Supplementary Materials

Materials and Methods

Culture of *C. elegans* and Strains. All strains were maintained at 20° on nematode-growth-medium NGM agar plates seeded with OP50 bacteria unless otherwise noted (*1*). The wild-type strain used for all experiments was N2 variant Bristol (*1*). CB4856 is the sequence polymorphic mapping strain used for SNP mapping (*2*). The integrated GFP-array bearing strains PD4251 containing [nls::pmyo-3::GFP] (*3*) and SK4005 containing zdIs4[pmec-4::GFP;lin-15(+)] (*4*) were kindly provided by Monica Driscoll and Scott Clark. All other strains were obtained from the *Caenorhabditis* Genetics Center funded by the NIH NCRR. Construction of the double mutants *rrt-1(gc47);Pmyo-3::GFP* and *rrt-1(gc47);Pmec-4::GFP* were generated by crossing *gc47/+* males into each of the integrated GFP-array bearing strains. F_2 progeny segregating 100% green progeny were kept and phenotyped for hypoxia resistance.

EMS Mutagenesis screen. In a clonal screen of 3884 genomes, the progeny of single F2 from EMS mutagenized P0 animals (*5*) were screened for survival following exposure to hypoxia. Fourteen hypoxia resistant mutants falling into 13 complementation groups were isolated. *gc47* was identified as a strongly resistant mutant and was outcrossed twice. This outcrossed strain was used in all subsequent experiments.

Hypoxic incubations. Hypoxic incubations were performed as previously described except the hypoxic incubation temperature was 26.5° (*6, 7*) . Hypoxia-treated animals were scored after a 24-hour recovery period on seeded NGM agar plates in room air at

20°. Animals without spontaneous or evoked movement (touching with a platinum wire) and without pharyngeal pumping were scored as dead.

Mapping of the Hyp phenotype to the *rrt-1* locus. Chromosome and interval mapping of *hyp-3(gc47)* was performed as described by Davis et al (*2*). Briefly, *hyp-3(gc47)* males were crossed into CB4856 and 28 hypoxia resistant (Hyp) animals were recovered among the self-progeny of *gc47*/CB4856 heterozygous hermaphrodites. These 28 Hyp mutant animals and 28 phenotypically wild-type animals were pooled into separate tubes and genomic DNA was prepared. Analysis of the divergent SNPs indicated an enrichment of Bristol bands in the mutant lanes and an enrichment of Hawaiian DNA in the non-mutant lanes for SNPs lying between -12 and +12 mu on Chromosome III. Subsequent genotyping of individual potential recombinants between *gc47* and CB4856 (HA) SNPs on chromosome III revealed that 12/22 mutants were homozygous Bristol at all four SNPs (-12, -7, -1, +4 mu), and the remaining 10 were Hawaiian at one or more SNPs. 5/10 with Hawaiian (HA) at -12 mu; 2/10 at -7; 1/10 at -1; and 6/10 at +4 mu. This placed *gc47* between -7 and +4 mu near – 2 mu. The Hyp phenotype of *gc47* was then mapped relative to *unc-93* (-5.02 mu) and *dpy-17* (-2.15 mu) on chromosome III (Fig. 2a). *gc47* males were crossed with *unc-93(e1500) dpy-17(e164)* animals and the resultant heterozygotes produced 11/14 Unc non-Dpy and 2/16 non-Unc Dpy recombinants that were Hyp, placing *gc47* just to the left of *dpy-17*. An *unc-93 gc47 dpy-17* triple mutant was constructed and then crossed with CB4856. 61/63 non-Unc Dpy and 4/67 Unc non-Dpy recombinants were hypoxia resistant. SNP analysis of these Hyp recombinants was carried out with confirmed SNPs (as reported on the Exilixis SNP Database) or those

found by us by random sequencing in the interval (Fig 2a and Table S3). The SNP genotype of each Hyp recombinant between CE3-140 and snpLA3 was as follows: for the Unc Hyp non-Dpy recombinants, 1/4 were HA positive with the leftmost HA snp being snpLA3; for the non-Unc Hyp Dpy recombinants, 6/61 were HA positive with all six HA at CE3-140, 3 out of the six were HA positive at both snpLA1, and snpLA2 and 1 out of the six was HA at CE3-141. This placed *gc47* in a 106 kb interval between CE3-141 and snpLA3.

