Supplementary Materials:

Materials and Methods

Figures S1-S8

Materials and Methods:

Strains

All strains were maintained as described previously (*29*). The following strains were used in this work: wild-type (N2), *hsf-1*(sy441), RW1596 (*myo-3*(st386); stEx30(*myo-3p*::GFP; *rol-6*(su1006))), GS1912 (arIs37(*myo-3*p::ssGFP); *dpy-20*(e1282)), AGD710 (uthIs235(*sur-5*p::*hsf-1*; *myo-2*p::tdTomato)), AGD794 (*hsf-1*(sy441); uthIs225(*sur-5*p::*hsf-1(CT)*; *myo-2*p::tdTomato)), AGD864 (uthEx627(*sur-5*p::*hsf-1(CT-DBD*Δ*)*; *myo-2*p::tdTomato)), AGD1101 (uthIs372(*sur-5*p::*pat-10*; *myo-2*p::tdTomato)), AGD1224 (arIs37(*myo-3*p::ssGFP); uthIs372(*sur-5*p::*pat-10*; *myo-2*p::tdTomato)), AGD1227 (stEx30(*myo-3*p::GFP; *rol-6*(su1006)); uthIs372(*sur-5*p::*pat-10*; *myo-2*p::tdTomato)).

Wild-type (N2), *hsf-1*(sy441), RW1596 (*myo-3*(st386); stEx30(*myo-3*p::GFP; *rol-6*(su1006))), GS1912 (arIs37(*myo-3*p::ssGFP); *dpy-20*(e1282)) strains were obtained from the *Caenorhabditis* Genetics Center (Minneapolis, MN).

For generation of overexpression strains the all-tissue promoter *sur-5* was used. *hsf-1(CT)* was made by truncating the gene product with an early stop codon after amino acid 587. The *hsf-1(CT-DBD*Δ*)* was made by removing amino acids 56-108 from the *hsf-1(CT)* plasmid using the restriction enzyme MfeI (New England Biolabs). Full length *hsf-1* and *pat-10*, as well as *hsf-1(CT)* and *hsf-1(CT-DBD*Δ*)* DNA plasmid constructs were injected at 50 ng/µl along with a co-injection marker (*myo-2p*::tdTomato) at 10 ng/µl to make transgenic overexpression worms.

Western Blot Analysis

Age synchronized worms were cultivated on nematode growth (NG) plates containing OP50 bacteria at 20°C until day 1 adulthood. Worm were washed off the plate with M9 buffer pre-heated to 34°C, collected and incubated in a 34°C water bath for 15 mins. Worms were centrifuged at 1000 x g for 30 sec and move back to NG plates seeded with OP50 bacteria at 20°C. Worms were allowed 1.5 hrs of recovery at 20°C before worms were collected and frozen in liquid nitrogen for further processing.

Worm extracts were generated by glass bead disruption in non-denaturing lysis buffer [150 mM NaCl, 50 mM Hepes at pH 7.4, 1mM EDTA, 1% Triton X100, protease inhibitor cocktail III (Calbiochem)]. Crude lysates were subject to centrifugation at 10,000 x g at 4°C for 5 mins. The supernatant was supplemented with 2x SDS sample buffer containing [50mM Tris-Cl at pH 6.8, 2 mM EDTA, 4% glycerol, 2% SDS, Coomassie Blue, protease inhibitor cocktail III (Calbiochem)]. Samples were boiled for 15 mins and resolved by SDS-PAGE. Proteins levels were monitored by standard immuno- blotting procedures with α-Hsp-16.2 (kind gift from Lithgow Lab) and α-tubulin (Sigma T6074) antibodies.

Transcript Analysis

Total RNA was isolated from synchronized populations at day 1 of adulthood using Qiazol (Qiagen) and then further purified with the RNeasy mini kit (Qiagen). cDNA was synthesized using the QuantiTect kit (Qiagen). SybrGreen was used for quantitative PCR as described in the SsoAdvanced SYBR Green Supermix protocol (BioRad). Experiments were repeated with three biological repeats and analyzed using the comparative Ct method. Internal controls utilized a geometric mean of *cdc-42*, *pmp-3* and *Y45F10D.4*, which were determined to be optimal control genes for worm qPCR (*30*). The Roche Universal ProbeFinder online tool was used to design primers. Primer sequences as follows:

