Supporting Information

Henis-Korenblit et al. 10.1073/pnas.1002575107

SI Materials and Methods

Strains. Single mutants were as follows: CF1037: *daf-16(mu86) I*, CF2172: *isp-1(qm150) IV*, CF1908: *eat-2(ad1116) II*, CF1041: *daf-2(e1370) III*, DR1572: *daf-2(e1368) III*, CF2472: *xbp-1(zc12) III* [generated by crossing out an integrated *Phsp-4::gfp* reporter from the original SJ17 *xbp-1(zc12) III; zcIs4 V* strain (1), gift of David Ron, New York University], CF2473: *ire-1(ok799) II*, CF2012 *pek-1(ok275) X*, CF2988: *atf-6(ok551) X*.

Multiple mutants were as follows: CF512: *fer-15(b26) II; fem-1 (hc17) IV*, CF1844: *fer-15(b26) II; daf-2(mu150) III; fem-1(hc17) IV*, CF1085: *daf-16(mu86)I; daf-2(e1370) III*, CF2851: *ire-1(ok799) eat-2(ad1116) II*, CF2503: *daf-2(e1370) xbp-1(zc12) III*, CF3075: *daf-2(e1368) xbp-1(zc12) III*, CF2504: *ire-1(ok799) II; daf-2(e1370) III*, CF3076: *ire-1(ok799) II; daf-2(e1368) III*, CF2990: *daf-2 (e1370) III; atf-6(ok551) X*, CF2991: *daf-2(e1368) III; atf-6(ok551) X*, CF2299: *daf-2(e1370) III; pek-1(ok275) X*, CF2764: *daf-2(e1368) III; pek-1(ok275) X*, CF3077: *ire-1(ok799) II; isp-1(qm150) IV*, CF3281 *ire-1(ok799) II; daf-2(e1370) xbp-1(zc12) III*.

Transgenic strains were as follows: CF2260: *zcIs4 [Phsp-4::gfp] V* [generated by crossing the integrated *Phsp-4::gfp* reporter from the original SJ4005 *zcIs4 V* strain (1), gift of David Ron (New York University), to the Kenyon lab N2 strain], CF2510: *daf-2(e1370) III; zcIs4 [Phsp-4::gfp] V*, CF3507 *daf-16(mu86) II; daf-2(e3170) III; zcIs4 [Phsp-4::gfp] V*.

CF3508: *[Pdox-1::gfp]* [generated by crossing UL2146 (2), gift of Ian Hope (University of Leeds, UK), to the Kenyon lab N2 strain]. CF3508 was crossed to the following backgrounds: *daf-16 (mu86) I, xbp-1(tm2457) III, xbp-1(zc12) III, daf-2(e1368) III, daf-2(e1370) III, daf-2(e1368) xbp-1(tm2457) III, daf-2(e1370) xbp-1(zc12) III and daf-16(mu86) I; daf-2(e1370) III*.

ire-1(ok799) is a null mutation in which three exons containing the kinase and endonuclease domains are deleted. *ire-1(ok799)* deletion mutants were smaller and paler than control animals, and many displayed a bagging (matricide as a result of internal hatching of progeny) and Unc (uncoordination) phenotypes. These phenotypes were not seen in *ire-1(RNAi)* animals, which are predicted to have higher residual gene activity.

xbp-1(zc12) is a putative null mutation, a nonsense mutation at codon 11 (1), terminating it before its functional domains. Animals carrying *zc12* are sensitive to ER stress relative to normal animals and unable to induce transcription through the *hsp-4* promoter in response to ER stress.

atf-6(ok551) is a putative null mutation deleting 1,900 bp of genomic sequence, resulting in a protein missing the leucine zipper portion of the bZIP domain, the transmembrane domain, and the ER luminal domain (3).

pek-1(ok275) is a putative null mutation in which more than five exons are missing, resulting in the loss of a critical transmembrane domain (4).

