Supporting Information

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SI Materials and Methods

Nematode Strains. *C. elegans* strains were cultivated as described by Brenner (1). The mutant alleles and *gfp* transgenes used in this work were *selb-1*(*sv36*) *I* (this work), *rrf-3*(*pk1426*) *II* (2), *gcs-1*(*ok436*), *trxr-1*(*sv43*) *III*, *trxr-1*(*sv47*) *III* (this work), *zIs356*[*daf-16::gfp rol-6*(*su1006*)] (3), *zcIs4*[*hsp-4*::*gfp*] (4), *muIs84*[pAD76 (*sod-3*::*gfp*)] (5), *svEx267*[P_{gsr-1}::*gsr-1*::*gfp*], *svEx277*[P_{trxr-1}::*gfp*], *svEx285*[P_{selb-1}::*selb-1*::*gfp*], *svEx741*[*gcs-1*::*gfp*] (this work), *chEx1677*[*qua-1*::*gfp*] (6), *svIs36*[P_{lon-3}::*lon-3*::*gfp*]. *svIs36* was generated from the extrachromosomal array, *svEx90* (7). The *gcs-1*::*gfp* plasmid is described in An et al. (8). *gcs-1*(*ok436*) was generated by the *C. elegans* gene knockout consortium. All strains were derived from the N2 wild-type strain (1).

Isolation of *sv36*, *sv43,* and *sv47 C. elegans* Deletion Mutants. A deletion library of wild-type N2 worms mutagenized with EMS was screened for deletions in the *selb-1* and *trxr-1* genes. Pooled genomic DNA samples representing a total of 400,000 haploid genomes were used as templates for nested PCR reactions. For *selb-1*, the first round of PCR was performed with primers with the sequences 5′-GGA CCT CTC AAT CTA GGC ATTC-3′and 5′-CCT TCG ATT TTT CCA CGT GGA C-3′. The second round was performed with primers with the sequences 5′-TTA ACA CGT CGA ATC GCC GAA C-3′ and 5′-GTT TCG GCC TTG AAC ATT CCA G-3′. One mutation, *selb-1*(*sv36*), was isolated. During backcrossing, nematodes were genotyped by single worm duplex PCR. The sequences of the primers used were 5′-TTA TCG ATT GCC CTG GGC ATT C-3′, 5′-CTG GGA CAG TGA TAC GTG GAG-3′and 5′-GAA ATT CGC GCC AAT TTC TTT TC-3′. To screen for mutations in the *trxr-1* gene, PCR was performed first with primers having the sequences 5′-GAT GGC ATG AAA TCT TCT ACC-3′and 5′-GGA GGC GCA TCA GAC CA GAG-3. The second round of PCR was performed with primers with the sequences 5′-GAC AGC CGA GAC AAC AAG AAG-3′and 5′-CTC TACTAG GCA CCA CCG A-3. Two deletion alleles were isolated, *sv43* and *sv47*. The sequences of the primers used to genotype worms during backcrossing of *sv43* were 5′-GAT GGC ATG AAA TCT TCT ACC-3′, 5′-CAT TTA TGA CGT AAC AAC TG-3′and 5′-CAT TCA GGA GTT TGT CGA AC-3′. The sequences of the primers used to genotype worms during backcrossing of *sv47* were 5′-GTC ACT ATC TTG TAT TTC CG-3′, 5′-TTG AGT TCT AAA TTG AAG GC-3′and 5′-TCA GAA TCG TGT TAT ATT CC-3′. The *sv47* deletion removes base pairs 721–2383 of sequence, where 1 is the A of the *trxr-1* start codon. Analysis of the mRNA produced from the *sv47* allele by RT-PCR revealed that it is generated by the splicing of exon 1 directly to exon 5. The exon 5 sequence is out of frame, and the message is predicted to encode a severely truncated protein of just 29 amino acids. The *sv43* deletion removes base pairs 370–2764. In the message generated, exon 1 sequences are spliced to a cryptic splice site in intron 1 at position 249 so that the message has 121 bases of intron sequence between exon 1 and sequences in exon 5. An in-frame stop codon is generated by the splicing event, and the exon 5 sequence is out of frame: the message encodes a protein of 58 amino acids. The *sv36* deletion removes base pairs 853–2427 of genomic *selb-1* sequence. In the message generated, exon 1 is spliced directly to exon 6 resulting in disruption of the reading frame. The message encodes a protein of 143 amino acids that lacks motifs at the C terminus known to be essential for the ability of *E. coli* SelB to bind to selenocysteine insertion elements (SECIS; ref. 9).

