibit decreased protein translation. Upon exhaustion of the available carbon source, cells stop proliferating by arresting their cell cycle in G0 and enter SP. (*B*) Alignment of the protein sequences of the yeast DJ-1/ThiJ/PfpI superfamily members:

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Hsp31, Hsp32, Hsp33, and Hsp34. Hsp32, Hsp33, and Hsp34 share the highest homology among the family members. Hsp32 shares ~99.6% protein identity with Hsp33, as they differ by only one amino acid. Hsp33 has two differing amino acids from Hsp34 (~99.2% amino acid identity) and Hsp34 differs in only one amino acid from Hsp32 (~99.6% identity). These three proteins are most diverged from Hsp31, sharing ~69% identity. (*C*) Midlog-phase cultures were normalized to an optical density at 600 nm of 0.2, serially diluted (fivefold), and spotted onto solid media containing various concentrations of hydrogen peroxide (H_2O_2), as shown. (*D*) Cultures were subjected to a heat shock of 50 °C for 5, 10, or 20 min and subsequently spotted onto YPD plates. (*E*) Superoxide levels were measured using the oxidation-sensitive dye DHE (*F*), and hydrogen peroxide levels were measured using the dye DHR 123. Stained cells were scored by flow cytometry. Dead cells were excluded before analysis based upon propidium iodide staining. (*G*) Overexpression of Hsp31, Hsp33, and Hsp34 complements reduced CLS in *HSP* gene-deletion strains. *hsp31*Δ, *hsp33*Δ, and *hsp34*Δ cells were transformed with a centromeric empty vector (p416GPD-HA) or with a vector encoding an HA-tagged version of the respective deleted protein (p416GPD/*HSP31*-HA, p416GPD/*HSP33*-HA and p416GPD/*HSP34*-HA). Cultures were grown on selective rich media (SC-URA) until reaching SP, considered day 0. An aliquot of each culture was serially diluted (fivefold) and spotted onto solid YPD media. A representative spotting plate after 4 d in culture is depicted. (*H*) *HSP31*, *HSP33*, and *HSP34* expression was confirmed after 1 and 4 d of growth in SP using an antibody against the HA tag. (*I*) Overexpression of either *HSP31*, *HSP32*, *HSP33*, or *HSP34* complements the lack of *hsp31*Δ thermotolerance. Cultures were grown until midlog phase and were subjected to a 50 °C shock for 30 min. A representative plate is shown. (*J*) GAL1 promotor repression is stronger in hsp31Δ cells. BY4741 and *hsp31*Δ cells were transformed with a vector expressing Leu2 under control of the GAL1 promoter (BG1805). The strains were grown until midlog phase in media containing glucose (GAL1 repression) and then shifted to media containing galactose (GAL1 induction). Cultures were retrieved after 1, 2, and 4 h of growth in galactose.

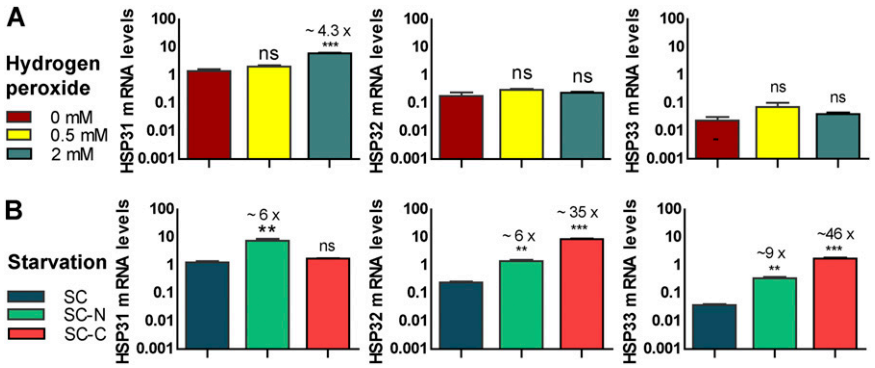

Fig. S2. Expression levels of *HSP31*, *HSP32*, and *HSP33* under oxidative stress and starvation conditions. Parental strain BY4741 was grown until midlog phase on synthetic complete media (SC) and was incubated with (*A*) hydrogen peroxide at two different concentrations (*B*) or starved for carbon (SC-C) or nitrogen (SC-N); mRNA was isolated and gene expression levels were quantified via qRT-PCR and normalized relative to *TAF10* and *UBC6* expression. Error bars represent SD of three independent experiments. Statistical analysis was performed using Student *t* test (***$P < 0.001$; **$P < 0.01$; NS, not significant).

