Supporting Online Material for

Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons

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Supporting Online Material

Materials and Methods

C. elegans strains

The following _C. elegans_ strains were used: _C. elegans_ Bristol wild-type

N2, _gcy-8 (oy44)_ IV, _gcy-23 (nj37)_ IV (_1_), PR678 _tax-4 (p678)_ III (_2_), PR767 _ttx-1_

_(p767)_ V (_3_), FK134 _ttx-3 (ks5)_ X (_4_), PS3551 _hsf-1 (sy441)_ I (_5_), _ocr-2 (ak47)_ IV

(_6_)_,_ _unc-54 (e1092)_ I (7), and the _hsp70_ (C12C8.1) promoter GFP heat shock

reporter (_8_). The _hsp70_ (C12C8.1) promoter GFP heat shock reporter transgenic

strain also contained a _rol-6_ co-injection marker: animals displayed a roller

phenotype, and adults are impaired in motility. To generate _gcy-8_ and _ttx-3_

mutant animals carrying the _hsp70_ (C12C8.1) promoter GFP heat shock reporter,

_gcy-8 (oy44)_IV or _ttx-3 (ks5)_ X animals were crossed with those carrying the GFP

reporter and progeny were selected for their roller phenotype. The genotypes

were verified by PCR when necessary. The following _gcy-8 (oy44)_ IV primers

that have been previously described (_1_) were used:

gcy8KF1: 5′-ATGGGTCTTCCCACGAGAAAAC;

gcy8KR1: 5′-GAGAACATTGGATTCACGGACC

gcy-8 (oy44) and _gcy-23 (nj37)_ were obtained from Dr. I. Mori, Nagoya

University, Nagoya, Japan. The remaining strains were obtained from the

Caenorhabditis Genetics Center (CGC).

All the thermosensory mutations (*gcy-8 (oy44), ttx-3 (ks5), gcy-23 (nj37)*, and *tax-4 (p678)*) except *ttx-1 (p676)* are either loss of function mutations or protein nulls, as specifically described below:

The *gcy-8 (oy44)* mutation is a deletion affecting the kinase homology and cyclase regions of the guanylyl cyclase protein, and is likely to be a functional null (*1*). The AFD-specific expression of *gcy-8* gene product was established by expressing transcriptional fusion constructs (*gcy-8*promoter::GFP fusion) in *C. elegans* (*9*). The *gcy-8* promoter chosen for these studies extended approximately 2kb upstream until the nearest predicted gene. Subsequently, AFD-specific expression of *gcy-8* has been confirmed by studies that have used microarrays and expression profiling to identify neuronally expressed genes (*10, 11*). The *gcy-8* protein fusion, made using full length genomic DNA fused to GFP, has been expressed in *C. elegans* and localizes exclusively to the sensory endings of AFD neurons (*1*). Thermotaxis assays suggest that while the *gcy-8 (oy44)* mutation alone has a very mild cryophilic phenotype; along with mutations in the other guanylyl cyclases expressed in the AFD, such as *gcy-23,* it shows a thermotaxis defect (*1*). The *gcy-8* protein fusion when expressed in this background rescues this thermotaxis defects (*1*).

The *ttx-3 (ks5)* mutation is a point mutation in a splice donor site within the gene, and does not appear to express protein (*12*). The animals are cryophilic, mimicking ablations in the AFD or AIY neuron.

The *gcy-23 (nj37)* mutation is a deletion within the coding sequence and also thought to be a functional null (*1*).

The *tax-4* (p678) mutation (*2*) causes the conversion of glutamine (82) to a stop codon in the region near the NH_2-terminus, and is therefore expected to be a null mutation.

The *ttx-1* (p767) alters splicing in some but not all transcribed messenger RNAs, and is likely not a molecular null. (*3*). However, the mutants show cryophilic thermotaxis behavior suggesting that AFD function is affected in a manner similar to that in the other mutants.

The *ocr-2 (ak47)* mutation does not affect the thermosensory function of the AFD neuron, but instead affects the sensory function of the four other neurons: ADF, AWA, ASH and ADL (*6*).

Growth conditions

The general methods for growing *C. elegans* were as described (*13*). The quality of bacterial food, and population densities of *C. elegans*, greatly

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influenced the outcome of all experiments so extreme care was taken to consistently expose the different *C. elegans* strains to bacterial lawns similarly grown and to maintain the animals at low population densities throughout their development and prior to and during the experiments.