cdc-42 forward 5'- AGGAACGTCTTCCTTGTCTCC -3'
cdc-42 reverse 5'- GGACATAGAAAGAAAAACACAGTCAC -3'
pmp-3 forward 5'- CGGTGTTAAAACTCACTGGAGA -3'
pmp-3 reverse 5'- TCGTGAAGTTCCATAACACGA -3'
Y45F10D.4 forward 5'- AAGCGTCGGAACAGGAATC -3'
Y45F10D.4 reverse 5'- TTTTTCCGTTATCGTCGACTC -3'
hsf-1 forward 5'- TTTGCATTTTCTCGTCTCTGTC -3'
hsf-1 reverse 5'- TCTATTTCCAGCACACCTCGT -3'
hsp-16.2 forward 5'- TCCATCTGAGTCTTCTGAGATTGTTA -3'
hsp-16.2 reverse 5'- TGGTTTAAACTGTGAGACGTTGA -3'
hsp-17 forward 5'- TAACCATGGCCGCAGATT -3'
hsp-17 reverse 5'- TTCACAACATCAATAGCATCTCC -3'
hsp-70a (C12C8.1) forward 5'- CGGTATTTATCAAAATGGAAAGGTT -3'
hsp-70a (C12C8.1) reverse 5'- TACGAGCGGCTTGATCTTTT -3'
hsp-70b (F44E5.4) forward 5'- TGCACCAATCTGGACAATCT -3'
hsp-70b (F44E5.4) reverse 5'- TCCAGCAGTTCCAGGATTTC -3'
pat-10 forward 5'- TCGAGGAGTTCTGGGAGTTG -3'
pat-10 reverse 5'- TTGTAGATCAGCGATTTTAAAGGA -3'
gfp forward 5'- CCACATGGTCCTTCTTGAGTTT -3'
gfp reverse 5'- ATAGTTCATCCATGCCATGTGTA -3'

RNA for sequencing analysis was prepared using Illumina TruSeq RNA Sample Prep Kit (Illumina). Paired-end sequencing was performed on an Illumina HiSeq 2000 and data was analyzed with CLC Genomics Workbench 6.5 software. Transcript abundance was normalized by reads per kilobase of transcript per million sequencing reads (RPKM) for each condition or genotype.

Lifespan analysis

Lifespan experiments were conducted at 20ºC as previously described (*31*). Lifespans were performed on worms fed OP50 *E. coli* or HT115 for RNAi, using the pre-fertile period of adulthood as day 0. Worms were transferred to fresh play every second day until day 12. Prism 6 software was used for statistical analysis to determine significance calculated using the log-rank (Mantel-Cox) method.

Thermotolerance assay

Synchronized day 1 adult worms were placed at 34ºC for 13 hrs on plates spotted with OP50 *E. coli* or HT115 for RNAi. Worms were then scored for viability. At least 80 worms were

used per genotype and experiments were repeated at least three times. Prism 6 software was used for statistical analysis.

RNAi feeding

Worms were fed from hatch HT115 *E. coli* containing an empty vector control or expressing double-stranded RNA. RNAi strains were taken from the Vidal library if present, or the Ahringer library if absent from the Vidal library.

Microarray Data Analysis

Raw expression data files were obtained for three replicates each of N2 (N), *hsf-1(sy441)* (S), *hsf-1(CT)* (Y), *hsf-1(FL)* (F) with the Affymetrix *C. elegans* Genome Array. A further three replicates for each of these genotypes were obtained under heat shock conditions. All microarray analysis was performed with Bioconductor (*32*). Standard data quality validation as suggested by Affymetrix was carried out with the 'simpleaffy' package, followed by 'affyPLM', which identified no problematic chips. The raw data were preprocessed according to the GC-RMA method (*33*) (implemented in 'gcrma'), which performs probe sequence based background adjustment, quantile normalization, and utilizes a robust multi-chip average to summarize information into single expression measurements for each probeset (exprs.xlsx). Prior to statistical testing, the data were submitted to a non-specific filter (via the package 'genefilter') that removed probesets with an expression interquartile range smaller than 0.5. To identify genes that were significantly differentially expressed between each mutant and its control and between each heat shock and its control, linear modeling and empirical Bayes analysis was performed using the 'limma' package (*34*). Limma computes an empirical Bayes adjustment for the t-test (moderated t-statistic), which is more robust than the standard two-sample t-test comparisons. To correct for multiple testing Benjamin and Hochberg's method to control for false discovery rate was employed (*35*). Genes with an adjusted p-value of 0.05 or smaller and a fold-change in expression larger than 2-fold were considered differentially expressed (PD110713_de_annot.xlsx).