RNAi. To perform RNAi (10), 10-mL liquid cultures of *E. coli* HT115(DE3) bacteria in 2× YT containing 50 μg/mL carbenicillin and 12.5 μg/mL tetracycline were established from frozen cultures and grown for 5–6 h at 37 °C. Small amount of the cultures were spread onto NGM agar worm culture plates supplemented with 50 μg/mL carbenicillin and 1 mM IPTG. The plates were left for 12 h or more for the bacterial lawn to grow and then seeded with five L4 larval worms of the appropriate genotype. The plates were left for 24 h at 20 °C after which time the worms were transferred to fresh RNAi plates. After a further 8 h, the parental worms were moved to fresh plates, allowed to lay eggs for 12 h, and then removed. Only eggs and larvae from the last set of plates were used in phenotypic tests. Larvae with reduced zygotic and maternal activity of *gcs-1* were generated by placing *gcs-1*(*ok436*)/*mIn1* hermaphrodies on *gcs-1* RNAi plates. The *gcs-1*(*ok436*) homozygotic progeny were examined for molting defects. When their mothers are grown on normal plates, *gcs-1* (*ok436*) homozygotes are maternally rescued and grow up to become sterile adults. *gcs-1*(*RNAi*) with N2 worms [or with the *rrf-3* (*pk1426*) strain] did not cause obvious defects at a high penetrance.

^{75}Se Labeling of *C. elegans* Worms. Worms were labeled with ^{75}Se by feeding them *E. coli* bacteria that had been grown in the presence of ^{75}Se. The protocol described by Buettner et al. (11) was used with minor modifications. Briefly, a 1.3 mL culture of *E. coli* strain OP50 (1) was grown overnight in LB medium supplemented with cysteine to a final concentration of 100 μg/mL and 20 μCi of ^{75}Se. A total of 200 μL of the overnight culture was spread onto an NGM agar *C. elegans* culture plate and allowed to grow for 24 h at room temperature (~21 °C). After this time, the plate was seeded with five L4 hermaphrodite worms of the appropriate strain. The worms were allowed to grow until they had just consumed all of the bacteria, and then washed off the plate with M9 buffer (22 mM KH_2PO_4, 42 mM Na_2HPO_4, 85 mM NaCl, 1 mM Mg_2SO_4). After at least three washes in M9 buffer, the worms were left at room temperature for 45 min to allow the digestion of any bacteria remaining in their intestines. The worms were then washed twice in ice-cold protein lysis buffer (150 mM NaCl, 50 mM Tris.Cl, pH 8.0, Complete protease inhibitor mix containing EDTA; Roche Diagnostics). The worms were broken open by sonication in this buffer.

Isolation of the *E. coli* HT115(DE3) Lacking selD. A protocol developed by Datsenko and Wanner was used to create the *selD* mutant strain (12). HT115(DE3) [Genotype F-, *mcrA*, *mcrB*, IN (*rrnD-rrnE*)1, *rnc14*::Tn10(DE3 lysogen: lavUV5 promoter -T7 polymerase)] was first transformed with pKD46, a plasmid encoding the λ Red recombinase under the control of an arabinose-inducible promoter (12). pKD3 plasmid DNA, which contains the chloramphenicol resistance marker (Cm^R) DNA was used as a template in PCR with the primers 5′-AAT TCA GAC ACT CTC ACT TAT CAC TTC ACG GAA TGA GGG TGT AGG CTG GAG CTG CTT C-3′and 5′-CGC GCG CCA GAC TCG GTT TTT CGG CAA TAA ACA ACC GCA TCA TAT GAA TAT CCT CCT TAG-3′. The PCR product, which contained Cm^R flanked by *E. coli selD* sequences, was digested with DpnI and then transformed in the presence of arabinose into HT115 (DE3) harboring pKD46. The colonies were selected on plates containing chloramphenicol and tetracycline at 37°C, at which temperature pKD46 cannot replicate. PCR with the primers 5′-ACG TCC CGG ACC CGA CGC CG-3′ and 5′-GCC TTT CCG GTG CGG TTT GG-3′ was used to screen the colonies for the

presence of the transformed fragment integrated at the genomic *selD* locus. To eliminate the Cm^R marker, cells grown at 37°C were transformed with pCP20, which encodes FLP recombinase under the control of a thermal-inducible promoter, confers resistance to carbenicillin (Cb^R), and is temperature sensitive for replication (12). Transformants were initially checked by PCR for the elimination of the Cm^R fragment and then for sensitivity to Cm and Cb. Finally, to generate a strain for RNAi of *C. elegans gsr-1*, a plasmid from the Ahringer library encoding double-stranded RNA from *gsr-1* was transformed into the HT115(DE3) Δ*selD* strain.