The bacteria Op50 was used for feeding *C. elegans* (*13*). Standard NGM plates (*13*) of 6cm in diameter with the thickness of the agar set at 6mm ensured similar rates of heat transfer. Plates were seeded with 200-500 μl of a stationary phase culture of Op50 grown in LB broth. The bacteria were allowed to establish a dense bacterial lawn at room temperature for 48 hours and no more than 72 hours before being plated with the appropriate *C. elegans* strains. Care was taken to prevent contamination with other bacteria.

To ensure that *C. elegans* used in experiments were exposed to low population densities and optimal growth conditions, five animals in the L4 stage were placed on Op50 seeded plates, allowed to reproduce at 20°C, and their progeny were allowed to develop for 48 to 72 hours before being transferred onto new, similarly seeded NGM plates for use in experiments. Typically 10 L4 progeny, grown as described, were transferred per plate, allowed to develop into adults for 22-24 hours at 20°C and corresponded to one sample in an experiment. Sufficient 'N' values were obtained by increasing the number of plates, and not by increasing the number of animals per plate.

Heat shock protocol

       C. elegans strains were grown as described above, and all animals were heat shocked at a population density of 10 adults per plate. Sufficient N values were obtained by repeating each heat shock experiment a minimum of 3 times, with 3 samples of 10 animals per plate, per experiment. Heat shock at 30°C or 34°C, for 15 minutes on agarose plates was achieved by sealing plates with parafilm, further sealing within zip-lock bags, and immersing in a water bath equilibrated to the appropriate temperature. Following heat shock, the parafilm was removed and the animals were allowed to recover at 20°C for the course of the experiment.

       We determined that this heat shock procedure resulted in the exposure of the somatic cells of both the wild-type and mutant animals to the same temperature, and that thermotaxis differences between the wild-type and thermosensory mutants did not confound our interpretation of data, by: (a) ensuring that the temperature equilibrated rapidly across the agarose plates and that there were no temperature gradients, (b) ensuring that the surface area of the mutant and wild-type animals was comparable and, (c) assaying heat shock gene induction of the wild-type and thermosensory mutants *gcy-8* and *ttx-3* in the *rol-6* genetic background that abolished their ability to migrate across the plate.

      In order to determine the rate of equilibration of the agarose plates, we directly measured the rate of temperature increase at 10 random but well spaced

points within the plate using a thermocouple (Fluke, 51 II Thermometer, Byram Labs, Everett, WA). Consistent and rapid equilibration of the heat shock temperature was attained at all points across the plate within the duration of heat shock (Supplemental Figures S1, A, B). The heat shock temperature of 30ºC was attained by 6 minutes (Supplemental Figure S1A), and 34ºC was attained at all points by 7 minutes (Supplemental Figure S1B). The temperature did not fluctuate within the range of detection of the thermocouple (0.01 ºC) during the remainder of the heat shock (Supplemental Figures S1, A, B).

We examined whether there were thermal gradients formed across the plate using a 0.008" thick thermochromic Liquid Crystal (LC) ink plastic film (Edmund Scientific, Liquid Crytal Mylar sheets, Calatog # 3072372; Supplemental Figures 1C-F). The LC sheet was calibrated using the thermocouple and produced color at wavelength in the red range at 30ºC (Supplemental Figure S1D), and blue at 34ºC (Supplemental Figure S1E). The LC sheets were then cut to the size of the agarose plates (6cm diameter) and applied onto the surface of the plates that had been seeded with Op50 bacteria. These plates were then subjected to the heat shock protocol described above and photographed immediately after. Plates that were immersed in the 30 ºC water bath turned red (Supplemental Figure S1D), while those immersed in a 34 ºC water bath, as described above, turned blue (Supplemental Figure S1E). This latter temperature was used for the majority of the experiments. We ensured that the LC sheets were indeed capable of detecting temperature gradients by

applying a gradient of 25-34 degrees to one of the plates, and obtaining a gradient of color change (Supplemental Figure S1F).