RNAi Experiments. RNAi bacterial strains were obtained from the Ahringer Library(*8*) and were grown overnight in 2xYT broth with 50 µg/ml ampicillin and 10 µg/ml tetracycline at 37° and then diluted 1:100 in 2xYT with the same antibiotics and grown at 37° with shaking (225 RPM) until reaching an OD of 0.4. A total of 150 ml of the RNAi bacteria plus 0.4 mM IPTG and 50 µg/ml carbenicillin was added to each NGM plate. After 2 days of bacterial growth at 23°, age-synchronized eggs were grown to young adulthood, unless otherwise noted, on the RNAi plates then assayed.

Transgenics. Fosmid clones, WRM0640cH03, WRM615bG07, WRM0616bD07, WRM065cA04, and WRM0632dC07, spanning the 106 kb interval were a gift from Mike Nonet and were from Geneservice Ltd – (http://www.geneservice.co.uk/home). DNA was prepared using a Qiagen midiprep protocol. Extrachromosomal transgenes were obtained by gonadal micro-injection of prepared fosmid DNA along with a GFP co-injection marker (pPHGFP, kindly provided by P. Hoppe) into the gonad of young adult N2 animals (*9*). *gc47* animals transformed with extrachromosomal arrays were generated by crossing *gc47* hermaphrodites into N2 males containing the transgene of interest and

looking for progeny segregating green animals. The *gc47* homozygous animals were identified by showing that all non-green progeny segregating from green animals were hypoxia resistant.

Quantitative Real-Time PCR Analysis. A synchronous population of wild-type animals was treated with the test RNAi for 3 days or until the worms reached adulthood. A random fraction of the plates were set aside, and the animals were tested for their hypoxia resistance and that data was included in Table S1. RNA was isolated from the remainder of the plates by a Trizol freeze-cracking method. cDNA was synthesized with a RETROscript random decamer kit (Ambion, Austin, TX) with 2 µg of total RNA as template. Quantitative real-time PCR was performed with SYBR green PCR master mix (Applied Biosystems, Foster City, CA) in an Applied Biosystems 7500-fast RT PCR instrument with a Rox passive-reference dye. Primers were constructed to amplify an approximately 100 bp fragment of the RNAi transcript. The ACT-3 β-actin transcript was used as the endogenous control (housekeeping gene); target primer sequences are available upon request. Standard PCR amplification with the primer sets produced single bands migrating at the correct size. Fold-expression changes were calculated with the formula $2^{-\Delta\Delta CT}$, where ΔΔCT is the RNAi ΔCT [test RNAi cycle threshold(CT) value – ACT-3 CT value] subtracted by the control ΔCT [L4440 cycle threshold(CT) value – ACT-3 CT value]. In all cases, raw SYBR fluorescence values were normalized to the passive-reference dye ROX.

Measurement of translation rate. This protocol is adapted from Hansen et al (*10*). A single 50 ml conical tube of OP50 bacteria was cultured at room temperature with

