



# XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity

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## SUMMARY

The ability to ensure proteostasis is critical for maintaining proper cell function and organismal viability but is mitigated by aging. We analyzed the role of the endoplasmic reticulum unfolded protein response (UPR<sup>ER</sup>) in aging of *C. elegans* and found that age-onset loss of ER proteostasis could be reversed by expression of a constitutively active form of XBP-1, XBP-1s. Neuronally derived XBP-1s was sufficient to rescue stress resistance, increase longevity, and activate the UPR<sup>ER</sup> in distal, non-neuronal cell types through a cell-nonautonomous mechanism. Loss of UPR<sup>ER</sup> signaling components in distal cells blocked cell-nonautonomous signaling from the nervous system, thereby blocking increased longevity of the entire animal. Reduction of small clear vesicle (SCV) release blocked nonautonomous signaling downstream of *xbp-1s*, suggesting that the release of neurotransmitters is required for this intertissue signaling event. Our findings point toward a secreted ER stress signal (SERSS) that promotes ER stress resistance and longevity.

## INTRODUCTION

Cells are faced throughout their lifetime with a variety of stresses that lead to protein misfolding and aggregation (Balch et al., 2008). The disruption of protein homeostasis (proteostasis) mechanisms that guard the proteome against these stresses can be catastrophic to cellular viability. To protect against proteotoxicity, cells have evolved compartment-specific stress responses that allow for the targeted induction of chaperone and degradation machineries within select organelles or subcellular locations. These stress responses include the heat shock response (HSR), the endoplasmic reticulum unfolded protein response (UPR<sup>ER</sup>), and the mitochondrial unfolded protein response (UPR<sup>mt</sup>) (Akerfelt et al., 2010; Ron and Walter, 2007; Haynes and Ron, 2010). These collective responses give the cell the capacity to manipulate its chaperone networks and protein degradation pathways in response to subcellular stress.

The UPR<sup>ER</sup> responds to the presence of misfolded proteins in the ER, an important site for folding and modification of many proteins, including those destined for secretion and membrane

localization (Ron and Walter, 2007). The UPR<sup>ER</sup> is comprised of three canonical branches. Each of these branches transmits information on ER proteostasis to the cytosol through a transmembrane regulator—PERK, IRE1, or ATF6—that activates a distinct downstream signal transduction pathway, culminating in a program that reduces ER load and increases the capacity of the organelle to deal with protein misfolding (Ron and Walter, 2007). Activation of the ancestral IRE1 pathway leads to the regulated splicing of a transcription factor, XBP1, by the IRE1 endoribonuclease activity (Sidrauski and Walter, 1997). Thus activated, spliced XBP1, XBP1s, is translated and regulates a range of transcriptional targets required for ER proteostasis (Shen et al., 2005; Acosta-Alvear et al., 2007).