To confirm that the mutant and wild-type animals subjected to the temperature stress had a comparable surface area, we measured the surface area of 30 images of wild-type, *gcy-8 (oy44)* and *ttx-3 (ks-5)* animals using Image J. Both the surface area measurements (depicted as pixel number) and the variation seen amongst different animals of each strain were very similar between wild-type and thermosensory mutant animals (Supplemental Figure S1G)

To ensure that that motility across the agarose did not affect heat shock dependent gene induction, we compared endogenous *hsp70* (C12C8.1) mRNA levels in the *hsp70p* (C12C8.1):: GFP; *rol-6* heat shock reporter strains that were wild-type with those carrying a mutation in their *gcy-8* and *ttx-3* genes (Supporting Online Material, Legend to Table SI)

RNA extraction and quantitative RT-PCR

mRNA was prepared using the "Absolutely RNA$^{®}$ Nanoprep Kit" (Stratagene, Catalog #400753). The manufacturer's protocol was adapted to achieve maximal lysis of worms. Briefly, 5-10 adult animals were picked either from the control or experimental plates into 100 µl of buffer made up by mixing 7 µl β-ME (instead of the recommended 0.7 µl β-ME), with 100 µl Lysis Buffer

provided by the manufacturer. The suspension was subjected to numerous cycles of freeze-thawing in liquid nitrogen and vortexing until the animals were completely lysed. RNA was then purified as detailed in the manufacturer's protocol. mRNA was reverse transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad, Catalog # 170-8891). Quantitative PCR was performed using iQ™ SYBR® Green Supermix (Bio-Rad, Catalog # 170-8880), in the iCycler system (Bio-Rad) at a 25 µl sample volume, in thin wall 200µl PCR plates (Cat. No. 223-9441) sealed with the optical quality sealing tape (Cat. No. 223-9444).

The relative amounts of *hsp* mRNA were determined using the Comparative C_T Method for quantitation (*14*). The levels of *hsp* mRNA levels within an experiment were determined relative to actin mRNA, which was used as the internal control. The range of input of RNA was determined using serial dilutions of the cDNA that yielded a C_T value of <30, for both the target cDNA and actin was used in all experiments. This typically corresponded to 1 µl of the total cDNA obtained per sample. C_T values were obtained in triplicate for each sample (technical triplicate), and three samples were used per experiment. Each experiment was then repeated a minimum of three times. All relative changes of *hsp* mRNA in the mutant strains were normalized to maximal wild-type values, except where otherwise noted.

The heat shock time course data shown in Figures 1D-F are representative for the indicated *hsp* genes over 6 hours. Each time point depicts

the average of three technical triplicates from three samples, in one experiment. During the course of recovery, different experimental replicates, which reflected different biological samples, all showed a consistent decrease in the maximal induction of *hsp* mRNA in the mutant strains compared to wild-type values. However, the levels of *hsp* genes between different biological samples were variable, especially during the later time points.

The primers used for the PCR analysis were:

hsp70 (C12C8.1)

    Forward: 5'-ACT CAT GTG TCG GTA TTT ATC-3'

    Reverse: 5'- ACG GGC TTT CCT TGT TTT-3'

hsp70 (F44E5.4)

    Forward: 5'-AAT GAA CCA ACT GCT GCT GCT CTT-3'

    Reverse: 5'-TGT CCT TTC CGG TCT TCC TTT TG-3'

hsp16.2

    Forward: 5'-ACT TTA CCA CTA TTT CCG TCC AGC-3'

    Reverse: 5'-CCT TGA ACC GCT TCT TTC TTT G-3'

Actin

    Forward: 5'-ATC ACC GCT CTT GCC CCA TC-3'

    Reverse: 5'-GGC CGG ACT CGT CGT ATT CTT G-3'

hsf-1

    Forward: 5'-GTC TTG TTG CGG CTG AGC CAT TTG CC-3'

Reverse: 5'-CAC AGA TTA AGA GAA AGC AAT CGG CA-3'

daf-16

Forward: 5'-CGC CGG ATG GAA GAA CTC GAT CCG T-3'

Reverse: 5'-GCA ATT GGT TCC TTA ATC GGC TTC GA-3'

hsp-1

Forward: 5'-CTC GAG TCA TAC GCC TTC AAC CTT A-3'

Reverse: 5'-GGC CAA TCC TTC CAA ATC CTT CTG-3'

daf-21

Forward: 5'-CGC TAC CAG GCA CTC ACC GAG-3'

Reverse: 5'-GGA CAA GCT CTT GTA GAA CTC AG-3'

cdr-1

Forward: 5'-TCT TCT CTC AAT TGG CAA CTG-3'

