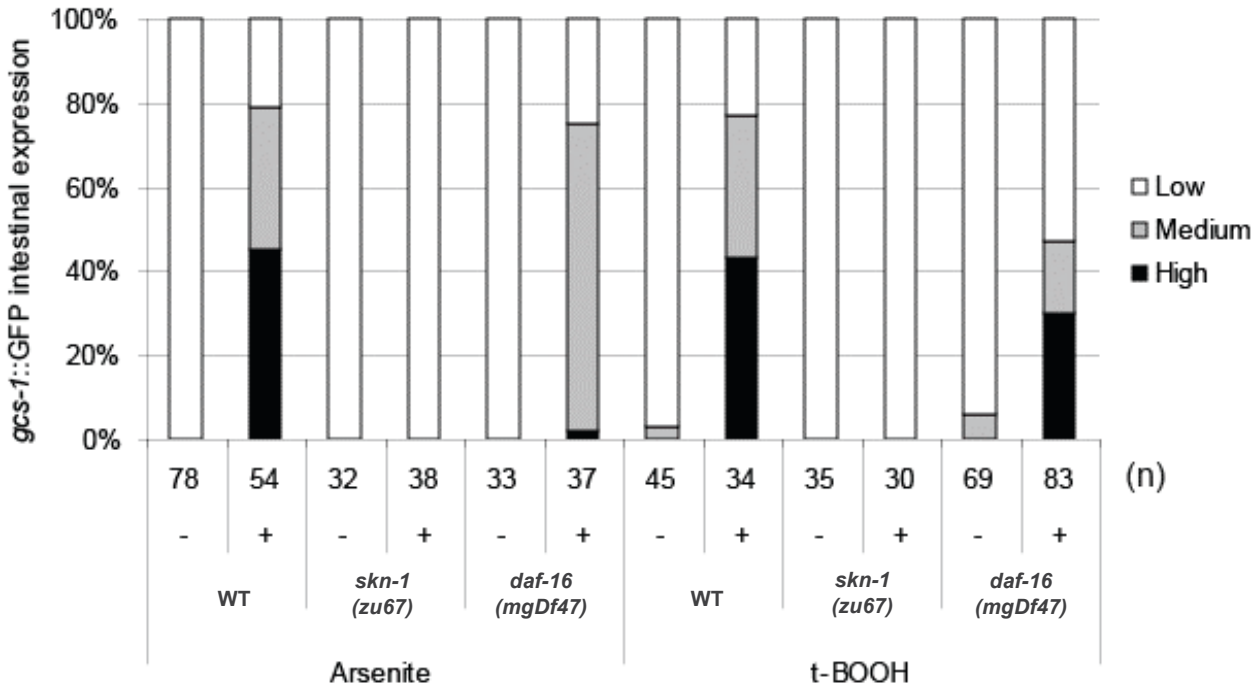
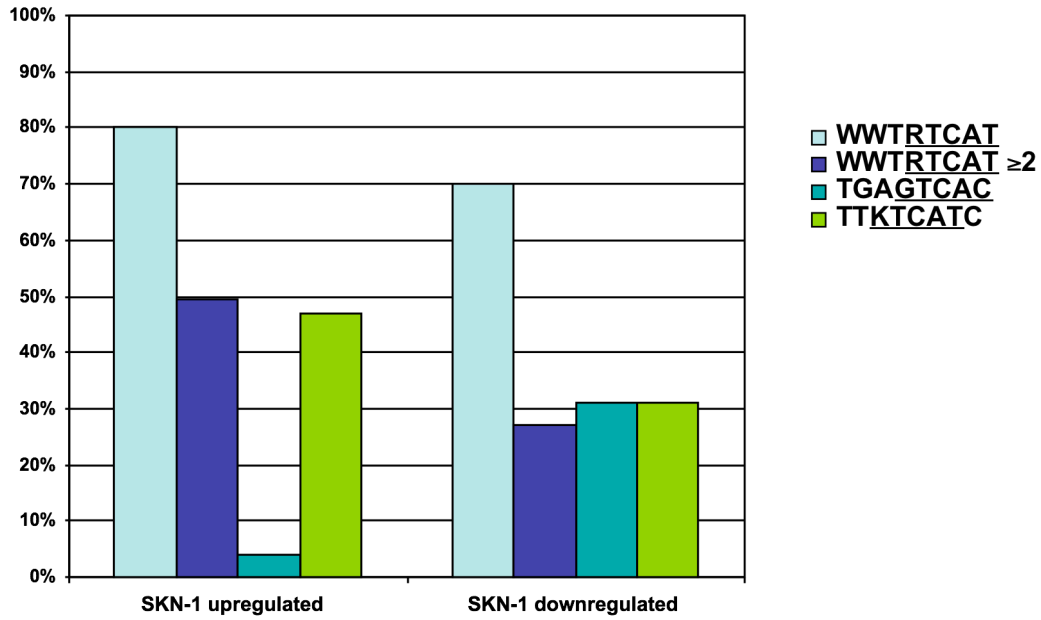
Supplemental Figure S1