shaking overnight in LB broth containing ^{35}S-methionine (Perkin-Elmer, Cat# NEG-709A) 10μCi/ml culture. The radiolabeled OP50 was pelleted, resuspended in 0.1 volumes of LB, and 100 μl was aliquoted into 1.5 ml eppendorf tubes. Synchronous cultures of N2 or *rrt-1(gc47)* L4 stage worms were raised from eggs to adult on uncrowded agar plates seeded with unlabeled OP50 for the *rrt-1(gc47)* strain only, L4440, *rrt-1(RNAi), mrs-1(RNAi),* or *prs-2(RNAi)* bacteria. Approximately 5000 worms/genotype or condition were washed from the plates with S-basal buffer, pelleted, and washed two more times. For each condition, worms were then aliquotted into 3 tubes with the radiolabeled OP50 bacteria and incubated for 3 hours at 22° with constant rotation. To determine the level of contamination by unincorporated ^{35}S-methioine, a separate aliquot was incubated for only 1 minute in the radiolabeled OP50. Worms were then pelleted, washed 3 times with S-basal, and incubated with unlabelled OP50 bacteria for 30 minutes. Worms were then pelleted, washed 3 times with S-basal, and the pellet flash frozen in liquid nitrogen, thawed then resuspended in 100 μl of 1% SDS and boiled for 15 minutes with periodic vortexing. The tubes were centrifuged at 16,000 g, and the supernatant was removed into a new tube with equal volume ice cold 10% trichloroacetic acid and incubated for 1 hour on ice. The TCA precipitate was pelleted at 16,000 g and washed twice with ice-cold ethanol and allowed to air dry. The pellet was resuspended in 100 μl of 1% SDS, 0.1M Tris-HCl, pH=8.0 and boiled for 30 minutes with periodic vortexing. Upon cooling, triplicate aliquots were used for determination of [protein] (BCA protein assay kit, prod#23227, Thermo Scientific) and radioactivity incorporation

by liquid scintillation (Beckman LS 6000IC). The level of contamination by unincorporated ^{35}S-methioine was less than 4%.

Imaging Phsp-4::GFP. N2, MC240 *rrt-1(gc47),* SJ4005 *zcIs4*[P*hsp-4*::GFP], SJ30 *ire-1(zc14);zcIs4,* and SJ17 *xbp-1(zc12);zcIs4* were the full genotypes of the strains used for these experiments. For measurement of P*hsp-4*::GFP induction under normoxic, hypoxic or tunicamycin conditions, age synchronous, 1-day post L4 adult animals raised on L4440 or *rrt-1(RNAi)* were incubated for 6 hours in M9 in normoxic or hypoxic conditions, or in M9 containing 25 μg/ml tunicamycin in room air. Animals were allowed to recover then a random sample of 10 animals/condition was transferred to agar pads and imaged. Pictures were taken at 5X with a Retiga Exi CCD camera using Q Capture Pro software with identical settings for all worms. After background subtraction and global calibration against a known standard OD step tablet, the integrated optical density for the entire worm was measured using ImageJ software (NIH). For measurement of the time course of Phsp-4::GFP induction, animals were grown on OP50 bacteria until one day post L4 then the worms still on the agar plates were placed into the hypoxic chamber or a 26.5° normoxic incubator for various times and allowed to recover for various times then imaged as described.

Pathological Scoring. L1 and L3 larvae underwent hypoxic incubations as described above. All pathologies were scored on surviving animals after 24 hr of recovery by an observer blinded to conditions. Muscle nuclei were visualized with a nuclear-localized GFP driven by the muscle-specific promoter, pmyo-3::GFP (*3*). Fragmented or missing nuclei were scored as pathologic. Axonal-beading pathology was scored in

mechanosensory neurons visualized with pmec-4::GFP (*4*). An axon with any beads was scored as positive for beading.

Lifespan and Brood Analysis. Worms were tightly synchronized on OP50 seeded NGM plates and eggs were incubated at 15 °C until they reached the L4 stage. Once L4 animals reached the L4 molt (t = 0 days), 80 worms of each strain were picked to several NGM plates (20 worms/plate). Animals were transferred to fresh OP50 plates every 1-2 days. Worms that crawled off the plate, bagged, or ruptured were censored. Kaplan Meier survival curves were generated and statistically compared using GraphPad Prism Software (GraphPad Software, Inc., San Diego, CA, USA). Brood size and embryonic lethality was determined by putting one L4 animal per plate and transferring to a fresh plate every 1-2 days and counting the total hatched and unhatched eggs one day post-transfer. Brood was calculated as the total number of hatched and unhatched animals. Embryonic lethality was calculated as the ratio of unhatched animals to the entire brood for each animal.

Measurement of locomotion rate. The speed of locomotion was measured by transferring 10 randomly-chosen young adults for each condition to an agar plate without bacteria. After a 5 minute quiescent period, 30 second digitized movies (1 frame/sec) were taken of each animal's unstimulated movement, and the distance traveled each second was measured and averaged over the 30 frames to obtain a mean speed for each animal.