Reverse: 5'-TTT GGG TAA ACT TCA TGA CGA-3'

Thermotolerance assay

Thermotolerance assays were conducted on wild type N2, *gcy-8 (oy44)* IV, *ttx-3(ks5)* X, and the *hsf-1 (sy441)* I animals grown as described above. Ten samples, each containing ten adult animals per plate, were used for one thermotolerance experiment, and three repetitions of the experiment were performed to obtain substantial 'N' values. Thermotolerance assays were conducted by immersing animals in a 35°C water bath for 7-9 hours. This duration of exposure was required to obtain 50% death of the wild-type N2 animals, and survivors were scored approximately 12 hours after recovery at 20°C.

RNAi experiments

Escherichia coli strain HT115 (DE3) harboring the appropriate dsRNA

expressing plasmid from the genomic RNAi library (J. Arhinger) were grown

overnight in LB broth containing ampicillin (100µg/ml) and tetracycline (12.5

µg/ml). 200-500 µl of bacteria was seeded onto NGM plates containing ampicillin

(100µg/ml) and tetracycline (12.5 µg/ml) and 0.4 mM Isopropyl β-d-

thiogalactosidase. Care was taken to ensure that the plates grew a healthy lawn

of RNAi bacteria by allowing the bacteria to grow for 2-4 days prior to use. For

each RNAi experiment, 10 animals were singled onto the RNAi plates as L4

larvae, and allowed to develop for 22-26 hours into adults prior to use. In all

cases, the knock-down of the appropriate RNA was confirmed by RT-PCR (not

shown). The RNAi constructs used were directed against either hsf-1 or daf-16.

Escherichia coli strain HT115 (DE3) harboring the RNAi plasmid vector L440

alone was used as the control.

Cadmium stress experiments

Sterile-filtered cadmium chloride was added to a final concentration of

50μM to standard, autoclaved, NGM medium, and used to make plates. OP50

was seeded onto the plates as described above. To assay cadmium-responsive

gene expression, wild-type N2 and gcy-8 mutant animals were grown on regular

NGM plates in the absence of cadmium as described above, and following their

development to adults, 10 animals were transferred onto the cadmium-plates for

a duration of 3 or 16 hours. These animals were then harvested for quantitative

RT-PCR. The levels of *hsp70* (C12C8.1) mRNA induction in both the wild-type

and *gcy-8* mutant animals after 3 hours is indicated in the text (Fig 2B). *hsp70*

(C12C8.1) mRNA was further induced more than 10- fold after 16 hours of

exposure.

To conduct RNAi experiments aimed at assessing the effects of *hsf-1*

knock-down on the induction of cadmium-responsive genes, animals were

exposed to both ds*hsf-1* and cadmium as L4 larvae for 24-28 hours. This was

done by growing the animals on RNAi plates containing cadmium, and seeded

with RNAi bacteria harboring the ds*hsf-1* plasmid. Animals were harvested for

RT-PCR 28 hours after being placed on the RNAi plates, and knock-down of *hsf-*

1 RNA was confirmed by RT-PCR.

Anesthesia experiments

The VA anesthetics used were 2-Bromo-2-chloro-1,1,1-trifluroethane

(Halothane, Fluka, catalog #16730) and Isoflurane (Webster Veterinary NDC#

14043-220-05). VA anesthetics were delivered as follows: lids of 1.5 ml

eppendorf tubes were cut off, VA was pipetted into the lids, and the lids

containing VAs were placed onto plates containing 10 adult wild-type or *gcy-8*

animals grown as described, and the plates were immediately sealed with

parafilm. To inhibit neuronal signaling during the course of heat shock, the VA

containing lids were placed on plates 5 minutes prior to the heat shock, and

retained during the heat shock treatment of 34°C for 15 minutes. The lids were

then removed 10 minutes post-heat shock during recovery at 20°C, when the

plates had equilibrated to 20°C. To inhibit neuronal signaling following the

administration of heat shock, animals were heat shocked, and then the lids

containing the same volume of VA was placed onto plates 20 minutes post-heat

shock, and removed after 30 minutes.

To control for non-specific effects of paralysis on heat shock gene

induction, we measured *hsp70* (C12C8.1) mRNA levels in animals with functional

AFDs, but paralyzed due to a partial deletion in their myosin gene (*unc-54)*.