Supplemental Figure S1.
Induction of the SKN-1 target *gcs-1* promoter by As and t-BOOH.

We optimized conditions for stress treatment by analyzing expression of the transgenic reporter *gcs-1::GFP*, in which the promoter for the stress-induced SKN-1 target gene *gcs-1* is fused to green fluorescent protein (GFP) . A range of concentrations of As and t-BOOH were tested to optimize expression (not shown). In the experiment shown, L3 to young adult animals were exposed to either 5 mM As in M9 (for 30 minutes) or t-BOOH that had been added to NGM agar at 12 mM (for 1 hour). After exposure, worms were allowed to recover for 1 additional hour on NGM agar before analysis. Under these conditions, sick or or dead worms were observed only at low frequency (0-5%) (data not shown).

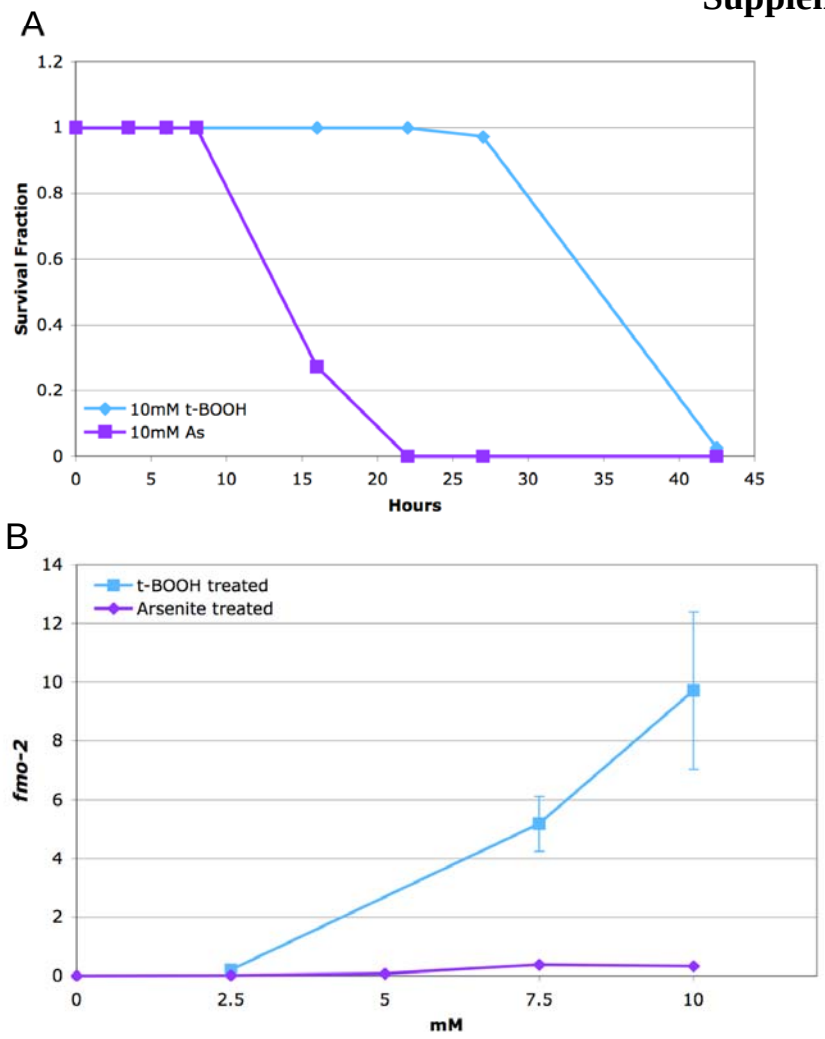
Each of these stresses induced robust *gcs-1::GFP* expression that was dependent upon *skn-1* and largely independent of *daf-16*. Expression of the *gcs-1::GFP* reporter was scored as in [14] . High indicates that fluorescence was present in high levels anteriorly and was detectable throughout most of the intestine, while medium refers to animals which fluorescence was present at high levels anteriorly or posteriorly but not in the rest of the intestine. Differential interference contrast (DIC) microscopy and fluorescence images were acquired with a Zeiss AxioSKOP2 microscope and an AxioCam cooled color digital camera. To discriminate intestinal autofluorescence from GFP epifluorescence, we used a triple-band emission filter set (Chroma 6100) in conjunction with a narrow-band excitation filter (484/14) as described previously [11]. This combination allows autofluorescence to be distinguished from GFP. Strains used were LD1002 N2 Ex003[*gcs-1::GFP*], LD1029 *skn-1(zu67)*;Ex003[*gcs-1::GFP*] [11] and LD1260 *daf-16(mgDf47)*; Ex003[*gcs-1::GFP*] [14] .



