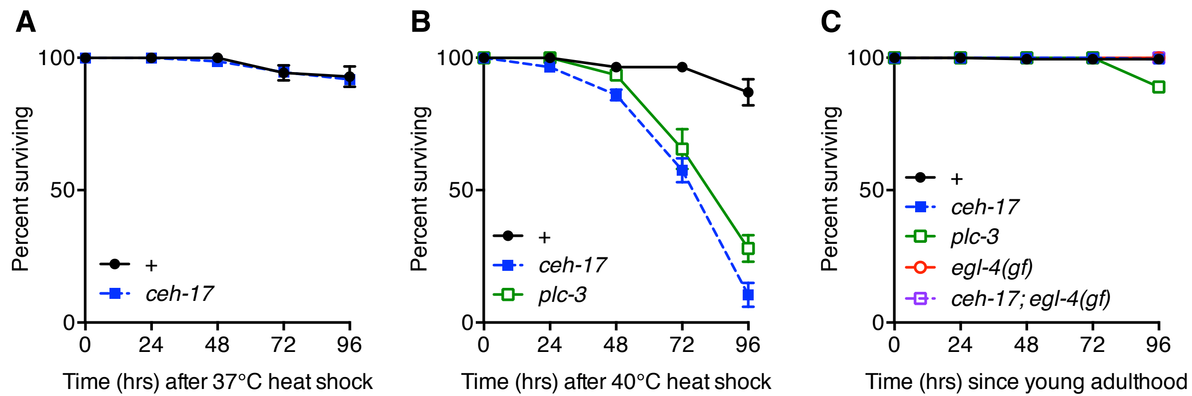

Figure S1. Quiescence-defective mutants are impaired for survival following 40˚C heat shock. Related to Figure 4. **A**, Percentage of wild-type and *ceh-17(np1)* adults surviving at daily intervals following a 30 minute 37 °C heat shock. *ceh-17* mutant animals show wild-type survival ($p = 0.766$; log rank test). **B**, Percentage of wild-type, *ceh-17(np1),* and *plc-3(tm1340)* adults surviving at daily intervals following a 20 minute 40 °C heat shock. Wild-type animals show greater survival than quiescence-defective *ceh-17* or *plc-3* mutant animals ($p < 0.001$, log rank test). **C**, Percentage of untreated wild-type, *ceh-17(np1)*, *plc-3(tm1340)*, *egl-4(ad450gf)*, and *ceh-17(np1); egl-4(ad450gf)* animals alive at daily intervals after reaching adulthood. *ceh-17*, *egl-4(gf)*, and *ceh-17; egl-4(gf)* animals show wild-type viability ($p = 0.323$ or greater, log rank test), whereas *plc-3* animals show a slight decrease in viability ($p = 0.013$, log rank test) due to a ruptured vulva phenotype. The magnitude of this effect cannot account for the decreased viability of heat shocked *plc-3* animals ($p < 0.001$, log rank test), and, notably, ruptured vulvae were not observed among heat shocked *plc-3* animals. Mean and SEM are shown.