These animals showed normal heat shock induction of *hsp70* (C12C8.1) (mRNA

levels in *unc-54* (e1092) animals relative to wild-type induction: wild-type=1.00,

unc-54 (e1092) =1.6 ± 0 5, measures 2 hours post-34°C for 15 minutes).

As has been described before, the effect of the VA is extremely variable in

any given population of *C. elegans* (*15, 16*) and is also influenced by other

environmental factors, such as population density, to which the animals are

exposed (*15*). Therefore it was necessary to titrate the amount of VA used for

each experiment. The volume of VA used was chosen as that which inhibited the

movement of 100% of the animals on a plate within the first 15 minutes following

exposure to the VA, did not cause any death over the course of the experiment,

and did not result in all the animals consistently moving off the bacterial lawn

following recovery from VA. Using *C. elegans* grown as described above, this corresponded to 5-15 µl for halothane and 10-25 µl for isoflurane. Animals were considered to have recovered from the effects of the anesthesia when they were actively moving on plates, and this occurred within 1 hour following VA exposure, when 100% of the animals had recovered.

Dauer pheromone experiments.

The effect of dauer pheromone on the heat shock response was tested using DAUMONE ((*17*) KDR Biotech. Co. Ltd. Cat # DA-1-010). Daumone stocks were prepared by dissolving daumone in ethanol (320 µg in 100 µl). *C. elegans* were grown as described above, and 5-10 minutes prior to heat shock, 10-50 µl of daumone was spotted onto the OP50 plate containing 10 adult *C. elegans*. Care was taken not to let the daumone touch the animals. 50 µl ethanol was used as controls. The animals were allowed to recover from heat shock on the same plates in the presence of daumone, after which they were harvested for mRNA..

Text

Supplemental Figure 1

The heat shock procedure resulted in the exposure of the somatic cells of both the wild-type and mutant animals to the same temperature.

Supplemental Figure 2

The *gcy-8* or *ttx-3* mutant animals continued to be impaired in *hsp70* (C12C8.1) promoter GFP reporter construct expression 24 hours following heat shock.

Supplemental Figure 3

The *gcy-8* and *ttx-3* mutant animals do not express less *hsf-1* compared to wild-type animals. In fact, *gcy-8* and *ttx-3* animals perhaps express more *hsf-1* mRNA relative to wild-type animals. The *gcy-8* animals do not express higher constitutive amounts of chaperones that negatively autoregulate HSF-1 activity, or other inhibitors HSF-1 (*18, 19*). Thus these explanations do not sufficiently explain the diminished heat shock dependent expression of HSP mRNA in the thermosenosry mutant animals.

Supplemental Figure 4

If AFD signaling is required for the heat shock response the inhibition of neuronal activity in wild-type animals should inhibit the transcription of genes encoding HSPs, mimicking the effect of AFD mutations. We used the volatile anesthetics (VAs) halothane and isoflurane which inhibit synaptic transmission to transiently and reversibly inhibit neuronal activity (*16*). Wild-type animals exposed to VAs for the full duration of the heat shock showed a marked decrease in *hsp70* (C12C8.1) expression 2 hours after recovery (Fig. S4). This was evident in the reduced levels of *hsp70* (C12C8.1) promoter GFP reporter

expression (Fig. S4 A-C), and the fraction of animals expressing GFP (20% versus 100% control; Fig. S4E), providing independent corroboration that the cellular heat shock response is neuronally regulated.

Surprisingly, wild-type animals inhibited in the induction of *hsp70* expression at 2 hours recovered *hsp70* mRNA expression as the anesthetic effect dissipated (Fig. S4E). This recovery required the normal functioning of the AFD neuron: *gcy-8* mutants subjected to VAs did not recover GFP reporter expression even after the anesthesia wore off (Fig. S4E). These data confirm that the expression of hsp70 (C12C8.1) mRNA in somatic cells requires active *gcy-8*-dependent neuronal signaling. In addition they may provide some clues into the mechanism of neuronal control of the cellular heat shock response, in which binding of HSF-1 to its promoter still requires an active neuronal signal to activate transcription.