Western Blotting with anti-CeTRXR-1 Antibodies. Samples for Western blot analysis were prepared by growing three plates for each strain until the worms had consumed nearly all of the bacteria. Worms were washed with M9 several times until there were no bacteria left. To the pellet of worms was added twice its volume of Nonidet P-40 lysis buffer (150 mM NaCl, 1% Nonidet P-40, 50 mM Tris[chemp]HCl pH 8) containing protease inhibitor mixture. The preparation was quickly frozen in liquid nitrogen. After thawing, the pellets were resuspended in SDS/PAGE sample buffer and then incubated for 10 min at 95 °C. Insoluble worm debris was cleared by centrifugation at 13,000 rpm in a microfuge. Protein samples were run in a 10% 0.75 mm SDS/PAGE gel at 120 V and then the blotted onto membrane for 1 h at 90 mA under semidry conditions. A 5% solution of nonfat dry milk blocking was used to block nonspecific binding. Western blots were probed with affinity purified anti-TRXR-1 antibody at a 1:1,000 dilution. Two bands are detected in extracts from wild-type worms, that are missing in the *trxr-1*(*sv47*) mutant. We do not at present know how the *trxr-1* gene gives rise to two proteins. RT-PCR analysis of *trxr-1* transcripts failed to provide evidence for alternative splicing or use of alternate promoters.

Previous work has shown that expression of a full length *C. elegans trxr-1* cDNA in mammalian cells leads to the expression of both isoforms (13). Because there are four in-frame ATG codons close to the start of the ORF, it is possible that the proteins result from translation initiation at two different start sites. Alternatively, TRXR-1 protein might be posttranslationally processed. The antibody also reacts nonspecifically with two more slowly migrating proteins present in all strains.

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6. Hao L, et al. (2006) The hedgehog-related gene *qua-1* is required for molting in *Caenorhabditis elegans*. *Dev Dyn* 235:1469–1481.
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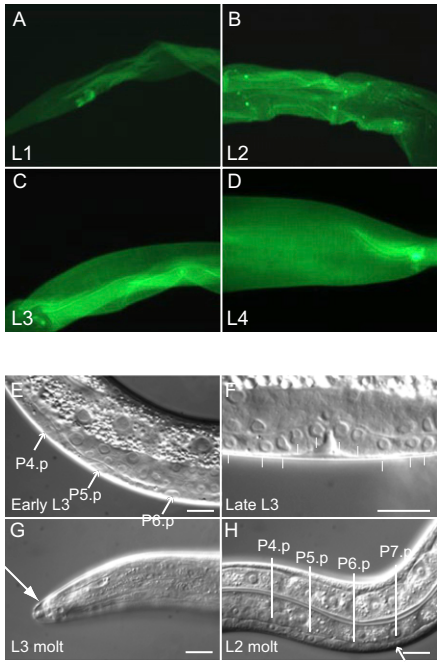

Fig. S1. Cuticle components are reduced during the L1–L4 molts, and inhibiting reduction inhibits molting. (*A*–*D*) Micrographs of shed cuticle from the indicated molts stained with Alexa C_5-maleimide. (*E*–*H*) Micrographs of worms treated with diamide viewed with DIC optics. Note that diamide prevents progression through the molt but does not block growth during the intermolt. (*E*–*G*) Images of the same worm at different time points that had been exposed to diamide directly after the L2 molt. Diamide did not prevent the divisions of cells in the vulval equivalence group (P3.p-P8.p) from occurring during the intermolt, but did cause a block at the L3 molt. P4.p, P5.p, and P6.p are indicated by arrows in *E*. The lines in *F* indicate descendents of P5.p, P6.p, and P7.p during their third round of division. The arrow in *G* indicates partially detached cuticle at the anterior of the worm arrested at the L3 molt. (*H*) A late L2 larval worm that had been exposed to diamide. P3.p-P8.p failed to divide, and the worm was blocked at the L2 molt.