Proteomic Data Analysis

Deionized water (Barnstead) was used for all preparations. Buffer A consists of 5% acetonitrile 0.1% formic acid, buffer B consists of 80% acetonitrile 0.1% formic acid, and buffer C consists of 500 mM ammonium acetate and 5% acetonitrile. Worms were placed in Clontech buffer, lysed using Precellys (Bertin Technologies) and centrifuged for 20 s at 18000 x g. Protein concentration of supernatants were determined by BCA (Pierce). 50 µg each of light and heavy proteins were mixed and brought to 200 ul with water. Proteins were precipitated with 60 µl TCA (Sigma-Aldrich) at 4ºC overnight. After 30 mins centrifugation at 18000 x g, protein pellets were washed 2 times with 500 µl ice-cold acetone. Air-dried pellets were dissolved in 8 M urea/ 100 mM Tris pH 8.5. Proteins were reduced with 5 mM Tris(2-carboxyethyl)phosphine hydrochloride (Sigma-Aldrich) and alkylated with 10 mM iodoacetamide (Sigma-Aldrich). Proteins were digested for 18 hr at 37ºC in 2 M urea 100 mM Tris pH 8.5, 1 mM $CaCl_2$ with 2 µg trypsin (Promega). Digestion was stopped with formic acid, 5% final concentration. Debris was removed by centrifugation, 30 mins 18000 x g.

A MudPIT microcolumn (*36*) was prepared by first creating a Kasil frit at one end of an undeactivated 250 µm ID/360 µm OD capillary (Agilent Technologies). The Kasil frit was prepared by briefly dipping a 20 - 30 cm capillary in well-mixed 300 µl Kasil 1624 (PQ

Corporation) and 100 µl formamide, curing at 100ºC for 4 hrs, and cutting the frit to ~2 mm in length. Strong cation exchange particles (SCX Luna, 5 µm dia., 125 Å pores, Phenomenex) were packed in-house from particle slurries in methanol to 2.5 cm. An additional 2.5 cm eversed phase particles (C18 Aqua, 3 µm dia., 125 Å pores, Phenomenex) were then similarly packed into the capillary to create a biphasic column. An analytical RPLC column was generated by pulling a 100 µm ID/360 µm OD capillary (Polymicro Technologies) to 5 µm ID tip. Reversed phase particles (Aqua C18, 3 µm dia., 125 Å pores, Phenomenex) were packed directly into the pulled column at 800 psi until 12 cm long. The MudPIT microcolumn was connected to an analytical column using a zero-dead volume union (Upchurch).

LC-MS/MS analysis was performed using an Eksigent nano lc pump and a Thermo LTQ-Orbitrap using an in-house built electrospray stage. MudPIT experiments were performed where each step corresponds to 0, 30, 50, 70 and 100% buffer C being run for 4 mins at the beginning of each gradient of buffer B. Electrospray was performed directly from the analytical column by applying the ESI voltage at a tee (150 µm ID, Upchurch Scientific).Electrospray directly from the LC column was done at 2.5 kV with an inlet capillary temperature of 250ºC. Data-dependent acquisition of MS/MS spectra with the LTQ -Orbitrap were performed with the following settings: MS/MS on the 6 most intense ions per precursor scan, 1 microscan, unassigned and charge state 1 reject; dynamic exclusion repeat count, 1, repeat duration, 30 second; exclusion list size 500; and exclusion duration, 180 second.

Protein and peptide identification and protein quantitation were done with Integrated Proteomics Pipeline - IP2 (Integrated Proteomics Applications). Tandem mass spectra were extracted from raw files using RawExtract 1.9.9 (*37*) and were searched against Wormbase protein database (WP180) with reversed sequences using ProLuCID (*38*, *39*). The search space included all fully-tryptic peptide candidates. Carbamidomethylation (+57.02146) of cysteine was considered as a static modification. Peptide candidates were filtered using DTASelect, with these parameters –p 1 –y 1 –trypstat –DM 10 –in (*37*, *40*). Quantitation was performed using Census (*41*).

Gene Ontology analysis

Candidate genes were annotated with the GO (*42*) Biological Process Ontology and clustered by shared GO terms using the DAVID web server (*43*). Processes and process clusters with fold enrichment larger than 1 and p-value smaller than 0.05 (using Fisher's exact test p-value for single terms, and the geometric mean of individual terms' p-values for the clusters) were considered significantly enriched.