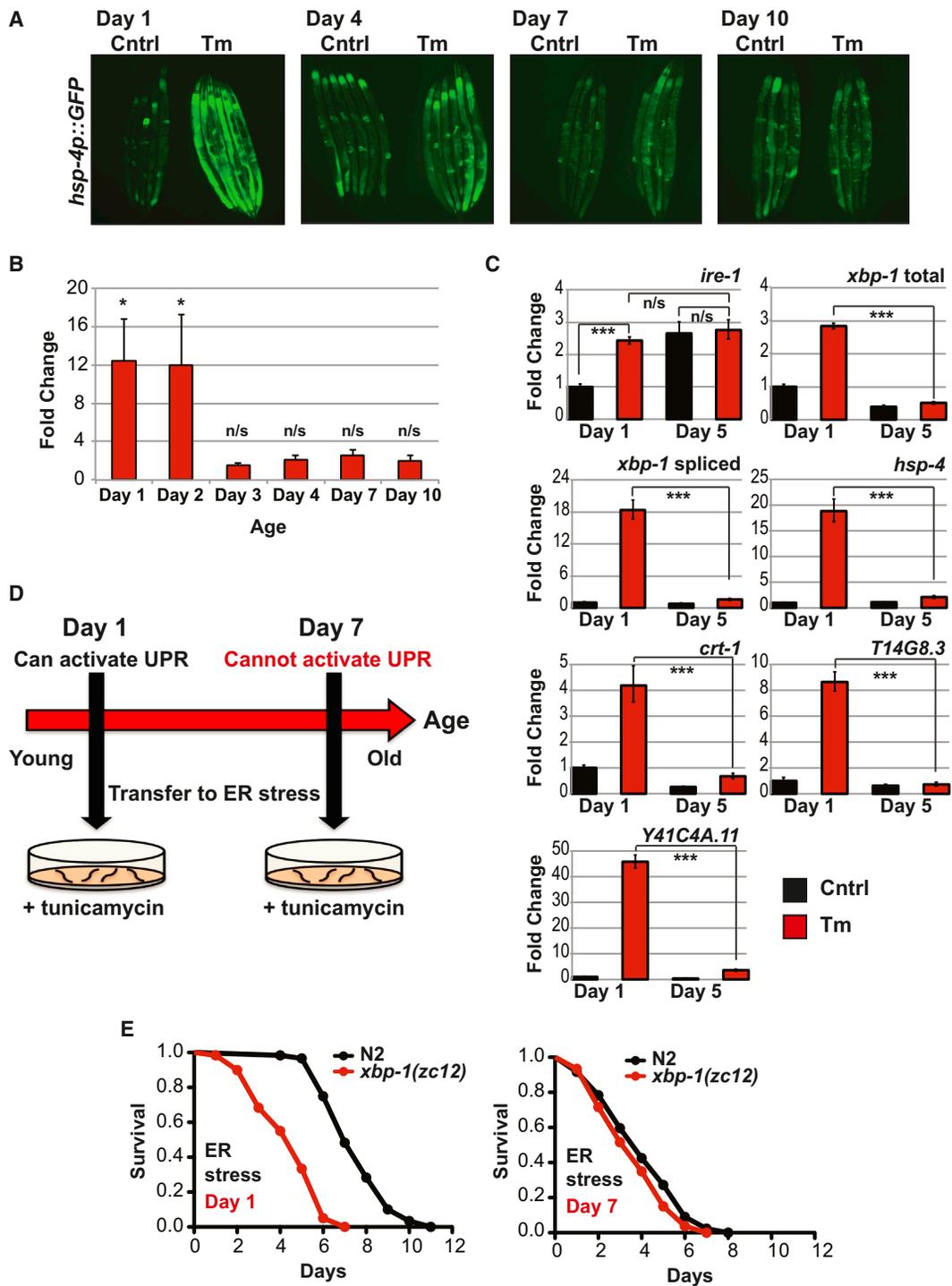
The maintenance of proteostasis both within the ER and throughout the cell becomes increasingly difficult with age, as damaged, misfolded, and aggregated proteins progressively accrue. The accumulation of damaged proteins is exacerbated by what appears to be an organism-wide loss in the ability to mount cellular stress responses. As such, a decline in the capacity of the cell to protect its proteome has been highly correlated with age-onset disease. During aging, ER chaperones are down-regulated, and activation of the UPR<sup>ER</sup> upon ER stress may be abrogated with age (Ben-Zvi et al., 2009). Reduction of the IRE-1/XBP-1 UPR branch also shortens the long lifespan caused by reduced insulin/IGF1 signaling (IIS) in *C. elegans* (Henis-Korenblit et al., 2010). However, it is unknown whether ectopic induction of the UPR<sup>ER</sup> is sufficient to prevent age-onset decline of proteostasis and delay age-related pathologies.

We hypothesized that the controlled induction of the UPR<sup>ER</sup> might reverse the loss of ER proteostasis in aging. We find that age-onset loss in UPR<sup>ER</sup> function can be rescued by the expression of constitutively active *xbp-1s*. The expression of *xbp-1s* in the neurons initiates a cell-nonautonomous response in which the UPR<sup>ER</sup> is activated in non-neuronal tissue, protecting against ER stress and extending lifespan in the whole organism. Moreover, cell-nonautonomous UPR<sup>ER</sup> activation requires neurotransmitter release from small clear vesicles (SCVs). We therefore conclude that constitutive activation of XBP-1 in neurons can rescue stress resistance and extend lifespan through a cell-nonautonomous signaling mechanism.

## RESULTS

### The Ability to Activate the UPR<sup>ER</sup> Declines with Age

Previous studies indicate that the ability to activate protective stress responses declines early in adulthood (Ben-Zvi et al.,



**Figure 1. The Ability to Activate the UPR<sup>ER</sup> Declines with Age**

(A) *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms were treated at days 1, 4, 7, and 10 of adulthood with 25 ng/μl tunicamycin in M9 buffer, or buffer only, for 4 hr, and GFP fluorescence assessed.

(B) *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms were treated as above at days 1, 2, 3, 4, 7, and 10 of adulthood, and GFP expression measured by fluorimetry. Fold inductions relative to untreated animals are shown, with error bars indicating mean + standard error of the mean (SEM) (n = 3). A Student's t test was used to assess statistical significance of induction at each age: \*p < 0.05, n/s = not significant.

(C) N2 animals were treated as above at day 1 and day 5 of adulthood. Transcript levels of UPR regulators and targets in tunicamycin-treated (red) and untreated (black) animals were measured by quantitative RT-PCR, with error bars indicating mean ± standard deviation (SD). A Student's t test was used to assess significance: \*\*\*p < 0.005, n/s = not significant.

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2009; Dillin et al., 2002a, 2002b; Durieux et al., 2011). We hypothesized that the maintenance of the ER proteome would also decline with age in *C. elegans*. We used an *hsp-4p::GFP* strain as a reporter for UPR<sup>ER</sup> activation (Calfon et al., 2002). Transcription of *hsp-4*, the worm ortholog of the mammalian ER-localized Hsp70 chaperone BiP, is upregulated upon ER stress in a manner dependent upon the IRE-1 kinase/endoribonuclease and the XBP-1 transcription factor. We treated *hsp-4p::GFP* worms with tunicamycin, an inhibitor of N-linked glycosylation that induces ER stress, at a range of ages from day 1 to day 10 of adulthood. We found that activation of the *hsp-4p::GFP* reporter was dependent upon *xbp-1* and *ire-1* (Figure S1A available online and data not shown), consistent with Calfon et al. (2002). In a wild-type background, reporter activation was robust at days 1–2 of adulthood but reduced sharply by days 3–4 and was essentially nonexistent by days 7–10 of adulthood (Figures 1A and 1B).