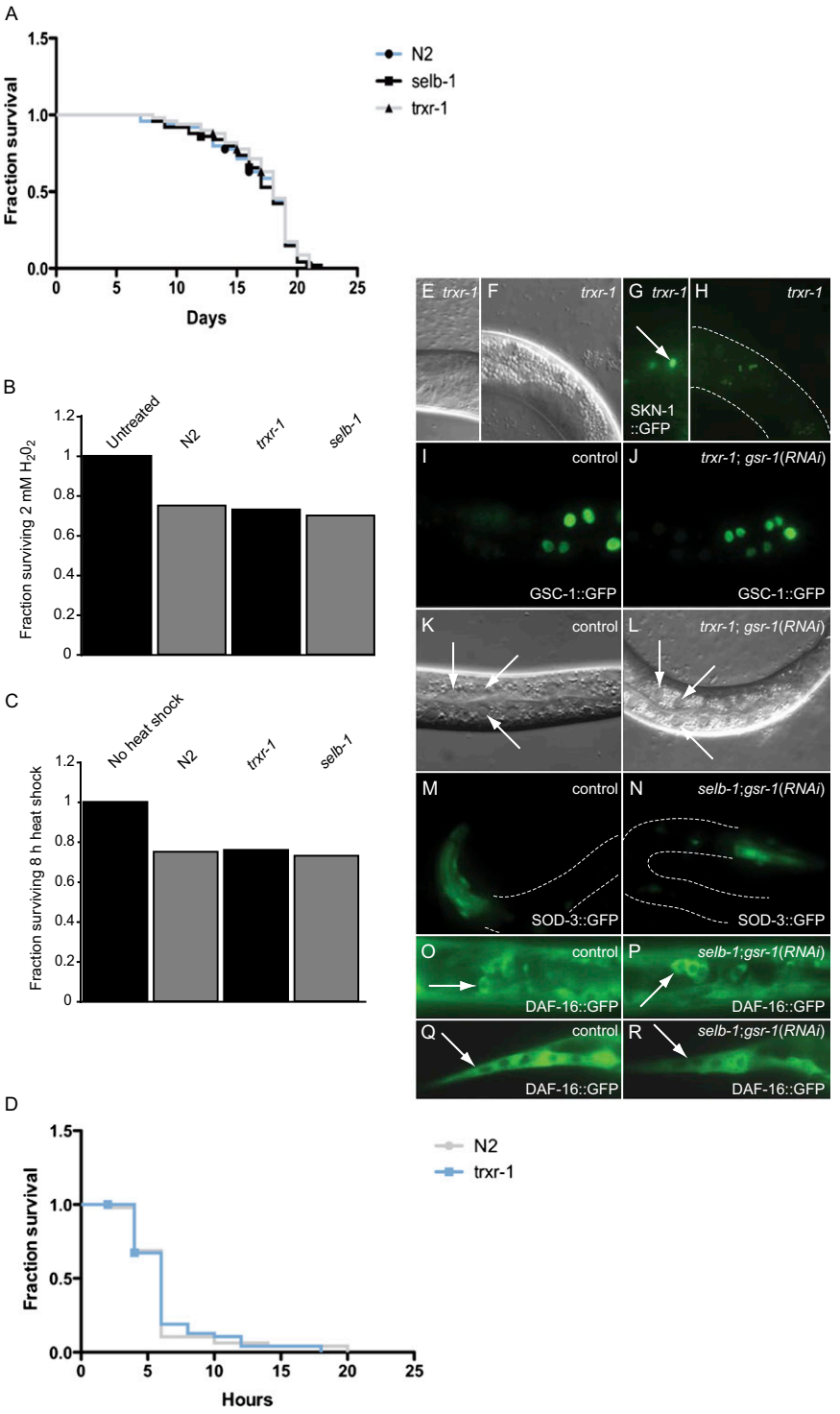

Fig. S2. *trxr-1* and *selb-1* mutants are not short lived, do not show increased sensitivity to acute oxidative stress, and do not display induction of molecular markers for the oxidative stress response. (*A*) Graph showing the proportions of *trxr-1* and *selb-1* worms grown at 20 °C surviving over time. Time 0 denotes the L4 to adult molt. (*B*) Fraction of *trxr-1* and *selb-1* worms surviving after incubation in 2 mM H_2O_2 for 2 h. (*C*) Fraction of worms surviving after heat shock for 8 h at 33 °C. (*D*) Fraction of worms surviving at the indicated times after incubation in 6 mM t-butyl peroxide. (*E*–*R*) Worms harboring markers for oxidative stress. (*E*, *F*, *K*, and *L*) Micrographs of worms viewed with DIC optics. (*G*–*J* and *M*–*P*) Micrographs of worms viewed with fluorescence optics. *trxr-1* denotes *trxr-1*(*sv47*), and *selb-1* denotes *selb-1*(*sv36*). The SKN-1::GFP marker is constitutively expressed in ASI sensory neurons; expression is induced in the intestine upon oxidative stress (1). (*G*) The constitutive expression in ASI (indicated by an arrow) is normal in *trxr-1* mutant animals (the weak signal is background autofluorescence seen at long exposure times). (*H*) Expression in the intestine is not induced. The GSC-1::GFP marker is expressed in posterior intestinal cells in unstressed animals and throughout the intestine in stressed animals (1). In both the control worm shown and the *trxr-1*; *gsr-1*(*RNAi*) animal, only the posterior intestinal cells express the marker. The arrows in *K* and *L* indicate more anterior intestinal nuclei that do not show appreciable GFP fluorescence. The worms shown harbored *svEx741*, an array made by injecting *gsc-1*::*gfp* DNA kindly provided by J. H. An and K. Blackwell (1). The SOD-3::GFP marker is widely expressed but most strongly in the pharynx (2). The level of expression is increased upon stress. The expression of the marker was not increased in *selb-1*; *gsr-1*(*RNAi*) animals, which have reduced *trxr-1* function (this study). SOD-3::GFP was encoded by *muIs84* (2). The DAF-16::GFP marker is predominantly cytoplasmic in unstressed animals, but pre-

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dominantly nuclear in animals exposed to oxidative stress (ref. 3; or those with reduced insulin signaling). No difference in the distribution of DAF-16::GFP was seen in *selb-1*; *gsr-1*(*RNAi*) mutant strain compared with the control. DAF-16::GFP was encoded by *zIs356* (3). The animals in *I–R* were homozygous for *rrf-3*(*pk1426*).