Binding Site analysis

We scanned the promoter region of *pat-10* (up to 5kb) and its first intron for matches to all HSF position weight matrices in Genomatix's MatBase (*7*, *8*) with the MatInspector program (*44*).

Microscopy and Fluorescence analysis

For fluorescence microscopy, worms were anesthetized with 10nM sodium azide and images were captured using a Leica DM6000 B microscope and Hamamatsu ORCA-ER camera. We also used a COPAS Biosort (Union Biometrica) to measure individual day 1 worm length, width and GFP fluorescence. At least 500 worms were measured per genotype and pooled in

three biological replicates. We normalized fluorescence by worm size to compare between genotypes.

FM 4-64FX dye was used as previously described (*18*). Briefly, worms were soaked in 0.4 mM FM 4-64FX dye (Molecular Probes) for 1 hr, washed, immobilized with 100 µg/ml levamisole and analyzed b fluorescence microscopy.

Motility Assays

Synchronized day 1 adult worms were placed at 34ºC for 9 hrs on plates spotted with OP50 *E. coli*. Worms were then allowed to recover for 12 hrs. Ten worms were then placed in a 30 µl drop of M9 buffer solution on an unseeded plate. Worms were then filmed for 20 seconds. Body bends were counted for each worm and averaged with the group. Three total biological replicates were performed for each genotype. Prism 6 software was used to assess statistical significance.

Actin Assembly Assay

Age synchronized worms were cultivated on 10 cm nematode growth (NG) plates containing OP50 bacteria at 20°C. For heat shock, stacked worm plates were shifted to 34°C for 8 hrs. Worms were washed off the plates with 34°C pre-heated M9 media and washed twice before worm pellets were collected and frozen in liquid nitrogen. Worm extracts were generated as described in the western blot sections and subject to 150,000 x g ultracentrifugation for 30 mins. Supernatant and pellet fractions were collected and resolved by SDS-PAGE. Standard western blotting procedures were used with α-actin antibody (Abcam ab3280).

Cell Culture Microscopy and Thermotolerance Assay

HEK293T cells were treated for 1 hr with 0.5µg/mL Latrunculin A (Cayman Chemical) or Cytochalasin D (Santa Cruz Biotechnology), both diluted in ethanol or only ethanol for the control. Formaldehyde fixed cells were treated with 5µM Alexa Fluor 568 Phalloidin (Life Technologies) to stain for actin for 20 min. Mounting buffer containing DAPI was used for final imaging.

For assaying thermotolerance, HEK293T cells were plated on 0.1% gelatin coated 24-well plate so they can reach 80-90% confluence the day of the experiment. Cells are grown in 37°C incubators, 5% CO_2. Cells were treated for 1 hr with 0.5µg/mL Latrunculin A (Cayman Chemical) or Cytochalasin D (Santa Cruz Biotechnology), both diluted in ethanol or only ethanol for the control. Cells were then transferred to a 45°C incubator for 2 hrs for the heat shock. Cells were collected, spun down 1 mins at 200g and resuspended in PBS. PBS containing Sytox Green (Invitrogen) was added to the cells reaching a final concentration of 1µM. Sytox Green binds the DNA of cells with compromised membrane and fluoresces. Cells were incubated at room temperature for 15 mins and analyzed using BD Fortessa cell analyzer. The percentage of dead vs. live cells was determined using density plot for the FIT-C channel. Two peaks were observed: one with very low signal and another at higher values corresponding to the uptake of the Sytox Green.