Lifespan Analysis. Lifespan analysis was performed as described previously (5). RNAi treatments were performed continuously from the time of hatching. Eggs were placed on plates seeded with the RNAi bacteria of interest. The chemical 2′-fluoro-5′-deoxyuridine (Sigma) was occasionally added to adult worms (70 μM) to prevent their progeny from developing. As a result of variability between experiments, related lifespans were performed concurrently or in overlapping time frames. In all experiments, the prefertile period of adulthood was used as t = 0 for lifespan analysis. Animals that ruptured, bagged (i.e., exhibited internal progeny hatching), or crawled off the plates were censored but included in the lifespan analysis as censored worms. Statview 5.0.1 software (SAS) was used for statistical analysis and to determine means. In all cases, *P* values were calculated using the log-rank (i.e., Mantel-Cox) method.

RNAi Lifespan Screen. A systematic RNAi lifespan screen was carried out using RNAi clones corresponding to the approximately 600 candidate genes identified in the described microarray study. For screening, we used the CF512 [*fer-15(b26); fem-1(hc17)*] and CF1844 [*fer-15(b26); daf-2(mu150); fem-1(hc17)*] sterile strains. Approximately 80 eggs were added to the RNAi plates and maintained at 25 °C from adulthood. Viability of worms on each plate was scored at weekly intervals until plates on which most worms were dead were identified at a time when most worms on the control plate were still alive. Plates were further screened at a later time points to identify RNAi plates on which most worms were alive at a time when most worms on the control plate were dead. Plates were censored as a result of events such as contamination or lack of bacterial growth. A quantitative survival analysis using both CF512 and CF1844 was performed with all of the bacterial RNAi strains that scored positively in the screen.

***xbp-1* and *ire-1* RNAi Clone Analysis.** The level of a expression of *Phsp-4::gfp*, a promoter fusion to an ER-stress response gene that is a target of *xbp-1*, in a wild-type background was used to assess the functionality of the *xbp-1* or *ire-1* RNAi clones. We confirmed that feeding worms with *xbp-1* or *ire-1* RNAi clones suppressed the expression of a *Phsp-4::gfp* reporter.

***xbp-1* RT-PCR.** A primer set encompassing the noncanonical intron of the *xbp-1* transcript was used, giving rise to two PCR products: an approximately 200 bp PCR product of amplified unspliced *xbp-1* transcript and an approximately 180 bp PCR product of amplified spliced *xbp-1* transcript. PCR products were visualized on an agarose gel stained with ethidium bromide. Gels were scanned and analyzed with NIH Image 1.63 software.

Primers Used in This Study. Top primer was *xbp-1*: 5′- TGCATGCATCTACCAGAACGTCGTCT –3′. Bottom primer was *xbp-1*: 5′- ATAGTTAGATACATATCCACACTG –3′.

Microarray Analysis. RNA from four biological replicates of matched *daf-2(e1370)* and *daf-2(e1370) xbp-1(zc12)* day-1 adults were hybridized competitively. RNA from four biological replicates of matched *daf-2(e1368)* and *daf-2(e1368) xbp-1(zc12)* day-1 adults were hybridized competitively. Analysis was performed in GeneSpring (Agilent Technologies) and Significance Analysis for Microarrays (SAM; Stanford University). Arrays were first filtered to remove features that were at or below average background signal. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO) (6) and are accessible through GEO Series accession number GSE20148 (http://www.ncbi.nlm.nih.gov/geo/). This list was also exported from GeneSpring and used for SAM analysis to identify those genes that were statistically significant based on a modified *t* test. The top 150 up-regulated or down-regulated genes from each SAM analysis list (one for each *daf-2* allele) were used to generate a list of approximately 600 candidate genes for further experiments (Table S2). Eighty additional genes that were significantly differentially expressed (with a false discovery rate of 2.5%) but ranked lower in the SAM analysis of the *daf-2(e1370)/ daf-2(e1370) xbp-1(−)* arrays were also investigated. These latter genes were selected because they were also differentially expressed in *daf-2(e1370)* versus N2 microarrays.

1. Calfon M, et al. (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415:92–96.
2. Reece-Hoyes JS, et al. (2007) Insight into transcription factor gene duplication from Caenorhabditis elegans Promoterome-driven expression patterns. *BMC Genomics* 8:27.
3. Shen X, Ellis RE, Sakaki K, Kaufman RJ (2005) Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in C. elegans. *PLoS Genet* 1:e37.
4. Shen X, et al. (2001) Complementary signaling pathways regulate the unfolded protein response and are required for C. elegans development. *Cell* 107:893–903.
5. Apfeld J, Kenyon C (1999) Regulation of lifespan by sensory perception in Caenorhabditis elegans. *Nature* 402:804–809.
6. Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 30:207–210.