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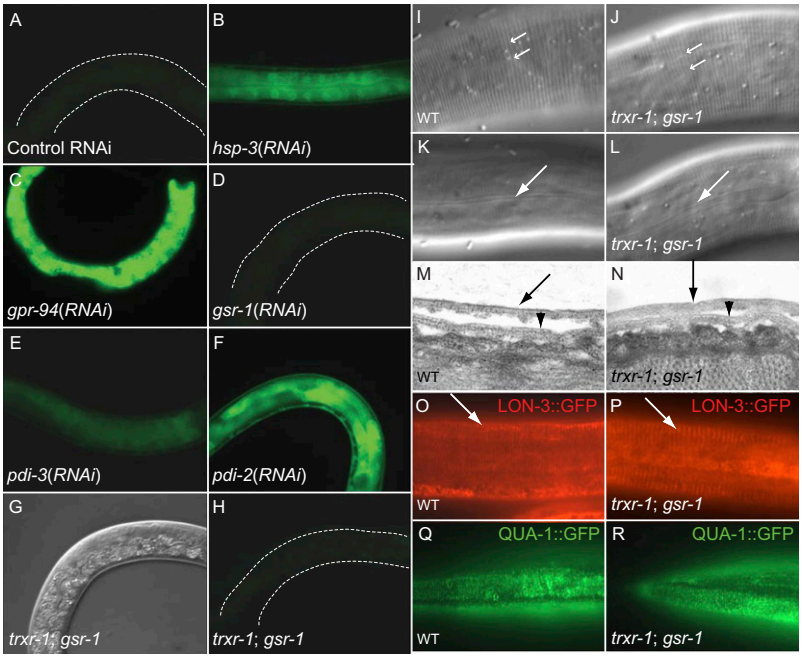

Fig. S3. The unfolded protein response is strongly induced in *pdi-2* mutant larvae but not in *trxr-1*; *gsr-1*(*RNAi*) larvae, which do not display cuticle synthesis defects. (*A–H*) Micrographs of L2 larvae harboring the *zcIs4* transgene array, which encodes *hsp-4*::*gfp*, a marker for the unfolded protein response (UPR; ref. 1). The larvae in *A–F* and *H* were viewed with fluorescence optics; the larva in *G* was viewed with DIC optics. *hsp-3*(*RNAi*) and *grp-94*(*RNAi*) are positive controls previously shown to cause induction of the UPR (2). Control RNAi in *A* was *zcIs4* worms cultured on HT115 (DE3), the *E. coli* RNAi strain, harboring empty vector, L4440. *pdi-2* or *pdi-3* encode proteins homologous to those in yeast that promote protein folding within the ER (3). *trxr-1*; *gsr-1* denotes *trxr-1* (*sv47*); *gsr-1*(*RNAi*). Note that whereas RNAi directed against *pdi-2* (and to a lesser extent *pdi-3*) caused a potent induction of *hsp-4*::*gfp*, no induction was seen in *trxr-1*; *gsr-1* animals. (*I–R*) Absence of cuticle synthesis defects in *trxr-1*; *gsr-1* larvae. (*I* and *J*) Annuli (indicated by arrows) in wild-type and *trxr-1*(*sv47*); *gsr-1* (*RNAi*) larvae. Annuli, circumferentially oriented parallel furrows in the cuticle, are disrupted in mutants with defects in cuticle synthesis or secretion (4). (*K* and *L*) Alae (indicated by arrows) in wild-type and *trxr-1*(*sv47*); *gsr-1*(*RNAi*) L1 larvae. Formation of alae, longitudinal ridges that run along either side of the animal in L1, dauer and adult worms is disrupted in animals with aberrant cuticle secretion (5). The alae are neither absent nor obviously abnormal in *trxr-1* mutants subjected to *gsr-1*(*RNAi*). (*M* and *N*) Transmission electron micrographs of cuticle in molting wild-type and *trxr-1*(*sv47*); *gsr-1*(*RNAi*) larvae. The arrows and arrowheads indicate the old and new cuticle, respectively. (*O* and *P*) Fluorescence micrographs of worms harboring a LON-3::GFP transgene stained with an anti-GFP antibody. LON-3 is a cuticle collagen (6, 7). LON-3::GFP in the mutants is localized to the annuli, indicated by arrows, as it is in wild type. (*Q* and *R*) Fluorescence micrographs of worms harboring a QUA-1::GFP transgene. QUA-1 is a cuticle protein that controls molting (8). With the exception of wild type, all strains were homozygous for *rrf-3*(*pk1426*).