Supplemental Figure S2.

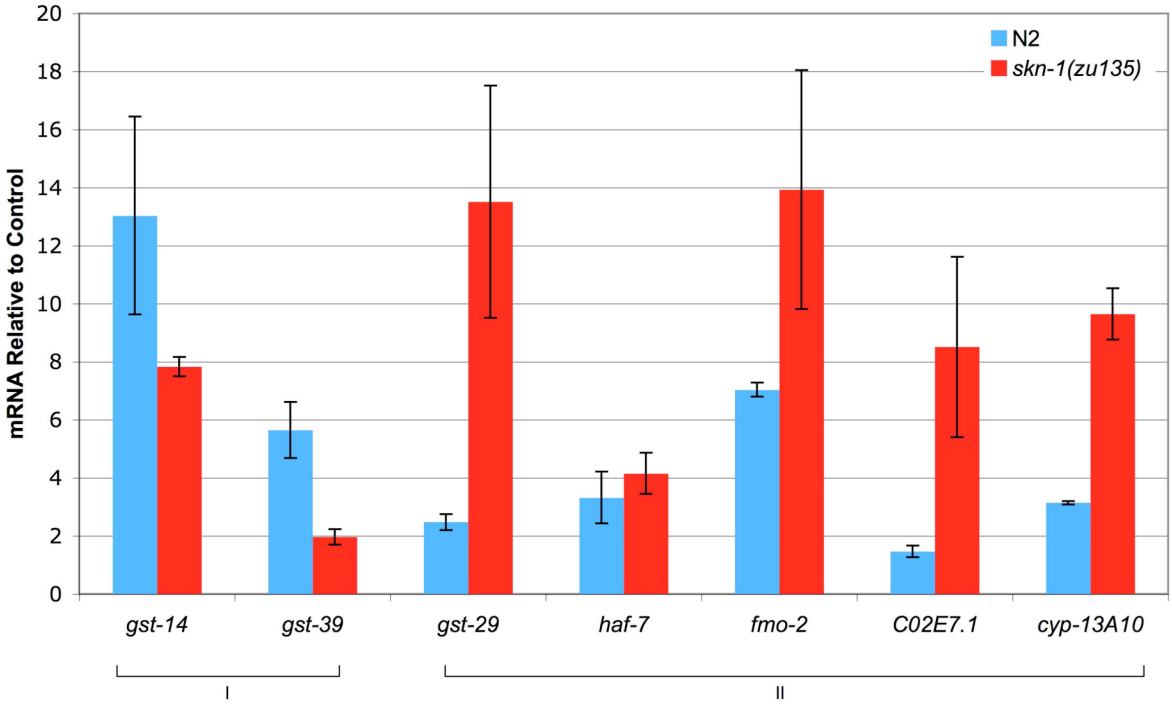
Motif occurrence in SKN-1-regulated gene promoters.