Figure Legends

Legend to Fig S1:. Both the wild-type and thermosensory mutant animals are exposed to the same temperature during heat shock. **(A)** The rate of temperature increase, averaged across 10 random, well spaced points on a 6 mm thick agarose plate used for the heat shock experiments when plates were transferred from 20 °C to a 30°C water bath for 15 minutes, and **(B)** when plates

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were transferred to a 34°C water bath for 15 minutes. **(C)** A photograph of a 0.008" thick thermochromic Liquid Crystal (LC) ink plastic film which changes colour to indicate temperature (red=30°C and blue=34°C) when applied to the surface of an agarose plate at 20 °C. **(D)** The LC film after applied to the surface of an agarose plate immersed uniformly in a 30°C water bath for 15 minutes. **(E)** The LC film after being applied to the surface of an agarose plate immersed uniformly in a 34°C water bath for 15 minutes. **(F)** The LC sheet when applied to the surface of an agarose plate exposed to a temperature gradient of 25-34°C for 15 minutes by immersing half of the plate in the 34°C water bath, while the other half remained at room temperature of 25°C. **(G)** The surface area of 30 images each of wild-type, *gcy-8* and *ttx-3* thermosensory mutant animals.

Legend to Fig S2: *hsp70* (C12C8.1) promoter-GFP reporter expression in **(A)** wild-type **(B)** g*cy-8* and **(C)** *ttx-3* mutant animals 24 hours post-heat shock (34°C;15 minutes).

Legend to Fig S3: A) Basal *hsf-1 mRNA* levels in wild-type and *gcy-8 and* ttx-3 mutants. **(B)** Basal mRNA levels of *daf-16*, *hsp90* (*daf-21*) and *hsp70* (*hsp-1*), in wild-type and *gcy-8* mutants. mRNA levels were measured relative to the wild-type strain, by quantitative RT-PCR

Legend to Fig. S4

Requirement of active neuronal signaling for heat shock gene expression. *hsp70* (C12C8.1) promoter-GFP reporter expression assayed 2 hours post-heat shock in **(A)** control, non-anesthetized wild-type worms, **(B)** wild-type worms anesthetized with VA during heat shock, and **(C)** wild-type worms anesthetized with VA following heat shock. **(D)** Total *hsp70* (C12C8.1) mRNA levels 2 hours post-heat shock in control non-anesthetized worms, and worms anesthetized with VAs (halothane and isoflurane) (pair-wise t-test ; P value=0.001 and 0.0001 respectively). **(E)** Percentage of wild-type or *gcy-8* mutant animals expressing the *hsp70* (C12C8.1) promoter-GFP reporter at various times post-heat shock. Heat shock in all experiments was 34°C for 15 minutes. mRNA levels were measured by quantitative RT-PCR and normalized to wild-type values.

Table SI

Strain	Baseline	30°C heat shock	34°C heat shock
Wild-type	1.4 ± 0.3	100.0	100.0
gcy-23	1.1 ± 0.3	18.4 ± 0.6	10.2 ± 0.7
gcy-8	3.6 ± 2.3	21.9 ± 12.0	12.2 ± 8.4
tax-4	0.9 ± 0.3	40.1 ± 9.7	43.1 ± 16.8
ttx-1	0.9 ± 0.0	42.0 ± 8.8	36.0 ± 12.8
ttx-3	2.8 ± 2.0	17.9 ± 8.4	6.7 ± 5.0

Legend to Table SI

hsp70 (C12C8.1) mRNA levels in wild-type, *gcy-8 (oy44)* IV, *gcy-23 (nj37)* IV (*1*), PR678 *tax-4 (p678)* III (*2*), PR767 *ttx-1(p767)* V (*3*) and FK134 *ttx-3(ks5)* X (*4*), prior to heat shock (column 2), 2 hours post-heat shock at 30°C for 15 minutes (column 3), and 2 hours post-heat shock at 34°C for 15 minutes (column 4). mRNA levels were measured by quantitative RT-PCR. Baseline *hsp70* (C12C8.1) mRNA values were normalized to the maximal wild-type induction following the 34°C heat shock. *hsp70* (C12C8.1) mRNA values following heat shock at either temperature was normalized to wild-type values at that temperature. In addition, *hsp70* (C12C8.1) mRNA levels in *ocr-2 (ak47)* mutant animals=90 ± 20.2, 2 hours post-heat shock at 34°C for 15 minutes. *hsp70* (C12C8.1) mRNA levels in the heat shock reporter transgene containing animals, 2 hours post-heat shock at 34°C for 15 minutes.: wild-type *hsp70p* (C12C8.1)::GFP; (*rol-6*)= 100, *gcy-8*; *hsp70p* (C12C8.1)::GFP; (*rol-6*) = 4.7 ± 2.1 and *ttx-3*; *hsp70p* (C12C8.1)::GFP; (*rol-6*) =26.5 ± 13.2. mRNA levels were