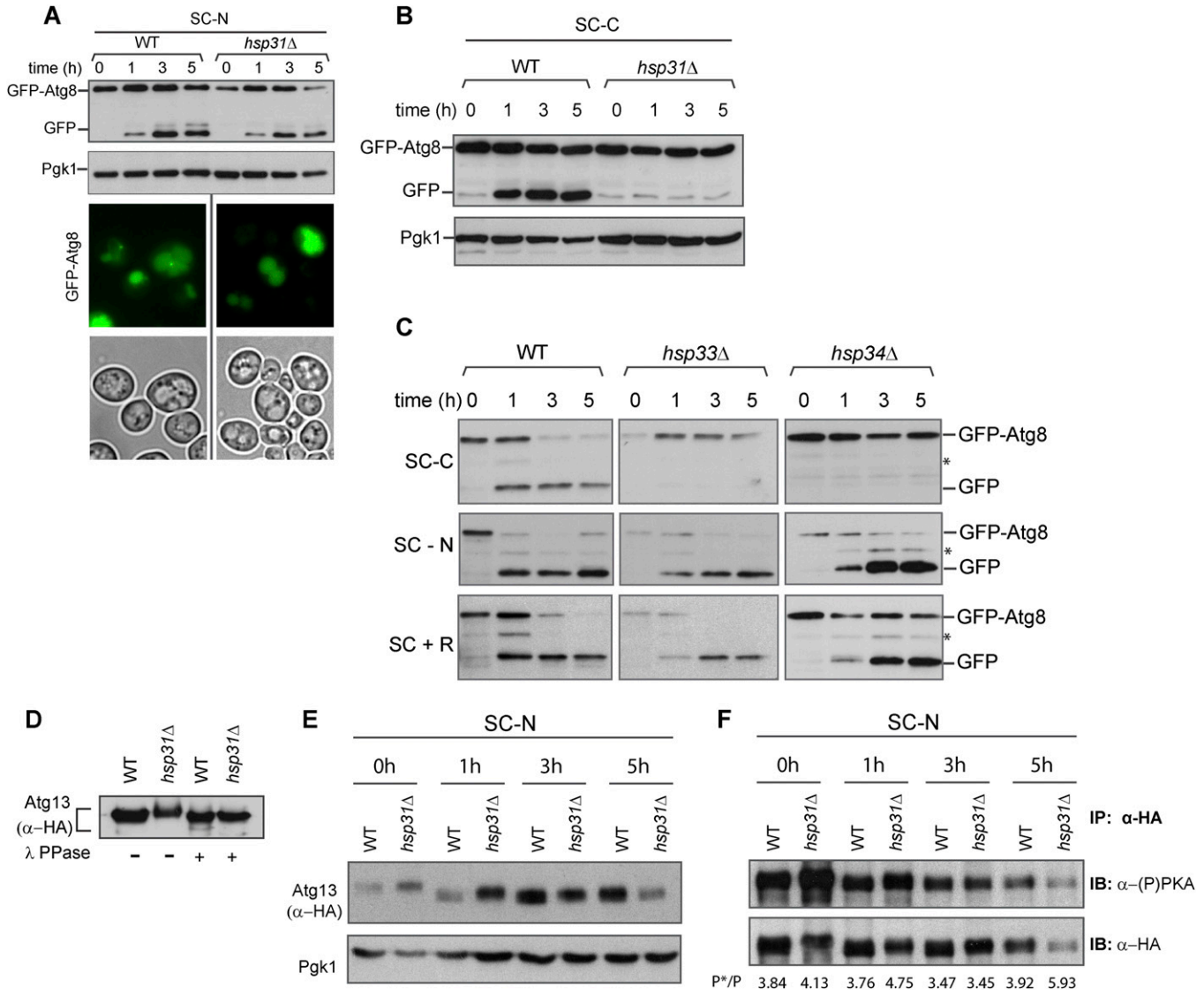

Fig. S3. The absence of Hsp31 minifamily members impairs autophagy. (*A–C*) Autophagy induction in hsp31Δ, hsp33Δ, and hsp34Δ strains requires a carbon source. WT, *hsp31*Δ, *hsp33*Δ, and *hsp34*Δ strains expressing GFP-Atg8 were grown until midlog phase and starved for carbon (SC-C) or nitrogen (SC-N) or treated with rapamycin (SC + R) for the specified time. Total lysates were analyzed by immunoblotting using antibodies against GFP or a housekeeping control Pgk1. (*A*) After 5-h nitrogen starvation, GFP-Atg8 localization was analyzed by fluorescence and light microscopy. (Magnification: 100×.) (*B*) A longer exposure of the film for the immunoblot shown in Fig. 3*A* in carbon starvation (SC-C) conditions is shown. (*D–F*) Analysis of Atg13 phosphorylation. (*D*) Differences in the electrophoretic mobility of HA-Atg13 from SP are a result of its phosphorylation. HA-Atg13 from SP cell lysates was immunoprecipitated with α-HA and treated with λ-phosphatase (Ppase) and then run on SDS-polyacrylamide gel to analyze its migration. (*E* and *F*) Yeast midlog-phase cultures were starved for nitrogen and harvested at different time points. (*E*) Migration of HA-Atg13 in SDS-polyacrylamide gels was assessed using an antibody against HA. An antibody for Pgk1 was used as a control for protein