The graph shows the frequency with which the indicated motifs were detected in the putative promoter regions (see Methods) of genes that were up- or down-regulated by SKN-1 under non-stressed conditions (Tables S1 and S2; see text). WWTRTCAT is the optimal SKN-1 binding site *in vitro* [28], and the other related motifs TGAGTCAC and TTKTCATC were identified in random searches for overrepresented sequences in SKN-1 downregulated and upregulated genes, respectively.



Supplemental Figure S3.
Stress responses are specific to stress type.

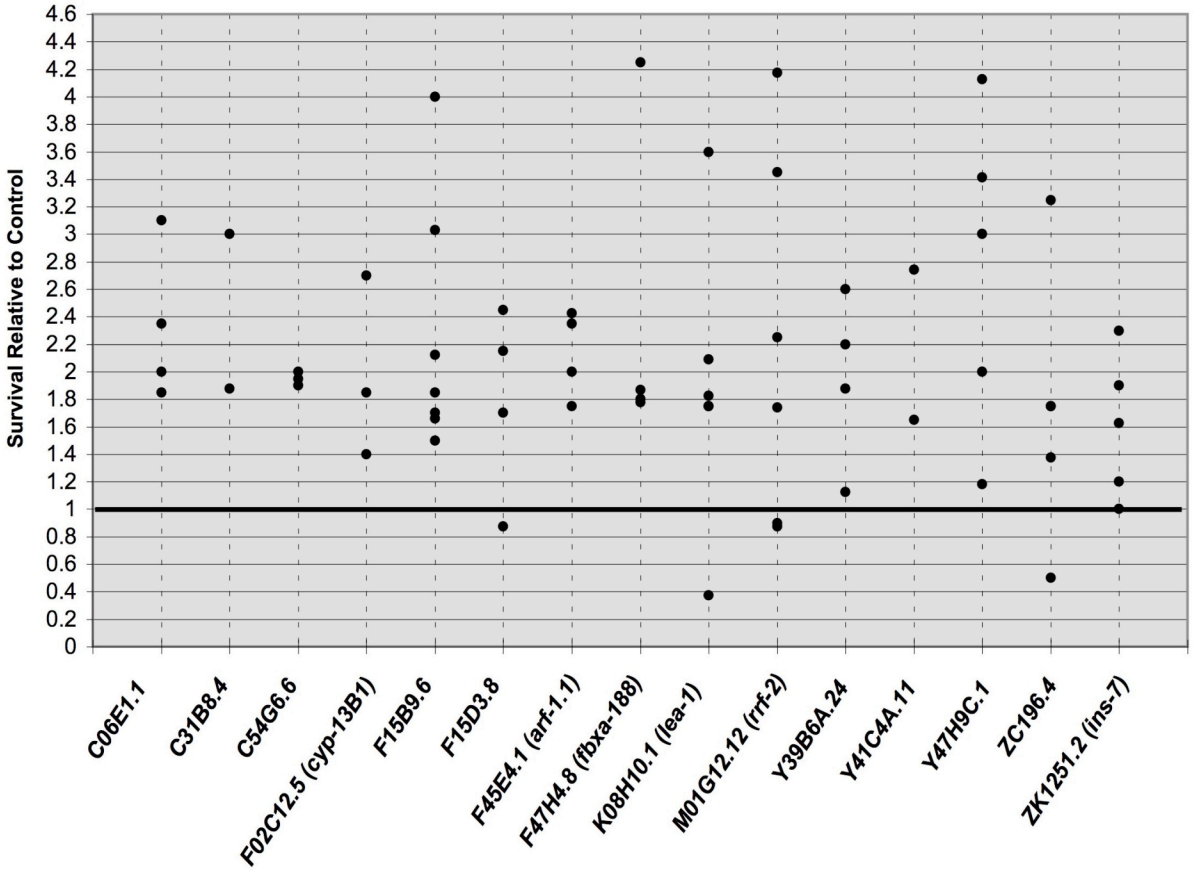
A) Representative survival fraction of young adult (pre-gravid) worms that were placed on NGM plates with 10mM arsenite (As) or tert-butyl hydroperoxide (t-BOOH). Worms were raised at 20°C and continually fed with control (*gfp*) RNAi from synchronized L1s. A parallel treatment with *skn-1* RNAi confirmed that *fmo-2* is induced by t-BOOH independently of SKN-1 (not shown). B) Expression of *fmo-2*, a TBHP-induced gene, under conditions of increasing t-BOOH or arsenite concentrations. Young adult (pre-gravid) worms were exposed to As or t-BOOH in seeded NGM plates for 3 hours. Worms were raised at 20°C and continually fed with control (*gfp*) RNAi from synchronized L1s. cDNA was synthesized and qPCR was performed with 2-3 biological replicates per data point. *fmo-2* expression was normalized to a standard curve and normalized to *act-1* expression. error bars = SEM