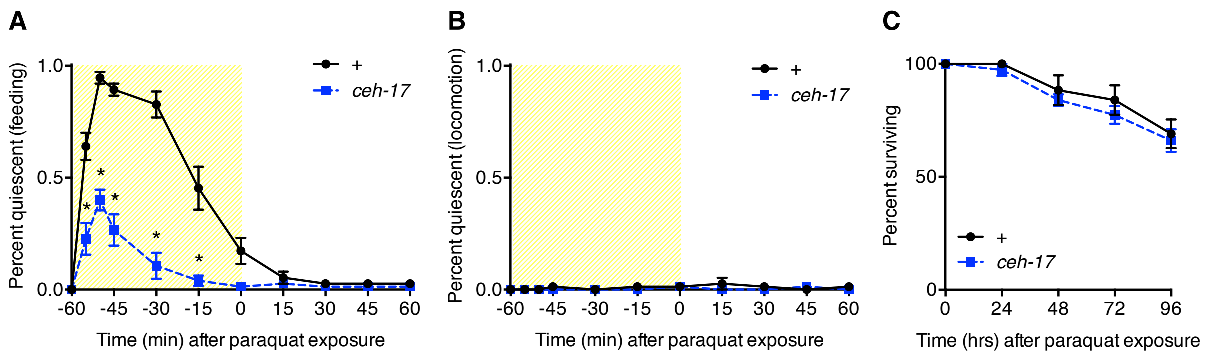

Figure S2. Wild-type and ALA-defective animals behave similarly after paraquat exposure. Related to Figure 4. **A** and **B**, Time course of feeding quiescence (A) and locomotor quiescence (B) in wild-type and *ceh-17(np1)* animals during and after 100 mM paraquat exposure. Shaded region represents time during paraquat exposure. During exposure, wild-type animals showed transient feeding quiescence, a fraction of which appears to be ALA-dependent. In contrast to the inhibition of feeding, paraquat did not inhibit locomotion during or following exposure. It is possible that paraquat exposure, while sufficiently harmful to reduce long-term viability (compare panel C to Figure S1C), leads to relatively weak ALA activation and reveals a lower threshold for feeding quiescence than for locomotor quiescence. **C**, Percentage of wild-type and *ceh-17(np1)* adults surviving at daily intervals following a 2 hr exposure to 100 mM paraquat. *ceh-17* animals do not show a survival deficit compared to wild type ($p = 0.688$, log rank test). Mean and SEM are shown. $^*p < 0.001$, Fisher's exact test.

Table S1. Inhibition of pharyngeal pumping by direct heat is quickly reversible and independent of ALA neuron function. Related to Figure 1.

	avg. temp at cessation of feeding (˚C)	*n*	p vs. wild type	avg. time to feeding recovery (sec)	*n*	p vs. wild type
Wild type N2	31.73 +/- 1.01	*24*		91.9 +/- 45.3	*21*	
ceh-17(np1)	31.70 +/- 1.74	*23*	0.94	75.0 +/- 30.6	*21*	0.16

A seeded NG agar plate containing a young adult animal was placed on a heated microscope stage. The temperature at which pharyngeal pumping ceased was measured using two in-agar K-type thermosensors linked to a digital thermometer. Immediately after cessation of pharyngeal pumping, the animal was transferred by wire pick to a 21˚C plate and the time until feeding resumed was noted. ALA-defective *ceh-17* mutant animals behaved similarly to wild type in response to this transient heat exposure. Two-tailed p values were calculated using an unpaired t test.

Table S2. *ceh-17* null mutant adults move at wild-type velocities. Related to Figure 1.

	mean velocity (mm sec^{-1})	Number of trials	sd	p vs. wild type
wild type N2	0.170	11	.014	
ceh-17(np1)	0.172	5	.031	.9073

Velocities of young adult animals in the absence of food were determined by automated locomotion analysis as described. Twenty animals were recorded per trial. Two-tailed p value was calculated using an unpaired t test with Welch correction for unequal variances.

Table S3. Settings used on the Multi-Worm Tracker. Related to Figures 1 and 2.

Setting	Value
Sample rate	4 frames per sec
Shortest valid track	12 frames
Interval for velocity calculations in Table S2	1 sec
Length of each recording for velocity calculations	60 sec
Length of each recording for quiescence calculations	30 sec
Length of each segment for quiescence calculations	6 sec
Segment interval shift for quiescence calculations	0.5 sec
Speed threshold for moving/still worms	0.03 mm/sec
Min size of single worm	35 pixels
Max size of single worm	220 pixels
Max distance between same worm in successive frames	35 pixels
Max size change by worm in successive frames	35 pixels