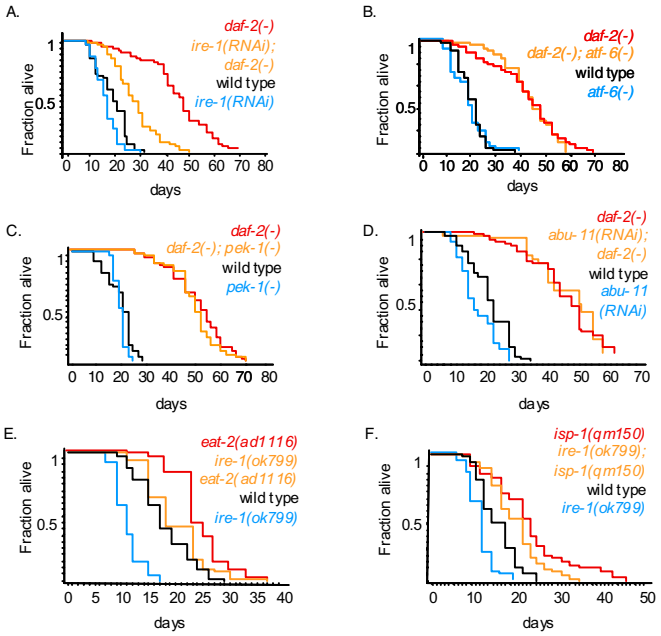

Fig. S1. Inactivation of ER stress–related genes in long-lived mutants. (*A*) Reduction of *ire-1* activity by RNAi shortened the lifespan of *daf-2(e1370)* mutants more than it shortened the lifespan of wild type. (*B* and *C*) Neither *atf-6(ok551)* nor *pek-1(ok275)* deletion shortened the lifespan of *daf-2(e1370)* mutants. (*D*) Inactivation of *abu-11* by RNAi did not shorten the lifespan of *daf-2* mutants [compare survival curves of *daf-2(–)* and *abu-11(RNAi); daf-2(–)* animals], whereas it did shorten the lifespan of wild type. *abu-11* is a gene required for lifespan extension by resveratrol (1), and is a member of a gene family that is up-regulated in response to ER stress when the XBP-1/IRE-1 UPR pathway is blocked (2). For additional lifespan data, see Table S1. (*E*) *ire-1(ok799)* deletion shortened the lifespan of *eat-2(ad1116)* mutants, but no more than it shortened wild-type lifespan. [Compare survival curves of *eat-2(–)* and *ire-1(ok799) eat-2(–)* animals versus those of wild-type and *ire-1(ok799)* animals.] (*F*) *ire-1(ok799)* deletion shortened the lifespan of *isp-1(qm150)* mutants, but no more than it shortened wild-type lifespan. [Compare survival curves of *isp-1(–)* and *ire-1(ok799); isp-1(–)* mutants versus those of wild-type and *ire-1(ok799)* animals.] For additional lifespan data, see Table S1.

1. Viswanathan M, Kim SK, Berdichevsky A, Guarente L (2005) A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span. *Dev Cell* 9:605–615.
2. Urano F, et al. (2002) A survival pathway for Caenorhabditis elegans with a blocked unfolded protein response. *J Cell Biol* 158:639–646.

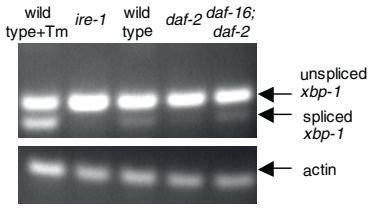

Fig. S2. The *ire-1/xbp-1* pathway is set at a lower level in *daf-2(–)* mutants. Representative RT-PCR of *xbp-1* from RNA isolated from day-1 animals. The arrows point at the unspliced *xbp-1* band, the spliced *xbp-1* band, and the loading control actin band. Control lane presents the RT-PCR products of animals treated with tunicamycin. The rest of the lanes present steady-state RT-PCR products in day-1 wild-type, *ire-1*, *daf-2*, and *daf-16; daf-2* animals. These animals have not been treated with tunicamycin.