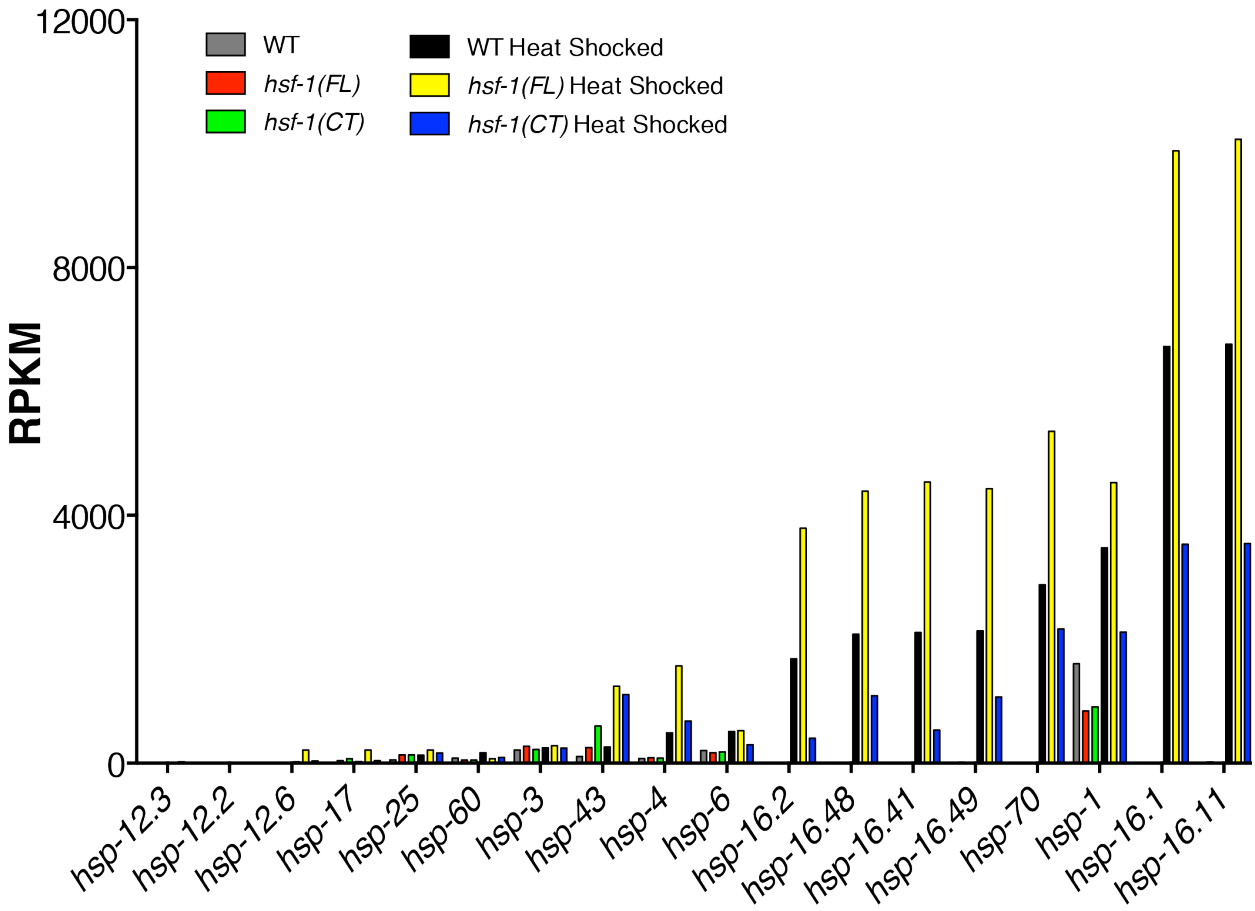

Fig. S1.

RNA-seq analysis shows heat induced upregulation of many HSPs is enhanced in *hsf-1(FL)* worms and inhibited in *hsf-1(CT)* worms. Transcript abundance was normalized by reads per kilobase of transcript per million sequencing reads (RPKM) for each condition or genotype.

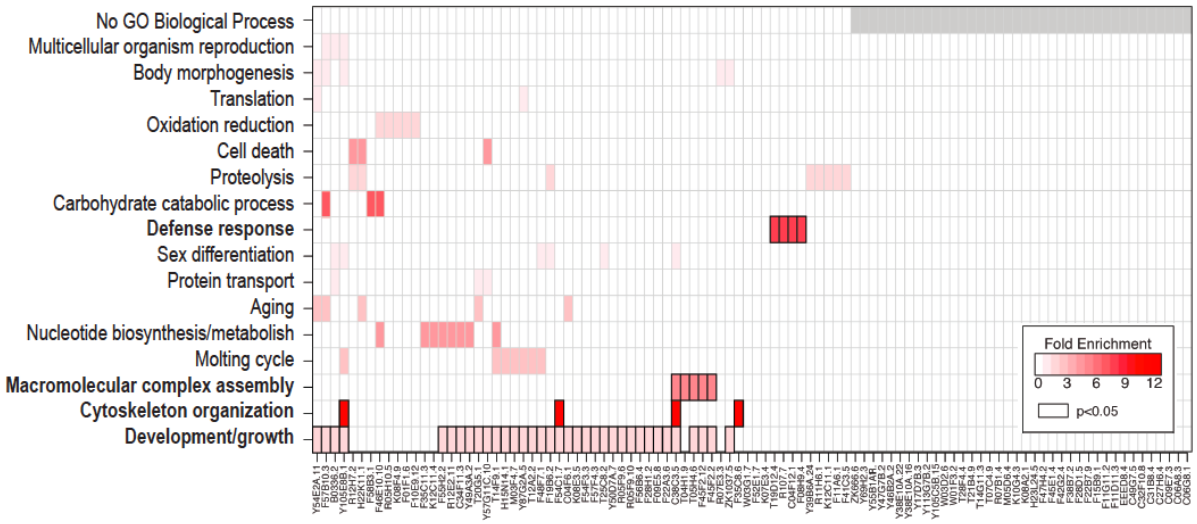

Fig. S2.