Supplemental Experimental Procedures

Strains

All *C. elegans* strains were maintained and assayed on nematode growth (NG) plates seeded with *Escherichia coli* strain OP50 [S1]. The following *C. elegans* strains were used: wild type N2, *ceh-17(np1)* IB16, *itr-1(sy290) lin-3(n1058)* PS1378, *let-23(sy10) unc-4(e120)*/mnC1 PS302, *plc-3(tm1340)*/mIn1[*mIs14(myo-2::gfp) dpy-10(e128)*] PS4886, *hsf-1(sy441)* PS3551, *daf-16(mu86)* CF1038, *hsp-4(gk514)* VC1099, zSi3001[*hsp-16.2::gfp*] TJ3001, *ceh-17(np1);* zSi3001[*hsp-16.2::gfp*] CVB7, zcIs4 [*hsp-4::gfp*] SJ4005, *ceh-17(np1); zcIs4*[*hsp-4::gfp*] CVB8, *egl-4(ad450gf)* DA521, *ceh-17(np1); egl-4(ad450gf)* CVB9. The *egl-4* gain-of-function allele also goes by the names *egl-4(ad450sd) and eat-7(ad450). ceh-17(np1)* and *plc-3(tm1340)* alleles are caused by deletions and are likely to represent null alleles. The *lin-3(n1058)* and *let-23(sy10)* alleles are caused by substitution mutations and behave as partial reduction-of-function alleles. The *lin-3(n1058)* fertility defect is rescued in PS1378 by the *itr-1(sy290)* mutation, which does not impair EGF-induced quiescence[S2]. The *let-23(sy10)* allele is linked to the *unc-4(e120)* mutation, which does not impair EGF-induced quiescence[S2]. The *plc-3(tm1340)* homozygotes are sterile due to ovulation defects and were selected as GFP negative animals from the balanced strain PS4886. All assays were performed on young adult animals, staged by growth from eggs for 3 days at 18-20˚C, or by hand selection of L4 larvae <18 hours prior to assay.

Heat shock

Heat shocks were performed using an LW Scientific circulating water bath and multiple total immersion thermometers to verify the water temperature. Seeded NG agar plates (55 mm diameter, 12ml Bacto agar) containing 20-30 young adults were wrapped in parafilm and floated in the water bath, agar side down, staggered by 2-3 min to allow scoring of multiple samples at specified time points after heat shock. Plates were quickly cooled to room temperature by 2 min incubation on ice. Cooling rate was determined using in-agar thermosensors. We noted that without incubation on ice, the plate temperature did not reach room temperature until approximately 20 minutes after heat shock, and that return to room temperature was critical for penetrant locomotor quiescence. When possible, all trials of a given experiment were performed on one day, to reduce variability associated with slight variations in water bath temperature as well as the temperature of the room in which behavioral analyses were performed, which varied between 21˚C and 24˚C. For an example of day to day variability, compare wild type 37˚C recovery curves in Figures 4A and 4C.

Analysis of feeding behavior and locomotion

Each adult animal was observed at high magnification on a stereomicroscope for 3-4 seconds and inspected for contraction of the posterior pharyngeal bulb. If no contractions were observed, the animal was considered quiescent for feeding behavior. For Figures 1B, 2E and Table S2, locomotion data was collected using an automated multi-worm tracker[S3]. Well-fed young adult animals were transferred with minimal OP50 to freshly and evenly seeded NG agar plates. Control animals were kept at room temperature and experimental animals were subjected to heat shock as described above. For analysis of locomotion during heat shock, plates were transferred from a 35˚C water bath to a heated microscope stage for tracking. Videos of worm movements were captured at 32x using a Unibrain Camera (Fire-i 580b) mounted on a Leica S6D microscope. Velocities in Table S2 were analyzed within MatLab (MathWorks) as previously described[S3]. A mean velocity was calculated for each multi-worm tracking experiment, and the mean of means for multiple experiments is presented. Fraction quiescence for each worm-track (approximating each animal) was calculated as the fraction of 6 second windows, staggered by 0.5 sec, in which instantaneous velocities did not exceed 0.03 mm sec^{-1}. Recordings were 30 sec long and velocities were sampled every 0.25 sec. For each experiment the average fraction quiescence of all worm-tracks is reported. Settings used for automated locomotion analysis are shown in Table S3. To assess reversibility of locomotor quiescence (Figure 2A), 'activity' was defined as at least a body-length of movement within 10 sec of observation by eye. Inactive animals were prodded once on the tail with a wire pick and assayed for activity again. For all subsequent stressor assays, categorical locomotion data were collected by direct observation immediately after the scoring of feeding behavior. The plate was left unperturbed for one minute and each animal in the field of view at low magnification was observed for 3-4 seconds. Animals were scored as quiescent only if they showed no detectable movement at all, not even side-to-side head movements.