Supplemental Figure S4.
qRT-PCR analysis of t-BOOH-induced genes.

Expression of selected genes was analyzed by qRT-PCR in N2 (WT) or *skn-1(zu135)* animals that had been treated with t-BOOH or mock-incubated (Control), under similar conditions used to obtain samples that were analyzed on microarrays. *skn-1(zu135)* is a likely null allele that causes a premature translation stop that would prevent DNA binding by all three SKN-1 isoforms [14]. We analyzed two categories of genes that had been defined by our microarray experiments: I) upregulated by t-BOOH independently of *skn-1*, but *skn-1*-dependent under normal or As treatment conditions (Tables S1, S5, S9), and II) upregulated by t-BOOH independently of *skn-1* and not identified in other experiments (Table S9). In each case, the results obtained were consistent with our microarray data. In *skn-1(zu135)* animals *gst-14* and *gst-39* were upregulated by t-BOOH 8- and 2-fold, respectively, although their induction was more robust in N2. Each gene in group II was upregulated by t-BOOH in *skn-1(zu135)*. For each group II gene induction in the *skn-1* mutant was more robust than in N2. qRT-PCR carried out as in Methods. Error bars = SEM.

Supplemental Figure S5



Increased oxidative stress resistance after inhibition of SKN-1-downregulated genes.
We investigated how a set of SKN-1-downregulated genes affects oxidative stress resistance by inhibiting their expression using RNAi, then assaying survival after As exposure as in Fig. 6. Given the variability inherent in stress-survival assays, we used a scatter plot to summarize the results of multiple analyses of As resistance in which we tested a range of young adult ages (2-9 hr into adult stage, Figure 6B, 6C, and not shown). Each data point corresponds to an experiment in which 30-60 worms were examined. All trials were performed in N2 (wild type) worms except for analyses of C31B8.4, C54G6.6 and Y41C4A.11, for which 2 of 3 trials were performed in the RNAi-sensitive strain *rrf-3(pk1426)*. The X-axis indicates the gene that was inhibited by RNAi. The Y-axis shows the relative survival of worms compared to control at the time when approximately 20% of the control sample was alive. This was determined from graphs such as that shown in Fig. 6C. Of 22 SKN-1 downregulated genes that were tested, RNAi inhibition of the 15 shown here increased survival greater than 1.2-fold compared to control in at least 2 of 3 trials. For most genes additional trials were performed, and in each case shown no more than a single trial failed to demonstrate stress protection. By these criteria, the seven genes for which RNAi did not result in significant stress protection were B0024.4, C17H1.7, F25B3.5, Y43C5A.3, Y46G5A.20, F47H4.10 (*skr-5*), Y75B8A.32 (not shown).