After filtering for genes significantly upregulated in heat protected strains but not in unprotected strains 98 genes were left. Gene ontology analysis found significant enrichment in development, cytoskeleton organization, complex assembly and immune defense response.

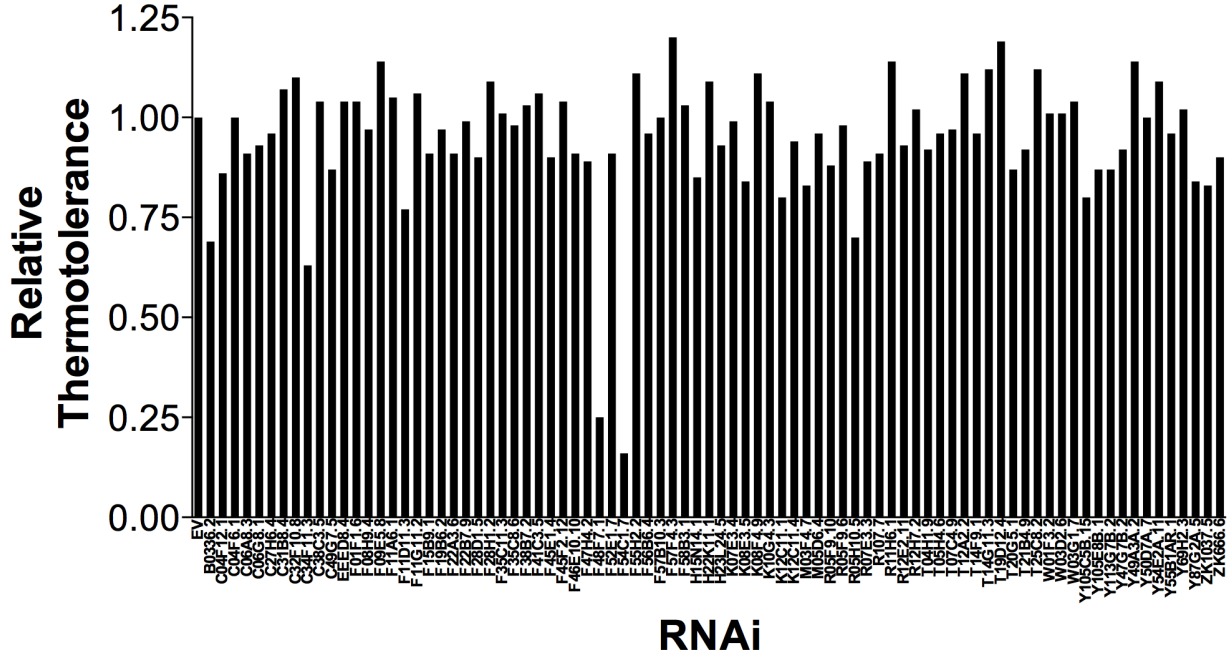

Fig. S3 98 unique genes passed filtering analysis for possible modifiers of thermotolerance. Screen results for thermotolerance of *hsf-1(CT)* on 84 RNAi treatments relative to control empty vector (EV). Fourteen RNAi treatments caused developmental arrest and could not be analyzed.