Measurement of sensory responsiveness

Responsiveness of animals was measured using two aversive stimuli: blue light and 1-octanol. For blue light stimulation, young adult animals on seeded plates were individually exposed to 3 seconds of blue light through the GFP filter of a fluorescence stereomicroscope at high magnification. A response was defined as a one-half body length movement with increased

velocity within 5 seconds immediately after 3-sec blue light exposure. Unresponsive animals were prodded on the tail with a wire pick to confirm that they were not paralyzed and could be awoken with a harsh stimulus; those that did not respond (3 out of 273 animals) were discarded from the analysis. For 1-octanol stimulation, an eyelash dipped in freshly prepared 30% 1-octanol (in ethanol) was waved one pharynx length in front of the noses of still or forward-moving animals. A response was defined as a pharynx-length equivalent of backwards movement within 5 seconds of exposure.

Hypertonic stress

For evaluation of behavior during and following hypertonic stress, 20-30 young adult animals were transferred by platinum wire to a drop of 500 mM NaCl in M9 buffer on a depression slide for 15 min, observed during exposure, then transferred by pipet with minimal liquid to seeded NG plates. Animals were gently removed from any remaining liquid and scored immediately for feeding and locomotion behavior, and then every 15 minutes for an hour.

Ethanol stress

For evaluation of behavior following ethanol stress, 20-30 young adult animals were transferred by platinum wire to a microfuge tube containing 50 ul 5% ethanol in M9 for 30 min, transferred by micropipet with <10 ul of liquid to seeded NG plates and scored immediately and every 15 minutes for an hour for feeding and locomotion. Control animals that had been transferred to M9 without ethanol displayed robust feeding and locomotion after removal from liquid. For evaluation of behavior during ethanol stress, animals were transferred to a 10 ul drop of 5% ethanol in M9 within a depression slide and observed for 30 min, adding 5 ul of fresh 5% ethanol solution every 10 min.

Cold shock

For evaluation of behavior following cold stress, a seeded NG agar plate (55 mm diameter, 12ml agar) containing 20-30 young adult animals was immersed in a frozen bead bath (Bath Armor) in a chest freezer (–14˚C to –16˚C) for 30 minutes. We noticed that the occasional plate did not show the rough agar surface indicative of freezing; these were discarded from the analysis. After cold shock, animals were scored immediately for feeding and locomotion behavior, and then quickly transferred to room temperature plates. Animals were then scored every 10 minutes for feeding and locomotion.

Cry5B toxin

A colony of JM103 bacteria containing an IPTG-inducible Cry5B protein expression plasmid[S4] was grown in LB broth for 6 hours and plated over the entire surface of NG plates containing carbenicillin and 1mM IPTG. These plates were incubated overnight at 37˚C, and used no sooner than 24 hours later for behavioral assays, as we noted that the plates were not as effective at inhibiting *C. elegans* locomotion before that time. Young adult animals were placed onto the Cry5B plates and scored every 5 minutes during exposure, transferred to regular NG plates seeded with OP50, then scored every 15-60 minutes for 5 hours. Behavior was quantified for a longer period than other stressors, as we had noticed a second quiescent bout at the end of the first hour of recovery.