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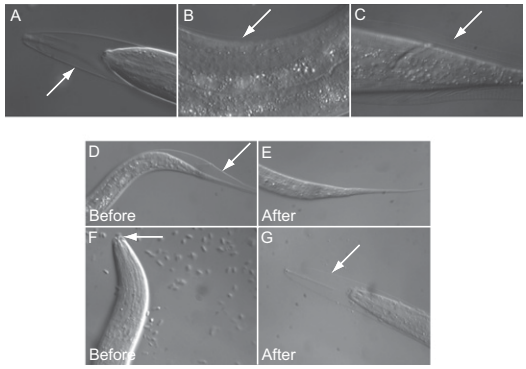

Fig. S4. GSH induces separation of the old and new cuticle in wild-type worms, and promotes apolysis and ecdysis in arrested *trxr-1*; *gsr-1* larvae. (*A*–*C*) Wild-type worm treated with 10 mM GSH for 45 min at 25 °C. (*D* and *F*) *rrf-3*(*pk1426*); *trxr-1*(*sv47*); *gsr-1*(*RNAi*) larvae arrested during molting. In *D*, extensive apolysis has occurred at the posterior of the worm; in *F*, the old cuticle remains attached to the new cuticle, except at the extreme anterior. (*E* and *G*) The same worms after treatment with 3 mM GSH for 45 min at 25°C. In *E*, the old cuticle has been completely removed; in *G*, GSH has induced separation of the old and new cuticle.