measured relative to the wild-type strain. mRNA levels in all cases was

measured by quantitative RT-PCR

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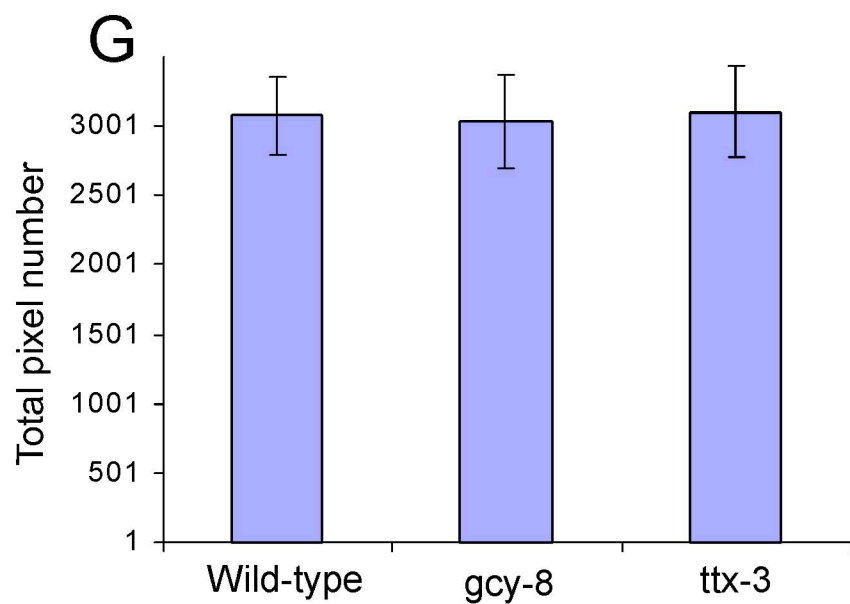