Table S1. Hypoxia resistant mutants from EMS clonal screen

Strain	% Dead	Other Phenotypes	Fails to Complement	Recessive
N2	96.4 ± 4.1			
hyp-3(gc47) MC240	1.2 ± 2.2	Mildly slow growing	*gc45*	yes
hyp-4(gc46) MC238	2.2 ± 2.9	None	Itself	yes
MC241	55.4 ± 7.7	None	MC249	yes
MC242	54.4 ± 15.5	None	Itself	yes
MC243	12.8 ± 10.8	Moderate Unc	Itself	yes
MC244	39.7 ± 20.6	None	Itself	yes
MC245	60.3 ± 15.3	Long body	Itself	yes
MC246	50.2 ± 18.6	Long body	Itself	yes
MC247	56.9 ± 26.9	Maybe be weak Daf-c	Itself	yes
MC248	37.2 ± 7.4	None	Itself	yes
MC249	18.1 ± 10.5	None	MC241	yes
MC251	21.9 ± 10.9	Long, slightly Unc, weak Daf-c	Itself	yes
MC252	46.0 ± 15.3	None	Itself	yes
MC253	36.4 ± 14.3	Slightly Dpy	Itself	yes

% Dead is mean ± SD of at least three trials; All strains are significantly different from N2 $p < 0.01$;

hyp is the provisional gene name given to a gene after preliminary mapping shows that it maps to a single locus

Table S2. RNAi knockdown and qRT-PCR of aaRSs.

Gene ID	Gene	aaRS class	cellular location	% Dead ± sem	# Trials	fold down-regulation by RNAi
T02G5.9	krs-1	II	ND	4.4 ± 2.5*	7	13.7
F26F4.10	rrt-1	I	cytoplasmic	6.2 ± 1.0*	16	15.7
F22D6.3	nrs-1	II	cytoplasmic	6.7 ± 2.4*	9	10.7
T11G6.1	hrs-1	II	ND	7.4 ± 1.6*	9	3.2
C47D12.6	trs-1	II	ND	7.6 ± 3.1*	4	2.5
R11A8.6	irs-1	I	cytoplasmic	7.5 ± 1.6*	9	3.6
Y80D3A.1	wrs-1	I	cytoplasmic	8.1 ± 4.7*	4	8.1
T10F2.1	grs-1	II	ND	9.1 ± 2.5*	8	4.7
R74.1	lrs-1	I	mitochondrial	13.0 ± 3.4*	11	16.1
F58B3.5	mrs-1	I	cytoplasmic	30.5 ± 8.4*	8	7.1
ZC513.4	vrs-1	I	cytoplasmic	37.7 ± 9.3*	6	1.9
K08F11.4	yrs-1	I	cytoplasmic	38.8 ± 2.8*	10	0.6
T08B2.9	frs-1	II	cytoplasmic	43.1 ± 6.5*	9	1.8
Y41E3.4	ers-1	I	cytoplasmic	44.1 ± 7.3*	8	1.2
T20H4.3	prs-1	II	cytoplasmic	62.2 ± 5.1*	6	4.2
C29H12.1	rrt-2	I	mitochondrial	62.2 ± 8.3*	9	1.2
F22B5.9	frs-2	II	cytoplasmic	66.7 ± 7.8*	10	0.6
W02B12.6	ars-1	II	mitochondrial	68.2 ± 9.4*	7	1.4
T07A9.2	ers-3	I	mitochondrial	71.5 ± 8.0*	11	0.8
C25A1.7	irs-2	I	mitochondrial	71.7 ± 5.4*	7	4.3
T27F6.5	prs-2	II	cytoplasmic	74.4 ± 7.1*	10	2.4
C34E10.4	wrs-2	I	cytoplasmic	75.6 ± 10.1*	7	1.2
W03B1.4	srs-1	II	mitochondrial	76.8 ± 12.3	8	2.2
	L4440			98.9 ± 0.7	20	

Cellular location is based on Wormbase annotation. ND, not determined based on RNAi transcript. Fold-expression changes were calculated using the $2^{-\Delta\Delta CT}$ method. Trials were independent and consisted of at least 20 worms per trial. * $P < 0.0004$ (one-tailed t test with Bonferroni correction). L4440 is the empty vector control.