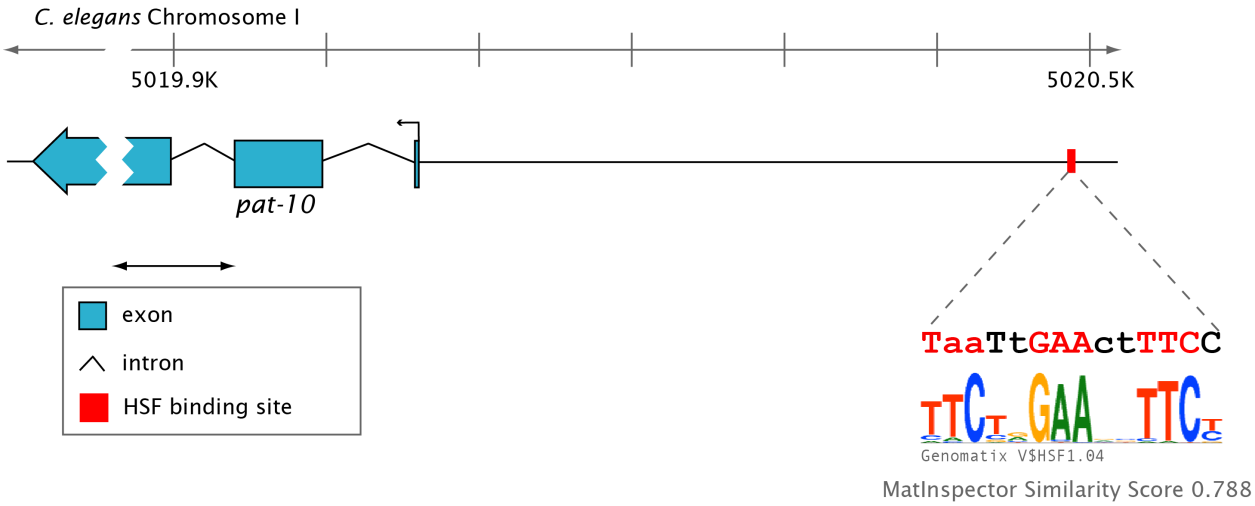

Fig. S4

pat-10 has a putative HSF-1 binding site upstream of the transcription start site. We identified a hit (position chrI:5,020,475-5,020,499, matrix match=0.8) to Genomatix's weight matrix V$HSF1.04 using the program MatInspector (*44*).

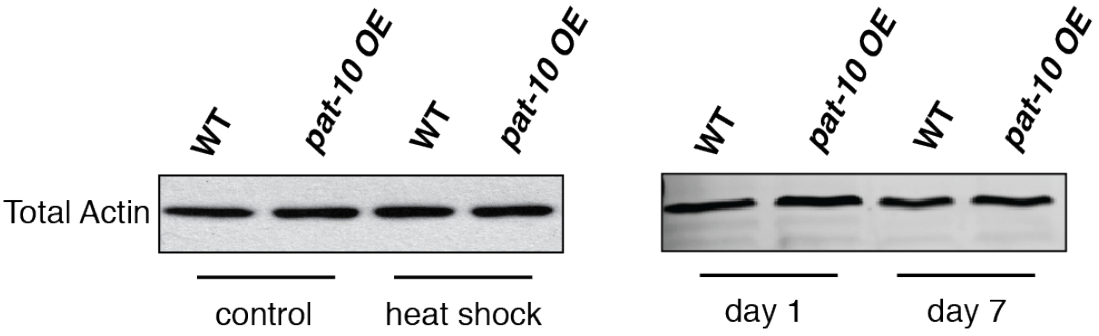

Fig. S5

Western blot of total actin shows equal amounts of actin under all conditions tested.