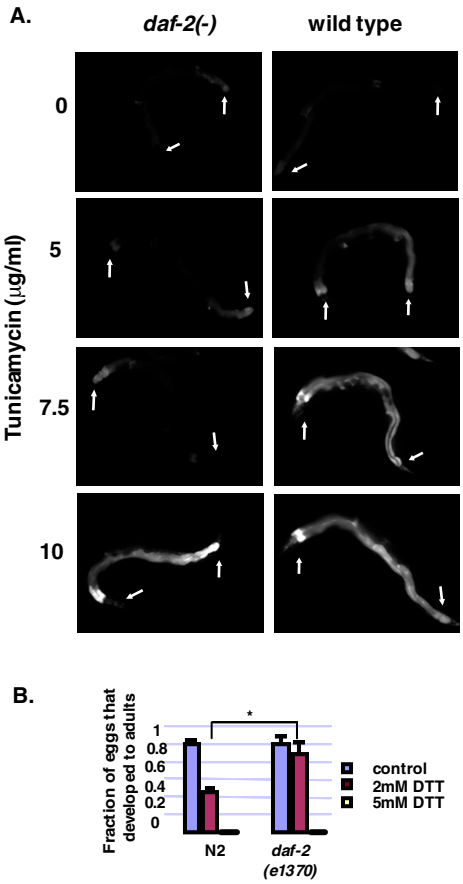

Fig. S3. *daf-2* mutants are resistant to ER stressors. (*A*) *daf-2(e1370)* mutants expressed less *Phsp-4::gfp* transgene than did wild type, even in the presence of tunicamycin. Representative micrographs of *Phsp-4::gfp* expression in wild-type and *daf-2* animals upon 5 h treatment with 0 to 10 μg/mL tunicamycin. Arrows point at anterior and posterior ends of the animals. (*B*) *daf-2(–)* mutants are resistant to DTT. Eggs from wild-type or *daf-2(e1370)* animals were grown in the presence of 0, 2, or 5 mM DTT. Percentage of eggs that developed into mature adults was scored. This experiment was repeated independently with similar effects. Each mutant was scored on three independent plates. Error bars represent the SEM for repeat plates within the experiment. Asterisks mark Student *t* test values of $P < 0.01$.

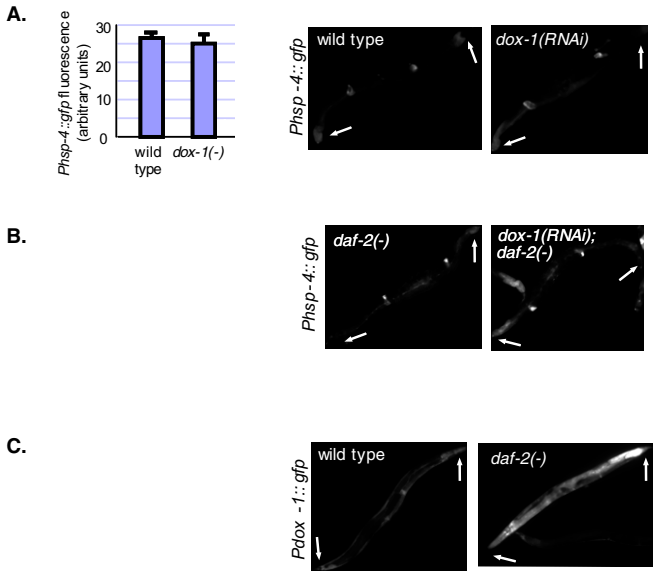

Fig. S4. Reduction of *dox-1* did not increase the set-point of the *ire-1/xbp-1* branch of the UPR. (*A* and *B*) Shown are representative fluorescence micrographs of day-2 transgenic wild-type adults (*A*) or *daf-2* transgenic adults (*B*) harboring a *Phsp-4::gfp* transgene upon reduction of *dox-1* activity by RNAi. Wild type, $n = 19$, relative mean fluorescence intensity (m) = 25.8 ± 1.7; *dox-1*(*RNAi*), $n = 18$, $m = 24.3 \pm 2.2$, $P = 0.61$. See Fig. 4*B* in the main text for the corresponding *daf-2* data and bar graph. (*C*) Animals expressing *Pdox-1::gfp* can be classified as expressing high levels or low levels of GFP. Shown are representative fluorescence micrographs of a wild-type animal expressing low levels of *Pdox-1::gfp* and a *daf-2*(*e1368*) mutants expressing high levels of the *Pdox-1::gfp* transgene in d transgenic adults. Animals expressing both low and high levels of *Pdox-1::gfp* can be found in wild-type animals and *daf-2* mutants, albeit at different redundancies. Arrows point at anterior and posterior ends of the animals.