loading. (*F*) Levels of PKA activity are similar in WT and *hsp31*Δ cells upon starvation. Atg13-HA was immunoprecipitated with α-HA antibody and levels of PKA phosphorylation were assessed. The numbers below the immunoblots indicate the ratio between phosphorylated Atg13 and total Atg13.

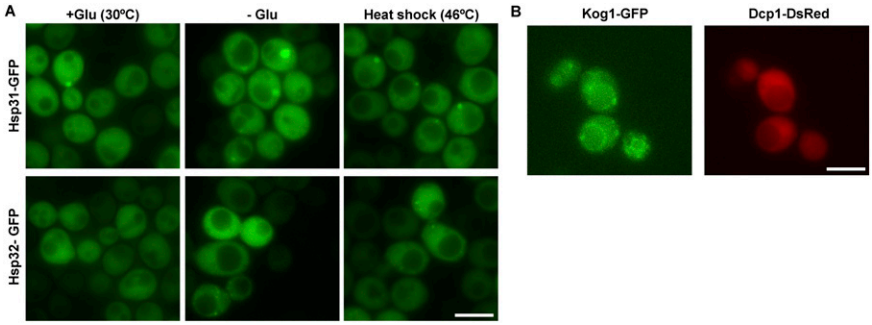

Fig. S4. Subcellular localization of Hsp31-GFP, Hsp32-GFP, Kog1-GFP, and DCP1-DsRed. (*A*) WT cells expressing Hsp31-GFP or Hsp32-GFP in midlog phase (+Glu) were starved for glucose 1 h (−Glu) or incubated at 46 °C for 30 min. (*B*) Subcellular localization of Kog1-GFP and Dcp1-DsRed in midlog phase. (Scale bar, 5 μm.)

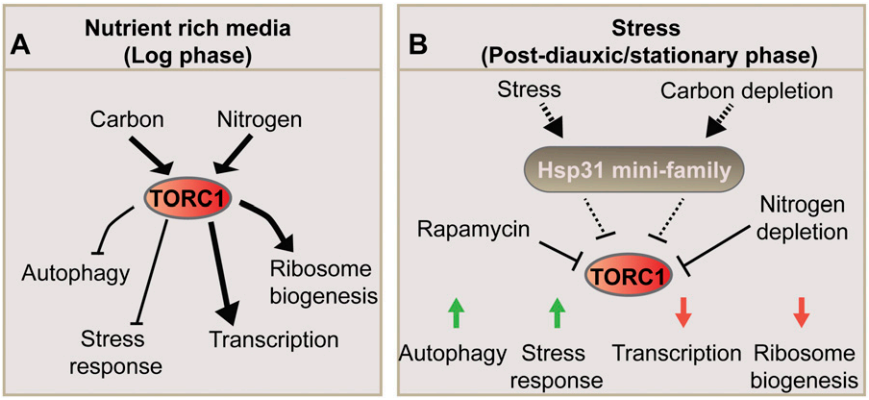

Fig. S5. Hsp31 minifamily members modulate target of rapamycin complex 1 (TORC1) activity. (*A*) Yeast cells grow exponentially (log phase) in the presence of nutrient-rich media containing glucose. TORC1, a conserved kinase, positively regulates processes that contribute to growth, such as ribosome biogenesis and transcription, and represses processes related to stress, including autophagy. (*B*) Upon stress, treatment with rapamycin, or nutrient deprivation (postdiauxic or SP), the activity of TORC1 decreases and, consequently autophagy and stress genes are induced. In parallel, ribosome biogenesis and transcription are repressed.