Oxidative stress

For analysis of behavior during and after paraquat exposure, 20-30 young adult animals were placed onto NG agar plates containing 100 mM paraquat (methyl viologen, Sigma 856177) that had been thinly seeded with OP50 *E. coli*. Animals were scored for pharyngeal pumping and locomotion every 15 minutes for an hour of exposure, transferred by wire pick to NG-OP50 plates, and scored again every 15 minutes for an hour of recovery.

Measurement of fluorescence

Two reporter genes were used to measure stress-responsive gene expression: *zSi3001*, a single-copy insertion of an *hsp-16.2::gfp* transgene [S5], and *zcIs4*, a multicopy insertion of an *hsp-4::gfp* transgene [S6]. Animals were mounted on 2% agarose pads in M9 containing 5 mM levamisole for immobilization. 12-bit fluorescence images of the anterior intestine of heat-shocked and untreated control animals were taken under identical exposure on a Zeiss Axio Imager A2 fluorescence microscope at 1000x. Mean pixel intensity over the brightest 80 um^2 circular region within the frame was determined using Zen software (Zeiss). At least ten animals of each genotype per condition were examined.

Survival assays

For analysis of survival in response to heat, seeded NG agar plates (55 mm diameter, 12ml agar) containing young adult animals (raised at 20˚C) were wrapped in parafilm and placed in a water bath at 35˚C or 37˚C for 30 minutes, or at 40˚C for 20 minutes. After heat shock, the plates were cooled to room temperature using a 2 minute incubation on ice, then returned to a 20˚C incubator. Animals were scored every 24 hours for fraction surviving, by visual inspection and prodding with a wire pick. An animal was considered to be dead when there was no visible reaction to any of 3 prods (to head, tail, and mid-body). For analysis of survival in response to oxidative stress, young adult animals were placed onto seeded NG plates containing 100 mM paraquat for 2 hours, removed to standard NG-OP50 plates at 20˚C, and scored every 24 hours for survival as above. Control survival curves for strains in standard (non-stressed) conditions were obtained by collecting young adult animals to NG-OP50 plates and scoring fraction surviving every 24 hours at 20˚C, transferring animals to new plates 2 days later to avoid confusion with the next generation. This transfer step was not required in the case of extreme heat shock, as this severely decreased fertility and the few progeny could be removed instead. We noted that a ruptured vulva phenotype led to lethality in a fraction of older *plc-3* animals, a phenotype that was notably absent in *plc-3* animals that had been heat shocked. The cause of this phenotype is not clear. For survival experiments involving the *egl-4(gf)* mutation (Figure 4E), all plates were allowed to recover in an isolated area at room temperature to avoid vibrations associated with the 20˚C incubator, because the *egl-4(ad450)* spontaneous sleep phenotype is highly sensitive to perturbation[S7]. Because of this sensitivity, only locomotion data was collected for Figure 4f, as this can be scored at low magnification. Analysis of pharyngeal pumping requires high magnification and hence frequent plate moving in order to score a large number of animals.

Statistics

For each experiment involving behavioral analysis, at least 3 independent trials of 20-30 worms were performed per genotype. The number of worms per trial was limited to 30 so that animals were at a low density on the plate, with minimal interaction, and it was possible to score

the behavior of each genotype within the allotted time frame. For survival assays, 30-50 animals per trial were used. Statistical analysis and figure preparation were performed using Prism software (Graph Pad). For categorical data, single time point analyses were performed using the Fisher's exact test, and curves were compared using the log-rank test (http://bioinf.wehi.edu.au/software/russell/logrank/). For quantitative data, (Figures 1B, 2E, 4D) a Student's t test was used. Animals were scored blind with respect to genotype for at least one trial of each experiment.

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