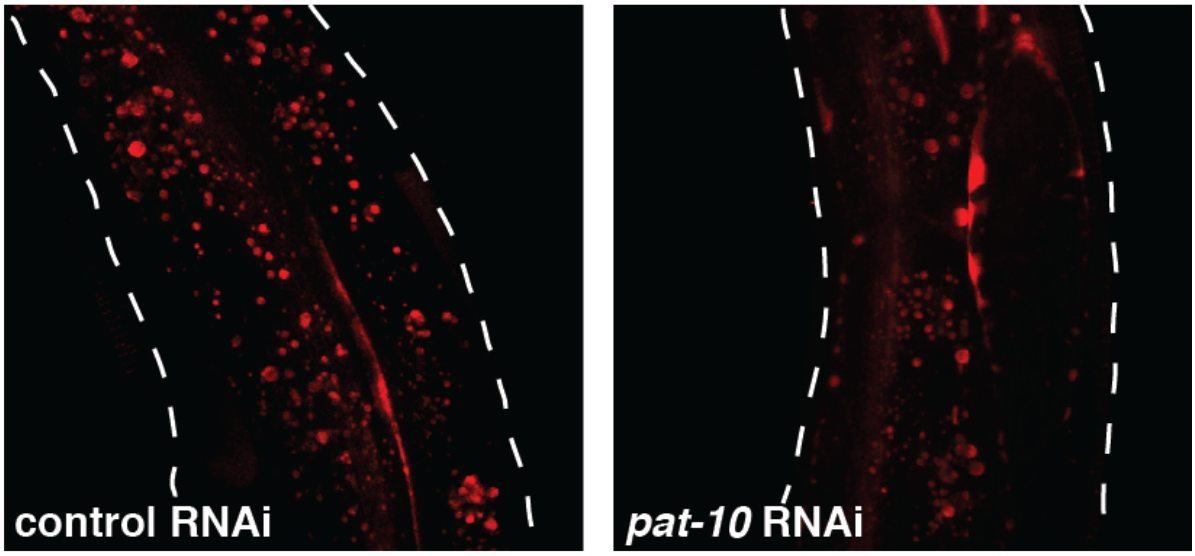

Fig. S6

pat-10 RNAi disrupted normal FM 4-64FX dye uptake and disposal, indicating impaired endocytosis. Further quantitative analysis of endocytosis was performed using a secreted GFP reporter.

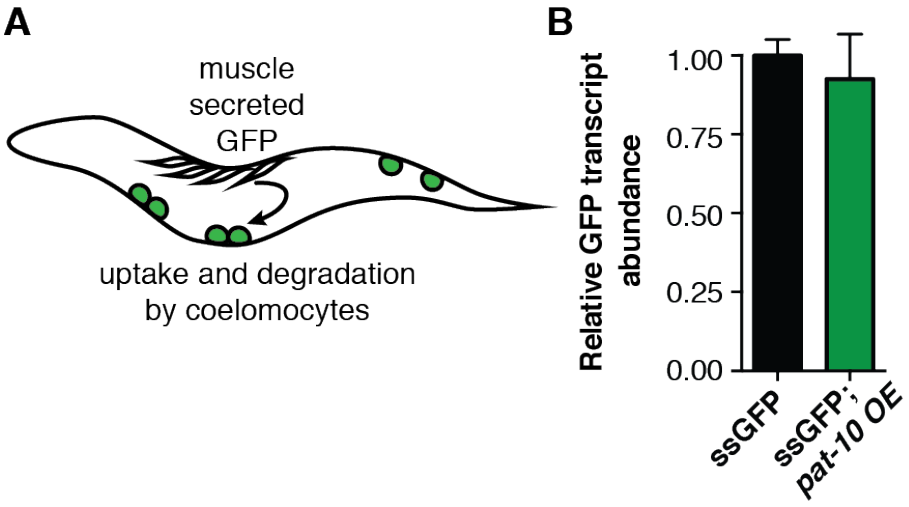

Fig. S7

(**A**) Schematic of reporter strain that secretes GFP (ssGFP) from muscle cells, which is then endocytosed by coelomocytes and degraded. (**B**) The decrease in fluorescence of the ssGFP; *pat-10 OE* strain is not due to a decrease in transcriptional expression of GFP, as determined by qPCR; error bars indicate SEM.

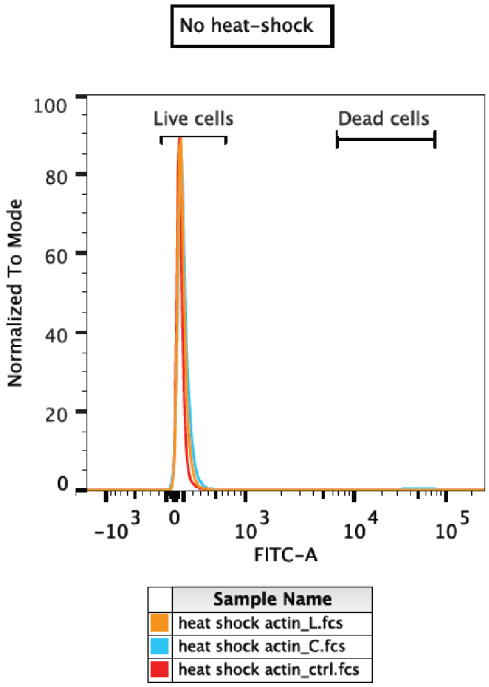

Fig. S8

HEK293T cells were treated for 1 hour with 0.5µg/mL Latrunculin A or Cytochalasin D, both diluted in pure ethanol or only pure ethanol for the control. After a 2 hour incubation at 37°C, cells were collected and treated with 1µM Sytox Green, which binds DNA of cells with compromised membrane and fluoresces. Using a BD Fortessa cell analyzer, it was determined that drug treatment did not cause cell death under control temperatures.