Table S3. Phenotypic analysis of *gc47*

genotype	Brood Size	n	Embryonic Lethality, %	n, embryos	Velocity	n
N2	270 ± 4.7	27	2.5 ± 0.3	7289	0.36 ± 0.02	12
gc47	111 ± 3.6*	19	9.7 ± 1.2*	2112	0.17 ± 0.02*	10

values are mean ± SEM; * - $p < 0.01$ (two-tailed t-test)

Table S4: Sequence of identified SNPs

SNP name	Physical location	CB4856	Bristol	Change	Context
CE3-140	III: 4,791,155	T	G	S	tgagacTtcggtt
snpLA1	III: 4,833,638	T	G	S	tacactTtttttt
snpLA2	III: 4,836,082	T	C	S	cactatTagtcaa
CE3-141	III: 4,877,043	T	A	S	tattaaTcttttt
snpLA4	III: 4,946,512	-	A	D	aaaaaa-ttcaaa
snpLA3	III: 4,983,114	T	-	I	ttttttTcaaata

For change, S – sequence, I – insertion, D- deletion. Context is given for CB4856 sequence with SNP indicated in uppercase.

Figure S1. Translation rate in aaRS reduction-of-function strains. ^{35}S-methionine incorporation was used to measure overall translation rate in wild type animals exposed to aaRS RNAi or L4440 and in *rrt-1(gc47)*. Incorporated cpm/μg protein were measured in triplicate samples and then normalized to the L4440 empty vector control. These data are plotted against animal death values from Tables S1 and S2. Values are mean ± sem; for some values the error bars are smaller than the data symbol. The regression line formula was y=1.85*x - 97.5, r^2 = 0.91. Slope p-value = 0.01.

Figure S2 Cycloheximide induces hypoxia resistance. Adult animals (20-30 per plate) were transferred to seeded NGM plates containing varying concentration of cycloheximide or solvent. Plates were prepared by spreading the appropriate concentration of cycloheximide (from a stock solution of 50 mg/ml in ethanol) onto NGM and then seeding with OP50 that was concentrated 10-fold and resuspended in M9 containing the appropriate cycloheximide dilution. Animals were incubated on cycloheximide plates for 24 hours, at which time they were then exposed to 20 hours of hypoxia.

Figure S3. ***gc47* is long-lived.** Median lifespan was 14 days for N2 (n=80) and 16 days for *gc47* (n=80), p = 0.0004 by Chi-quared, logrank test. Worms were synchronized and allowed to reach L4 stage at 15° at which point they were transferred to 20° (t=0). Animals that crawled off the plate, exploded, or bagged were censored and not included

in the total. The maximum lifespan for gc47 was 34 days compared to 26 days for wild type.

Figure S4. **RRT-1 functions in somatic cells to promote hypoxic sensitivity.** N2 (blue) or *rrf-1(pk1417)* (yellow) animals were fed *rrt-1* RNAi through adulthood, then exposed to varying durations of hypoxic insult. The control was N2 animals fed empty vector (black). mean ± s.e.m. of at least three independent trials, > 30 animals per trial. *p < 0.01 vs N2, two-tailed t-test

Figure S5. **Reduction of *rrt-1* function blocks hypoxia-induced morphologic cell defects.** L1 (pmec-4::GFP) or L3 (p-nls-myo-3::GFP) animals in an N2 or *rrt-1(gc47)* background were exposed to 22 hours of hypoxia (HYP) or normoxia (Norm). After 24 hours recovery, surviving animals were scored for cell morphological defects. Scale bars = 20 µm. **(A, C)** Protection of myocytes by *rrt-1(gc47).* Hypoxia produces fragmented myocyte nuclei (arrows) in wild-type animals. Nuclear morphology is preserved in hypoxia-treated *rrt-1(gc47).* *p < 0.01; n=144 (wild type) and 121 (*gc47*) animals. **(B, D)** Axonal pathology reduced by *rrt-1(gc47).* A single touch sensory neuron visualized with GFP is shown. Hypoxia induces an axonal beading morphology (arrow) which is reduced in *gc47* animals following hypoxia insult. *p < 0.01; n=148 (wildtype) and 180 (*gc47*) animals.