Table S1. Validation of microarrays by qRT-PCR

	hsp31Δ vs. WT			*hsp32Δ* vs. WT			*hsp33Δ* vs. WT		
	Microarrays	qRT-PCR		Microarrays	qRT-PCR		Microarrays	qRT-PCR	
Gene	Fold-change	Fold-change	*P* value	Fold-change	Fold-change	*P* value	Fold-change	Fold-change	*P* value
SOL4	0.14	0.13	0.02	0.04	0.07	0.02	0.12	0.09	0.02
SSA3	0.12	0.11	0.00	0.14	0.11	0.01	0.12	0.10	0.01
GTT1	0.24	0.18	0.00	0.08	0.08	0.01	0.15	0.15	0.01
TRX3	0.42	0.48	0.03	0.41	0.37	0.01	0.33	0.35	0.01
OM45	NC	0.24	0.01	0.26	0.09	0.01	0.36	0.16	0.00
POT1	0.15	0.20	0.01	0.04	0.05	0.02	0.08	0.07	0.01
HXK2	5.46	7.31	0.00	6.08	6.58	0.00	6.66	20.23	0.12
MIG2	6.49	4.50	0.26	8.38	2.84	0.00	7.94	6.27	0.29
HXT4	5.74	9.10	0.02	5.70	5.94	0.01	4.97	7.80	0.01
RPS24A	2.91	3.02	0.00	3.42	3.77	0.05	3.02	1.90	0.03
HEM13	NC	6.73	0.01	7.25	25.21	NS	4.43	7.42	NS

Eleven differentially expressed genes (DEGs) found in both the gene expression microarrays of *hsp32*Δ and hsp33Δ strains were validated by qRT-PCR. Among these 11 genes, 9 were also differentially expressed in *hsp31*Δ cells. The table indicates the fold-change obtained by microarray and qRT-PCR analyses. In addition, it shows the SD and *P* value obtained by qRT-PCR. (NC, genes that were not changed; NS, statistically not significant).

Table S2. Genes down-regulated in *hsp31*Δ, *hsp32*Δ, and *hsp33*Δ versus WT cells

Standard name	Systematic name	*Saccharomyces* Genome Database description (summarized)
Gluconeogenesis		
TDH3	*YGR192C*	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3; involved in glycolysis and gluconeogenesis
TDH1	*YJL052W*	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 1; involved in glycolysis and gluconeogenesis
TDH2	*YJR009C*	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 2; involved in glycolysis and gluconeogenesis
PCK1	*YKR097W*	Phosphoenolpyruvate carboxykinase, key enzyme in gluconeogenesis
FBP1	*YLR377C*	Fructose-1,6-bisphosphatase, key regulatory enzyme in the gluconeogenesis pathway
MDH2	*YOL126C*	Cytoplasmic malate dehydrogenase, involved in the glyoxylate cycle and gluconeogenesis during growth on two-carbon compounds
Reserve carbohydrate biosynthesis		
Glycogen biosynthetic process		
GLC3	*YEL011W*	Glycogen branching enzyme, involved in glycogen accumulation
GLG2	*YJL137C*	Glycogenin glucosyltransferase; self-glucosylating initiator of glycogen synthesis
GSY2	*YLR258W*	Glycogen synthase; expression induced by glucose limitation, nitrogen starvation, heat shock, and SP
PGM2	*YMR105C*	Phosphoglucomutase; catalyzes the conversion from glucose-1-phosphate to glucose-6-phosphate
GAC1	*YOR178C*	Regulatory subunit for Glc7p type-1 protein phosphatase (PP1), tethers Glc7p to Gsy2p glycogen synthase
GDB1	*YPR184W*	Glycogen debranching enzyme; contains glucanotranferase and alpha-1,6-amyloglucosidase activities; required for glycogen degradation
Trehalose biosynthetic process		
ATH1	*YPR026W*	Acid trehalase required for utilization of extracellular trehalose
HSP104	*YLL026W*	Disaggregase; heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70)
NTH1	*YDR001C*	Neutral trehalase, degrades trehalose
NTH2	*YBR001C*	Putative neutral trehalase, required for thermotolerance
PGM2	*YMR105C*	Phosphoglucomutase; catalyzes the conversion from glucose-1-phosphate to glucose-6-phosphate
TPS1	*YBR126C*	Synthase subunit of trehalose-6-P synthase/phosphatase complex; synthesizes the storage carbohydrate trehalose
TSL1	*YML100W*	Large subunit of trehalose 6-phosphate synthase/phosphatase complex; Tps1p-Tps2p complex converts uridine-5′-diphosphoglucose and glucose 6-phosphate to trehalose