Table S1. Lifespan analysis of long-lived mutants upon inactivation of ER stress–associated genes

Each boxed panel within the tables includes lifespan data of experiments performed in parallel. Statistical analysis and comparisons are done within each panel. White background represents experiments that did not involve RNAi. Gray background represents experiments that included RNAi. AO ("adult only") in comment column refers to animals transferred to bacteria expressing dsRNA on the first day of adulthood. Control refers to N2 for single mutants. Control refers to *daf-2(–)* for *daf-2(–)* double mutants in sections A–F. Control refers to *isp-1(–)* for *isp-1(–)* double mutants in section G. Control refers to *eat-2(–)* for *eat-2(–)* double mutants in section H. Events/observations represents the number of animals censored during the experiment, as described in *Materials and Methods* in the main text. Lifespan experiments were carried out at 20 °C unless indicated otherwise. See *Strain* in *Materials and Methods* in the main text for more information about the different mutations described.

Table S1 (DOC)

Table S2. SAM output for genes that were differentially expressed in *daf-2* and *daf-2 xbp-1(zc12)*

The table lists SAM output for genes that were differentially expressed in *daf-2(e1370)* and *daf-2(e1370) xbp-1(zc12)* with a false discovery rate of 2.5%. Likewise, the table lists SAM output for genes that were differentially expressed in *daf-2(e1368)* and *daf-2(e1368) xbp-1(zc12)* with a false discovery rate of 10.2%. Data are based on four biological replicates of matched *daf-2(–)* and *daf-2(–) xbp-1(–)* day-1 adults were hybridized competitively. RNAi clones encoding genes from this list were incorporated into an RNAi sublibrary to be tested as lifespan regulatory genes. These genes include the top 150 up-regulated or down-regulated genes based on SAM ranking. In addition, genes from this list that were also differentially expressed in *daf-2(e1370)* and N2 arrays were also incorporated into the library. Genes incorporated in the RNAi sublibrary are highlighted.

http://kenyonlab.ucsf.edu/Table_S4.xls

Table S3. Fold change of *dox-1* genes in *daf-2*(*e1370*) animals compared with N2 animals

The table shows the fold change in expression levels of the *dox-1* transcripts in microarrays profiling *daf-2* and wild-type animals on day-1 of adulthood. On average, the expression of *dox-1* was increased by a factor of 1.37 in *daf-2* mutants relative to wild type (*t* test, $P = 0.0282$). One sample *t* test was calculated for the log 2 of the gene expression ratios. The *P* values are for one-tailed tests.

Table S3 (DOC)

Table S4. Parametric analysis of gene set enrichment of XBP-1 target genes in *daf-2* and *daf-16; daf-2* mutants

A predefined gene set of 17 genes that were reported previously by Urano et al. (1) and Shen et al. (2) to be dependent on *xbp-1* signaling for induction upon ER stress was generated. This set of genes was analyzed by PAGE, a modified gene set enrichment analysis method (3) that tests for significantly changed biological themes from microarray data at the level of genes sets rather than individual genes. The gene set was examined in microarray data sets of *daf-2* and *daf-16; daf-2* animals (4). Based on PAGE analysis, this set of genes was significantly down-regulated in *daf-2* single mutants relative to *daf-16; daf-2* double mutants with a *P* value of 2.32E-06.

Table S4 (DOC)

1. Urano F, et al. (2002) A survival pathway for Caenorhabditis elegans with a blocked unfolded protein response. *J Cell Biol* 158:639–646.
2. Shen X, Ellis RE, Sakaki K, Kaufman RJ (2005) Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in C. elegans. *PLoS Genet* 1:e37.
3. Kim SY, Volsky DJ (2005) PAGE: parametric analysis of gene set enrichment. *BMC Bioinformatics* 6:144.
4. McElwee JJ, Schuster E, Blanc E, Thomas JH, Gems D (2004) Shared transcriptional signature in Caenorhabditis elegans Dauer larvae and long-lived daf-2 mutants implicates detoxification system in longevity assurance. *J Biol Chem* 279:44533–44543.