Figure S6. Hypoxia-induced paralysis is slower in *gc47* animals. Young-adult animals were washed with M9 from synchronized, seeded NGM agar plates into 1.5 ml eppendorf tubes and rinsed twice with M9. Approximately ten worms were transferred with 20 µl of M9 onto an unseeded NGM agar plate. The plates, with lids removed, were immediately placed into the hypoxia chamber. At 30 minute intervals, the covers were put on the plates and sealed with parafilm prior to removal from the chamber. Worms were immediately scored for movement, either spontaneous or after tapping the plate. Data points represent the mean ± s.e.m. of two independent experiments performed in triplicate > 10 animals per plate.

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Figure S1.

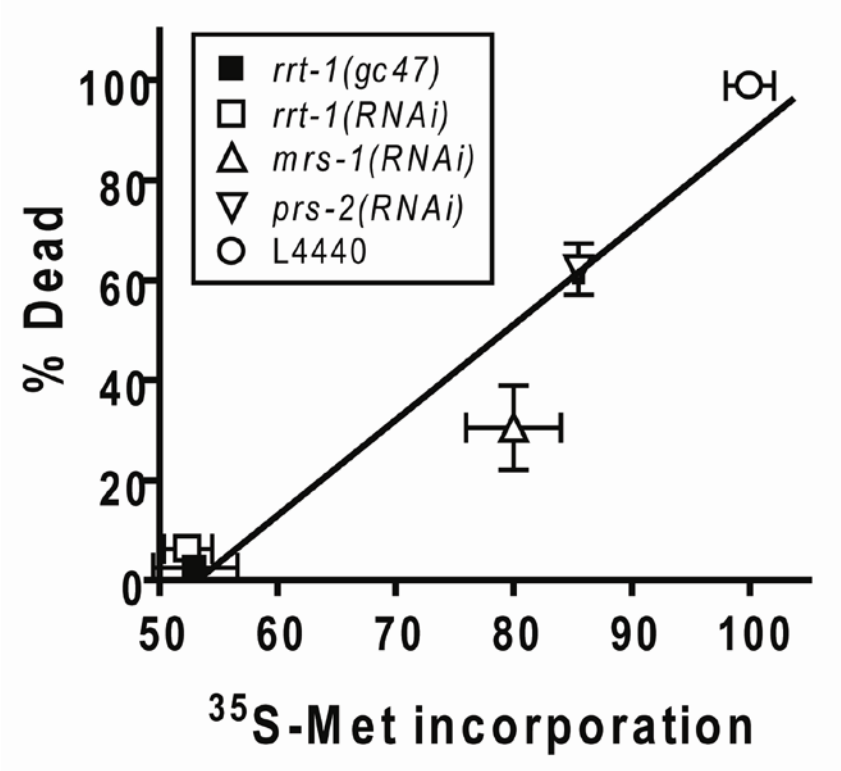
rrt-1(gc47)
rrt-1(RNAi)
mrs-1(RNAi)
prs-2(RNAi)
L4440
% Dead
100
80
60
40
20
0
50
60
70
80
90
100
35S-Met incorporation

Figure S2.