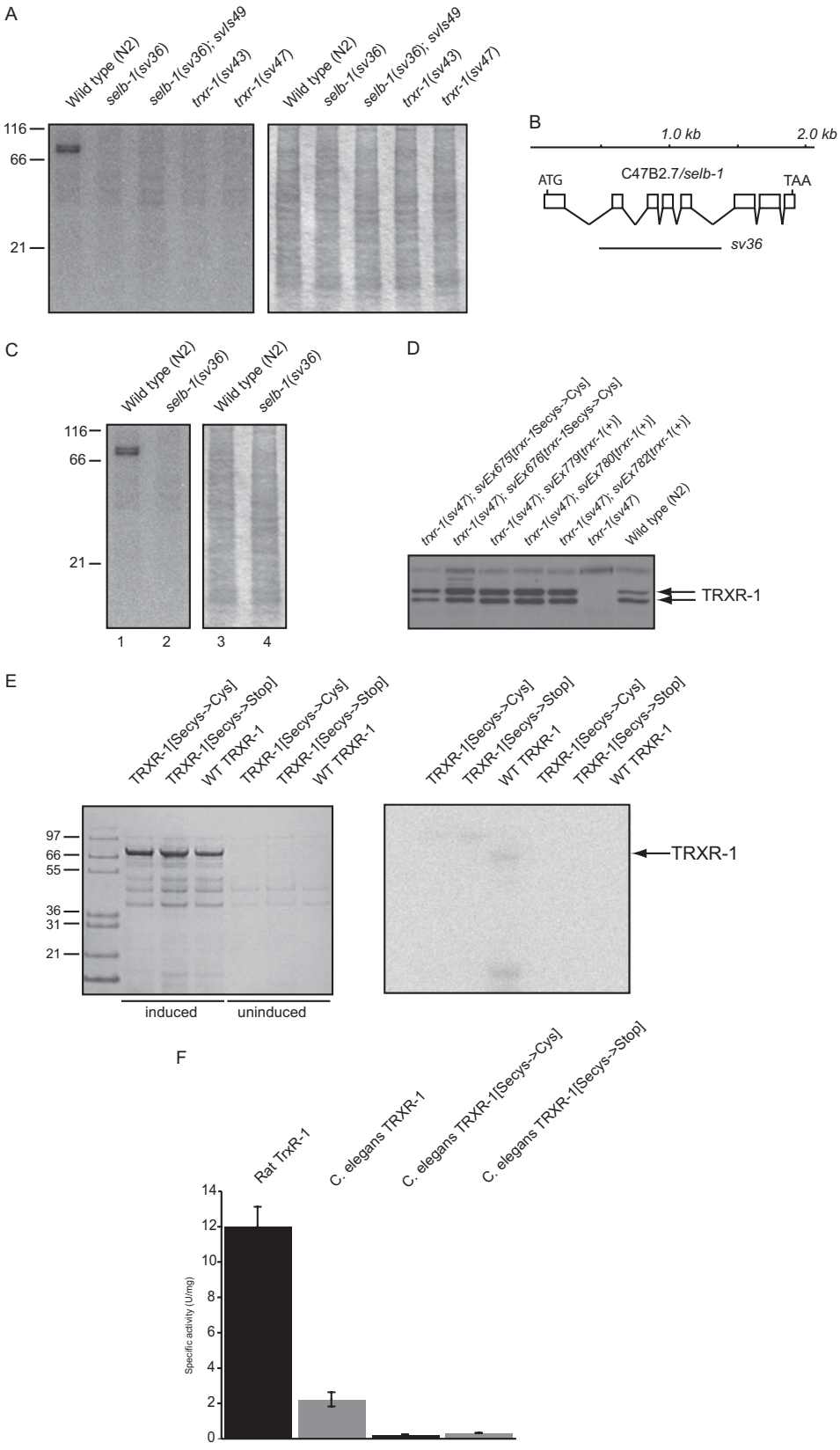

Fig. S5. TRXR-1 is the sole selenoprotein detected in *C. elegans* worms labeled with ^{75}Se, and production of *C. elegans* TRXR-1 proteins in vitro. (*A Left*) Autoradiogram of a gel containing protein extracts of worms raised on ^{75}Se-labeled *E. coli*. (*A Right*) The Coomassie-stained gel. (*B*) The *C. elegans selb-1* gene. Boxes represent exons, and lines represent introns. The line underneath denotes the region deleted in the *sv36* mutant allele. (*C Left*) Autoradiogram of a gel containing protein extracts of worms raised on ^{75}Se-labeled *E. coli*. (*C Right*) The Coomassie-stained gel. (*D*) Western blot of worms harboring transgenes encoding wild-type or mutant TRXR-1 probed with an antibody raised against TRXR-1. (*E*) Production of *C. elegans* TRXR-1 proteins in vitro. (*Right*) Coomassie-

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stained gel of protein extracts from *E. coli* strains harboring plasmids encoding wild-type or mutant TRXR-1. An *E. coli* SECIS element was present to the 3′ of the *trxr-1* coding region to allow incorporation of selenocysteine encoded by the UGA codon. *trxr-1* cDNA sequences were under the transcriptional control of the *E. coli lac* promoter. The three lanes on the left show protein extracts from cells in which *lac* promoter activity was induced with IPTG; those on the right are from untreated cells. All cells were grown in the presence of ^{75}Se. (*Right*) An autoradiogram of the gel shown at *Left*. The radioactive label is incorporated into wild-type TRXR-1 but not the proteins encoded by the cDNAs lacking the UGA codon. The protein produced corresponds in size to the larger of the two isoforms seen *C. elegans*. (*F*) Graph showing the activity of purified proteins in catalyzing the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to 5′-thionitrobenzoic acid (TNB; ref. 1). Error bars represent SEM.

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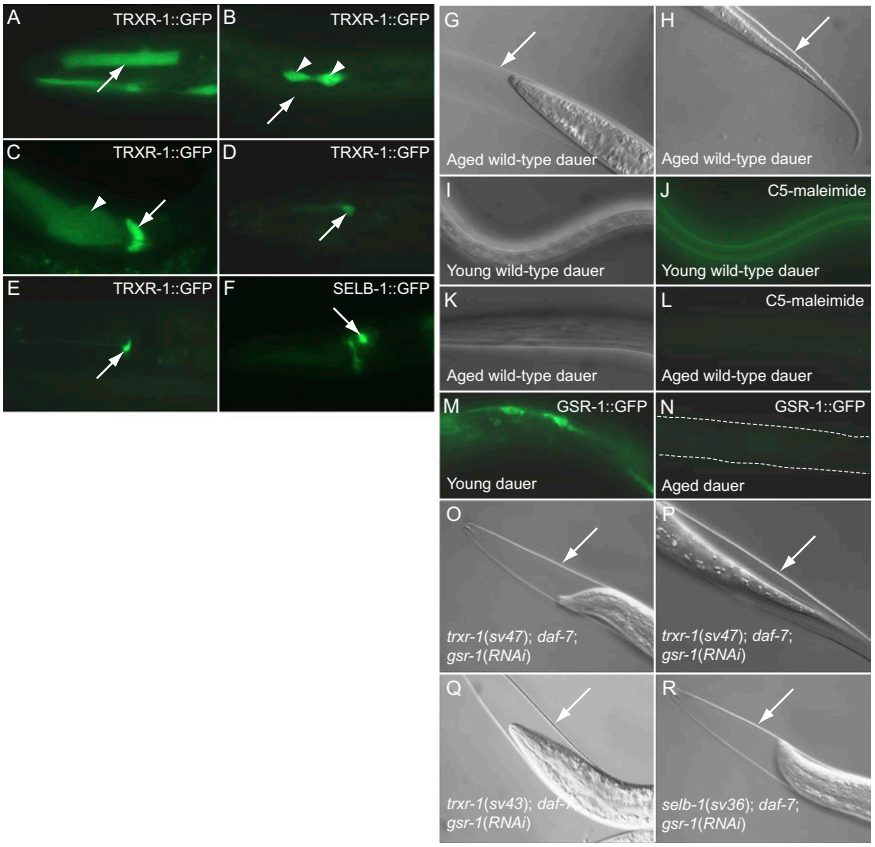