Table S3. DEGs involved in glucose transport and metabolism

Gene	*Saccharomyces* Genome Database description	*hsp31*Δ vs WT (fold-change)	*hsp32*Δ vs WT (fold-change)	*hsp33*Δ vs WT (fold-change)
HXT1	Low-affinity glucose transporter; induced by Hxk2p in the presence of glucose and repressed by Rgt1p when glucose is limiting	—	2.07	—
HXT3	Low-affinity glucose transporter; induced in low or high glucose conditions	3.19	3.44	4.29
HXT4	High-affinity glucose transporter, induced by low levels of glucose and repressed by high levels of glucose	5.74	5.7	5.17
HXT5	Moderate affinity for glucose, induced in the presence of non-fermentable carbon sources, induced by a decrease in growth rate	−3.8	−11.4	−6.33
MIG2	Zinc finger transcriptional repressor; cooperates with Mig1p in glucose-induced repression of many genes	6.49	8.38	7.94
HXK2	catalyzes phosphorylation of glucose in the cytosol; predominant hexokinase during growth on glucose; functions in the nucleus to repress expression of HXK1 and GLK1 and to induce expression of its own gene	5.46	6.08	6.66
GLK1	Glucokinase, catalyzes the phosphorylation of glucose at C6 in the first irreversible step of glucose metabolism; one of three glucose phosphorylating enzymes; expression regulated by non-fermentable carbon sources	—	−5.00	−3.34
RGT1	Glucose-responsive transcription factor that regulates expression of HXT genes in response to glucose; binds to promoters and acts both as a transcriptional activator and repressor	—	−2.61	−2.13
NRG2	Transcriptional repressor that mediates glucose repression and negatively regulates filamentous growth	3.32	2.82	3.32
SKS1	Putative serine/threonine protein kinase; involved in the adaptation to low concentrations of glucose independent of the SNF3 regulated pathway	3.24	6.89	3.78
STD1	Protein involved in control of glucose-regulated gene expression; interacts with kinase Snf1p, glucose sensors Snf3p and Rgt2p, TATA-binding Spt15p; regulator of transcription factor Rgt1p	—	3	2.22
SNF3	Plasma membrane low glucose sensor that regulates glucose transport; contains 12 predicted transmembrane segments and a long C-terminal tail required for induction of hexose transporters; also senses fructose and mannose; similar to Rgt2p	—	−2.36	—

Table S4. Components of stress granules and P-bodies identified in association with Hsp31 and Hsp32

Proteins	mRNP granule	Source
Proteins associated with Hsp31		
TIF4631/eiF4G1	SG or Pb	(1)
RPS23B (40S ribosomal subunit)	SG	(2, 3)
RPL34A (40S ribosomal subunit)	SG	(2, 3)
RPS26B (40S ribosomal subunit)	SG	(2, 3)
RPS9B (40S ribosomal subunit)	SG	(2, 3)
RPG1 (eIF3 subunit)	SG	(4)
TIF11 (eIF1A)	SG	(4)
Proteins associated with Hsp32		
Ygr250c	SG	(5)
Pbp1	SG	(5)
Pab1	SG or Pb	(1)
Tif35 (eIF3 subunit)	SG	(2)
Proteins associated with Hsp31 and Hsp32		
RPS28B (40S ribosomal subunit)	SG	(2)

Pb, P-body; RNP, RNA-protein; SG, stress granule.

1. Brengues M, Parker R (2007) Accumulation of polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-bodies in *Saccharomyces cerevisiae*. *Mol Biol Cell* 18(7):2592–2602.
2. Grousl T, et al. (2009) Robust heat shock induces eIF2alpha-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast, *Saccharomyces cerevisiae*. *J Cell Sci* 122(Pt 12):2078–2088.
3. Buchan JR, Parker R (2009) Eukaryotic stress granules: The ins and outs of translation. *Mol Cell* 36(6):932–941.
4. Buchan JR, Yoon J-H, Parker R (2011) Stress-specific composition, assembly and kinetics of stress granules in *Saccharomyces cerevisiae*. *J Cell Sci* 124(Pt 2):228–239.
5. Buchan JR, Muhlrad D, Parker R (2008) P bodies promote stress granule assembly in *Saccharomyces cerevisiae*. *J Cell Biol* 183(3):441–455.