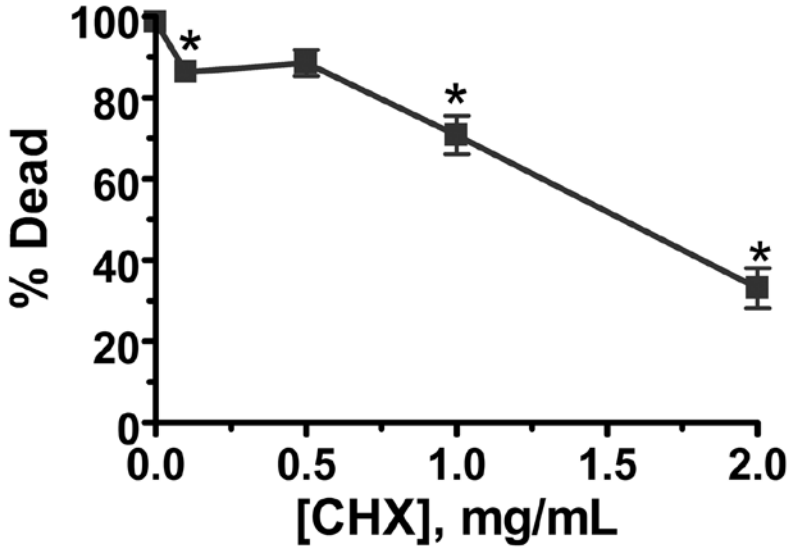

Figure S3.

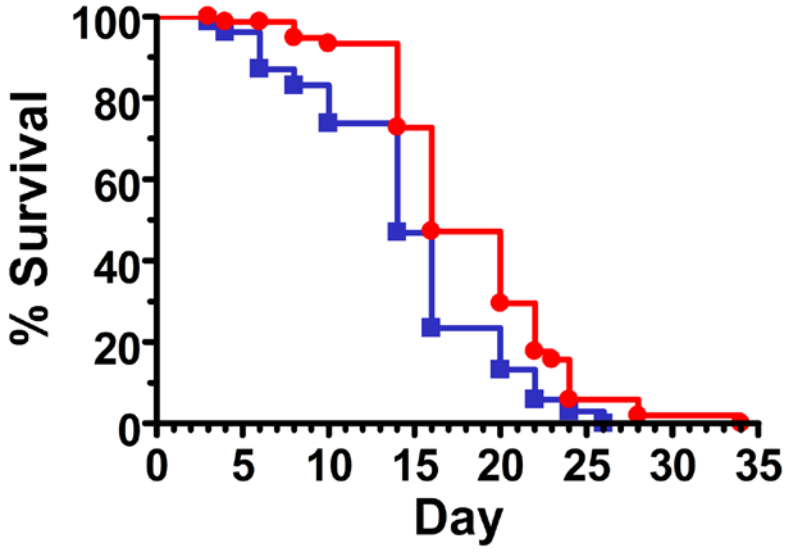

Figure S4.

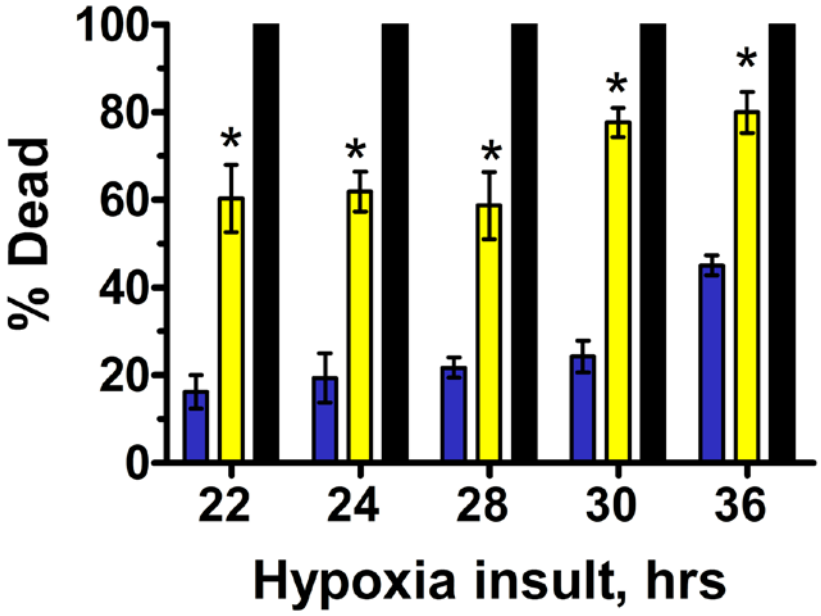

Figure S5.