Fig. S6. TRXR-1::GFP and SELB-1::GFP reporters are expressed in tissues implicated in molting; age and reduced *trxr-1* and *gsr-1* function compromise the efficiency of the dauer molt. Micrographs of hermaphrodite worms were viewed with DIC (*G–I*, *K*, and *O–R*) or fluorescence (*A–F*, *J*, *L–N*) optics. (*A–F*) Micrographs of worms carrying GFP reporters. The arrow in *A* indicates expression of TRXR-1::GFP in pharyngeal muscle. The arrowheads in *B* indicate expression in seam cells, and the arrow indicates weak expression in the hypodermis. The arrow in *C* indicates expression in rectal epithelial cells; the arrowhead indicates expression in the posterior part of the intestine. The arrow in *D* indicates one of a pair of cells that lie in the anterior head region that express the reporter. Their position and morphology are consistent with them being amphid sheath cells. The arrows in *E* and *F* indicate expression in neurons within the nerve ring. Both markers are strongly expressed in a pair of neurons. (*G* and *H*) Three month-old dauer worm arrested at the dauer/L4 molt. Arrows indicate old cuticle. (*I–L*) Staining of the dauer cuticle with C_5-maleimide decreases with age. (*M* and *N*) Expression of GSR-1::GFP in dauer larvae decreases with age. (*O–R*) Larvae of the indicated genotypes arrested at the dauer/L4 molt. The worms in the bottom four panels were homozygous for *rrf-3*(*pk1426*) and were <4 d old. *daf-7* denotes *daf-7*(*e1372*ts). Worms containing *daf-7*(*e1372*ts) were raised at the restrictive temperature, 25°C, until they had entered dauer, and then moved to the permissive temperature, 15°C, at which temperature *daf-7*(*e1372*ts) single mutant worms exit dauer and develop into adults.

Table S1. Brood sizes of *trxr-1* and *selb-1* mutants

Genotype	Average brood size* ± SD
Wild type N2	306.5 ± 36.5
trxr-1(*sv47*)	273.6 ± 29.6
trxr-1(*sv43*)	292.9 ± 32.5
selb-1(*sv36*)	304.1 ± 26.6

*The total numbers of progeny of 15 worms of each genotype were determined.

Table S2. Genes tested for synthetic growth defects with *trxr-1*

Gene category	Gene name	Gene sequence designation	Slow growth/larval arrest*
Thioredoxin reductase	*trxr-2*	ZK637.10	No
Catalases	*Ctl-1*	Y34D9A.6	No
	Ctl-2	Y54G11A5.1	No
Superoxide dismutases	*sod-1*	C15F1.19	No
	sod-2	F10D11.2	No
	sod-4	F55H2.1	No
	sod-5	ZK430.1	No
Glutaredoxins	*glrx-10*	Y34D9A.6	NA†
	glrx-21	ZK121.1	No
Glutathione peroxidases		C11E4.1	No
		C11E4.2	No
		F26E4.12	No
		T09A12.2	No
		R03G5.5	No
		Y94H6A.4	NA†
Thioredoxins		Y44E3A.3	No
	trx-1	B0228.5	No
	trx-2	B0024.9	No
		K02H11.6	No
	trx-3	M01H9.1	No
Methionine sulfoxide reductase	*msra-1*	F43E2.5	No
Glutathione reductase	*gsr-1*	C46F11.2	Yes

*All genes were tested by conducting RNAi with the indicated gene in *rrf-3*(*pk1426*), *rrf-3*(*pk1426*); *trxr-1*(*sv47*) and *selb-1*(*sv36*); *rrf-3* (*pk1246*) mutant backgrounds. *rrf-3* mutations increase the sensitivity to RNAi (1). For *trxr-2*, a possible interaction was also tested by generating a *trxr-2*(*tm2047*); *trxr-1*(*sv47*) double mutant strain.
†Not applicable. RNAi of these genes caused larval arrest of the *rrf-3*(*pk1426*) control strain.

1. Simmer F, et al. (2002) Loss of the putative RNA-directed RNA polymerase RRF-3 makes *C. elegans* hypersensitive to RNAi. *Curr Biol* 12:1317–1319.