Table S5. Strains used in this study

Strain	Genotype	Source
BY4741	MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0	Open Biosystems
BY4742	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0	Open Biosystems
*hsp32*Δ 2n	MATa/α his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0/*LYS2* met15Δ0/*MET15* can1Δ::*LEU2*-MFA1 pr-HIS3/CAN1+ hsp32::URA3MX/*HSP32*	Open Biosystems
*hsp31*Δ	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hsp31::kanMX4	(1)
*hsp32*Δ	MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 met15Δ0 can1Δ::*LEU2*+-MFA1pr-*HIS3* hsp32::*URA3*MX	Present study
*hsp33*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hsp33::NatMX	Present study
*hsp34*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hsp34::HygMX	Present study
Lx Wild-type	MATa ura3Δ0 leu2Δ0 lys2Δ0 his3Δ1 can1Δ::*LEU2*-MFA1pr-*HIS3*	Present study
Lx1 (*hsp31*Δ)	MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::*LEU2*-MFA1pr-*HIS3* hsp31::kanMX4	Present study
Lx2 (*hsp32*Δ)	MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::*LEU2*-MFA1pr-*HIS3* hsp32::URA3MX	Present study
Lx3 (*hsp33*Δ)	MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ ura3Δ0 can1Δ::*LEU2*-MFA1pr-*HIS3* hsp33::NatMX	Present study
Lx4 (*hsp34*Δ)	MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::*LEU2*-MFA1pr-*HIS3* hsp34::HygMX	Present study
*gis1*Δ	MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 gis1::kanMX4	Open Biosystems
*sod1*Δ	MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 sod1::kanMX4	Open Biosystems
*yap1*Δ	MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 yap1::kanMX4	Open Biosystems
W303	MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+]	Open Biosystems

1. Skoneczna A, Miciałkiewicz A, Skoneczny M (2007) *Saccharomyces cerevisiae* Hsp31p, a stress response protein conferring protection against reactive oxygen species. *Free Radic Biol Med* 42(9):1409–1420.

Table S6. Primers used in this study

Primer	Forward sequence (5′ to 3′)	Reverse sequence (5′ to 3′)	Comments
HSP33 flanking regions	ACACTTTTCTCCTTCATTCAAAA-AGAAAACTGGCCTTGCACGT-ACGCTGCAGGTCGAC	CATTAGAAGCCTCCCCTTCAACAATTCCCT-TGTCTTTTGTTATCGATGAATTCGAGCTCG	Excision of *HSP33* or *HSP34*
GIS1	TAACACCTCTGCAGCCGCATCTAT	TTGTCATCGTTATCGCCGCCTAGA	qRT-PCR
HSP31	ATCTGCAGGCCACGGTACCTTATT	TGCGACAACACCACCGTTAGCATA	qRT-PCR
HSP32	TTTTGCGGTCGTTCGATACT	GGTCCATGACAGATGGCA	qRT-PCR
HSP33	ATTTTGCGATCGTTCGATACA	GGTCCATGACAGATGGCA	qRT-PCR
TAF10	ATATTCCAGGATCAGGTCTTCCGTAGC	GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG	qRT-PCR - control
UBC6	AGCAGGCTCACAAGAGATTGACGA	TACCGTGATATTGACCGCCCTTGT	qRT-PCR - control
GTT1	TGGCAGACAAGATCAGTCAGGCAT	CCTCTGGTGCGGCAAACTTTCTTT	qRT-PCR - validate microarrays
HEM13	AAACTTGGTGGTTTGGTGGTGGTG	GCATCCTTGTGCAGTTGGTGGAAT	qRT-PCR - validate microarrays
HXK2	GGCCGATGTGCCAAAGGAATTGAT	TACCACCCTTCTTGGACAAACCCT	qRT-PCR - validate microarrays
HXT4	TAGGTCTAGGTTTCGCTTGGGCTT	TGGATCGTCTGCGCTGACCTTATT	qRT-PCR - validate microarrays
MIG2	CATTCATGCCTCCGCTGCATCTTT	TTGGCACAGAGCGTGGAGTGTTTA	qRT-PCR - validate microarrays
OM45	AAACAGCCGCTCAACTTTCCAAGG	ACTGTTTCAAGTCCTCCTTCGCCT	qRT-PCR - validate microarrays