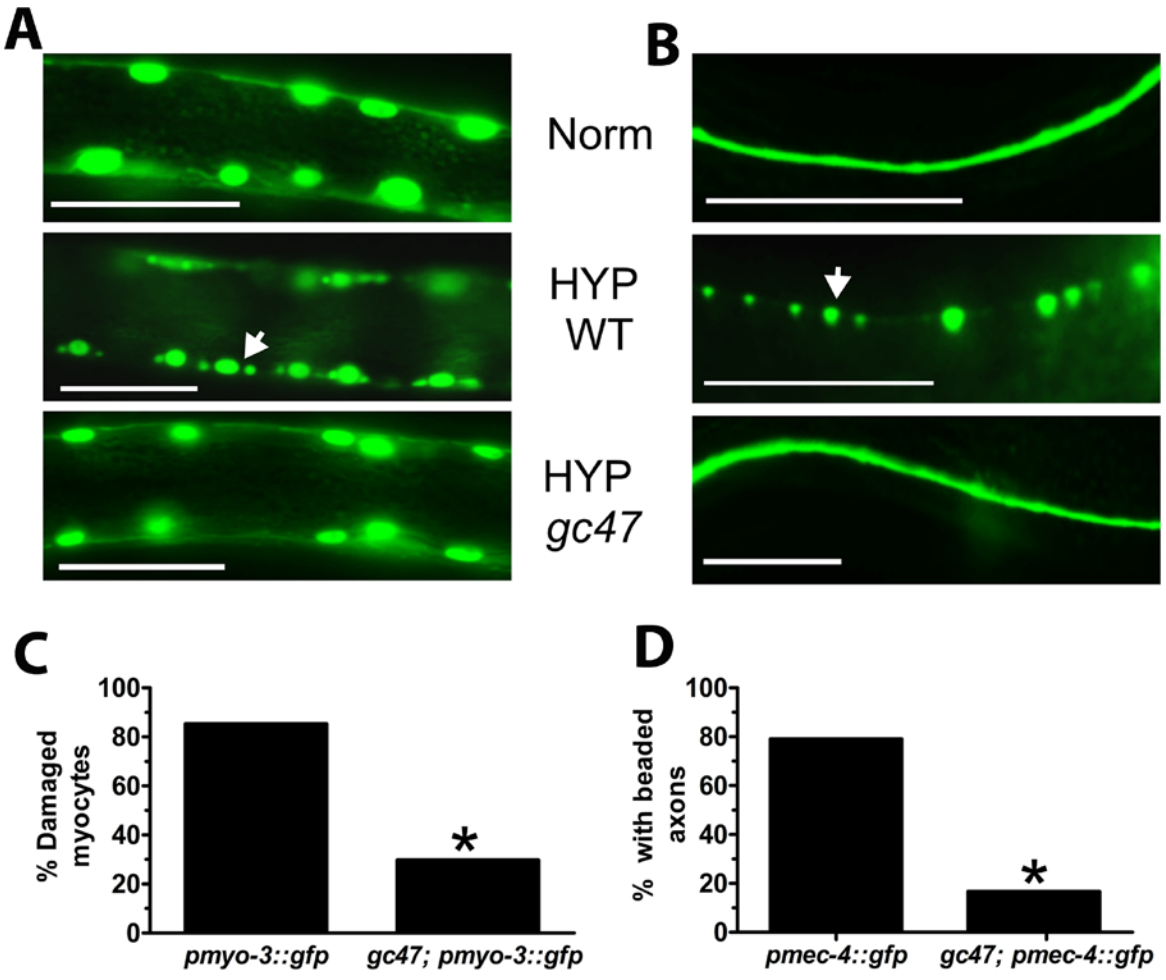
A
B
Norm
HYP
WT
HYP
gc47
C
% Damaged
myocytes
100
80
60
40
20
0
*
pmyo-3::gfp
gc47; pmyo-3::gfp
D
% with beaded
axons
100
80
60
40
20
0
*
pmec-4::gfp
gc47; pmec-4::gfp

Figure S6.