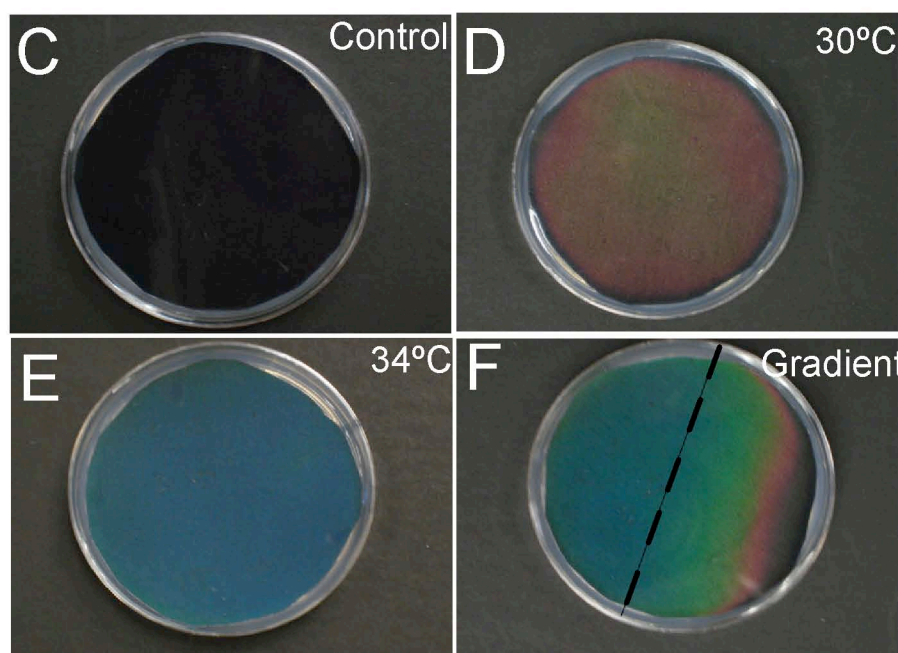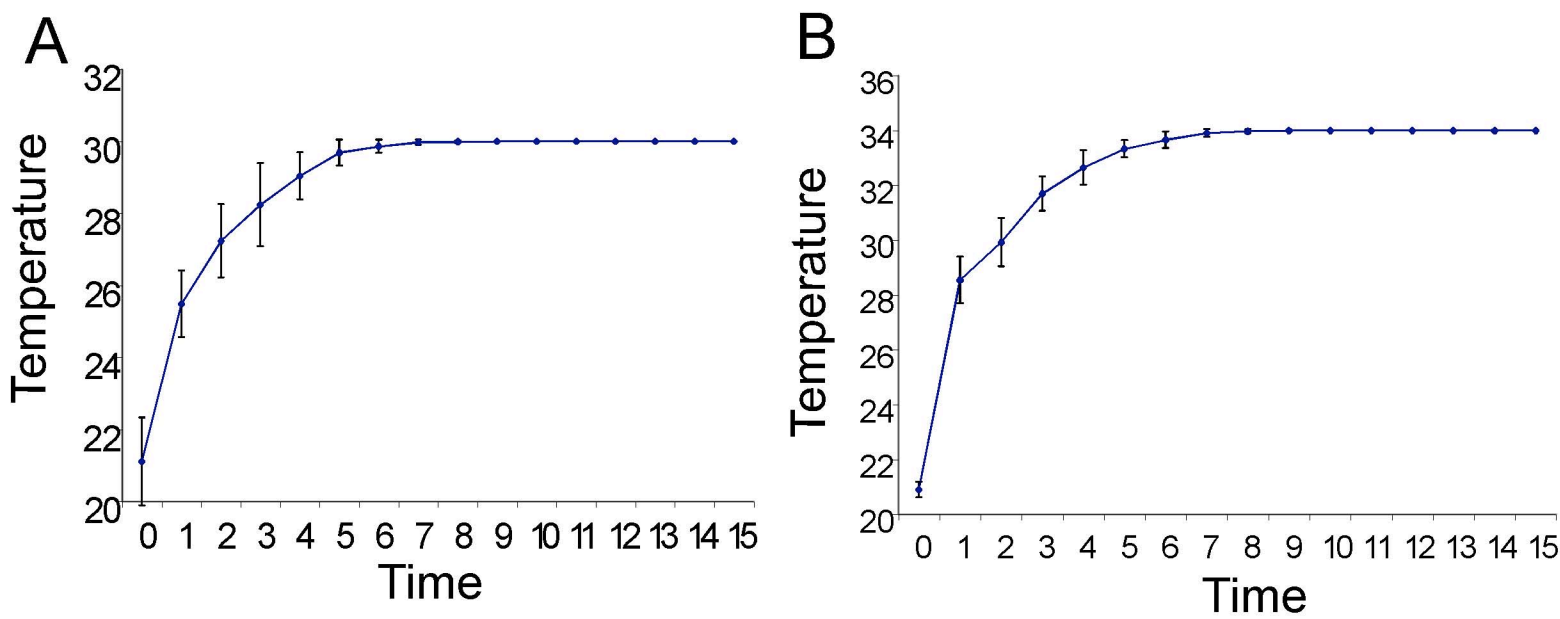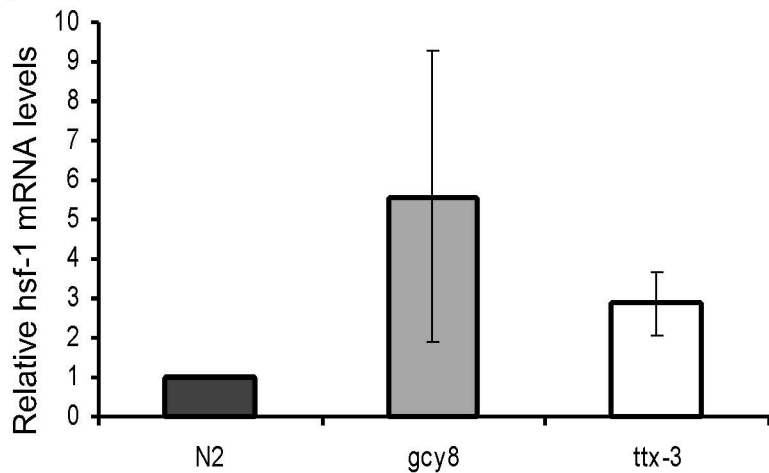
Figure S1

Figure S2

A. Wild-type

B. gcy-8

C. ttx-3

Figure S3

Figure S4