A similar loss in UPR<sup>ER</sup> activation was evident upon examination of transcript levels (Figure 1C). Levels of total *xbp-1* transcript increased 2-fold following tunicamycin treatment at day 1 of adulthood but were virtually unchanged upon tunicamycin treatment at day 5. Levels of spliced *xbp-1* transcript were increased around 20-fold by tunicamycin treatment at day 1, but very little increase in spliced transcript was observed by day 5. Transcriptional upregulation of UPR<sup>ER</sup> target genes *hsp-4*, *crt-1*, *T14G8.3*, and *Y41C4A.11* was also greatly reduced by day 5. Interestingly, whereas *ire-1* transcript levels were increased roughly 2-fold by ER stress at day 1, by day 5, basal transcript levels were increased around 2-fold relative to day 1 basal levels, and no further increase was seen upon ER stress. *xbp-1* splicing and UPR<sup>ER</sup>-induced transcriptional changes therefore seem to be abrogated with age, within early adulthood.

Tunicamycin treatment inhibits the formation of N-acetylglucosamine lipid intermediates, preventing the glycosylation of newly synthesized glycoproteins, and therefore requires active protein translation to induce ER stress (Heifetz et al., 1979). *C. elegans* are robustly translationally active through day 3 of adulthood (Hansen et al., 2007), whereas the ability of tunicamycin to activate the UPR<sup>ER</sup> is substantially reduced by day 3 (Figure 1B), suggesting that this diminished activation is not caused by a loss of translation. As an independent measure of ER stress that is not dependent upon active protein translation, we treated animals with thapsigargin, which is an inhibitor of the sarco-endoplasmic reticulum Ca<sup>2+</sup>-transport ATPase (SERCA) and induces ER stress by depletion of ER Ca<sup>2+</sup> stores (Lytton et al., 1991; Kijima et al., 1991). Treatment with thapsigargin at day 1 of adulthood induced the UPR<sup>ER</sup>, although to a lesser degree than treatment with tunicamycin, as previously shown (Figures S1B and S1C) (Caruso et al., 2008). However, UPR<sup>ER</sup> activation by thapsigargin was abolished by day 4 of adulthood with similar kinetics to the loss of UPR<sup>ER</sup> activation upon tunicamycin treatment.

In addition to tunicamycin and thapsigargin, heat stress and exposure to pathogenic bacteria also induce the UPR<sup>ER</sup> (Galbadage and Hartman, 2008; Richardson et al., 2010). We treated *hsp-4p::GFP* animals with exposure to pathogenic *Pseudomonas aeruginosa* strain PA14 or with a 34°C heat shock at day 1 and day 7 of adulthood. As seen with tunicamycin treatment, we observed upregulation of GFP fluorescence at day 1 but not at day 7, suggesting that the response of the UPR<sup>ER</sup> to these stresses also declines with age (Figures S1D and S1E). Taken together, loss of UPR<sup>ER</sup> activation with age is not simply a consequence of reduced rates of translation but rather reflects the ability of the animal to mount a response to ER stress.

### Protection against UPR<sup>ER</sup>-Inducing Stress Declines with Age

Because induction of the UPR<sup>ER</sup> declined with age, we hypothesized that physiological resistance to ER stress would also decline. We challenged wild-type N2 and *xbp-1(zc12)* mutant animals, which lack an intact UPR<sup>ER</sup>, to the ER stressor tunicamycin at day 1 and day 7 of adulthood and monitored their survival (Figure 1D). We found that when animals were transferred to tunicamycin at day 1, N2 worms survived substantially longer than their *xbp-1(zc12)* mutant counterparts (Figure 1E). However, when transferred at day 7, the survival of N2 animals was no longer than that of *xbp-1(zc12)* mutant animals, consistent with an age-onset loss of ER stress resistance. No difference in longevity between wild-type and *xbp-1* mutants was seen upon treatment with paraquat, an agent that increases oxidative stress and does not activate the UPR<sup>ER</sup>, demonstrating that *xbp-1* mutant animals are specifically susceptible to ER stress (Figures S2A and S2B).

We then asked whether the youthful susceptibility to ER stress that we observe in *xbp-1* animals affected longevity under standard culture conditions and found that the lifespan of *xbp-1* mutants was consistently shorter than that of N2 animals (Figure S2C). Importantly, pharyngeal pumping rates (indicative of food intake) were similar between these genotypes, and animals were still actively feeding at day 8 of adulthood (Figure S2D). These results suggest that the presence of functional XBP-1 offers a protection against ER stress in young animals that is no longer present in older animals, potentially due to a decline in XBP-1 activation and target regulation during early adulthood.

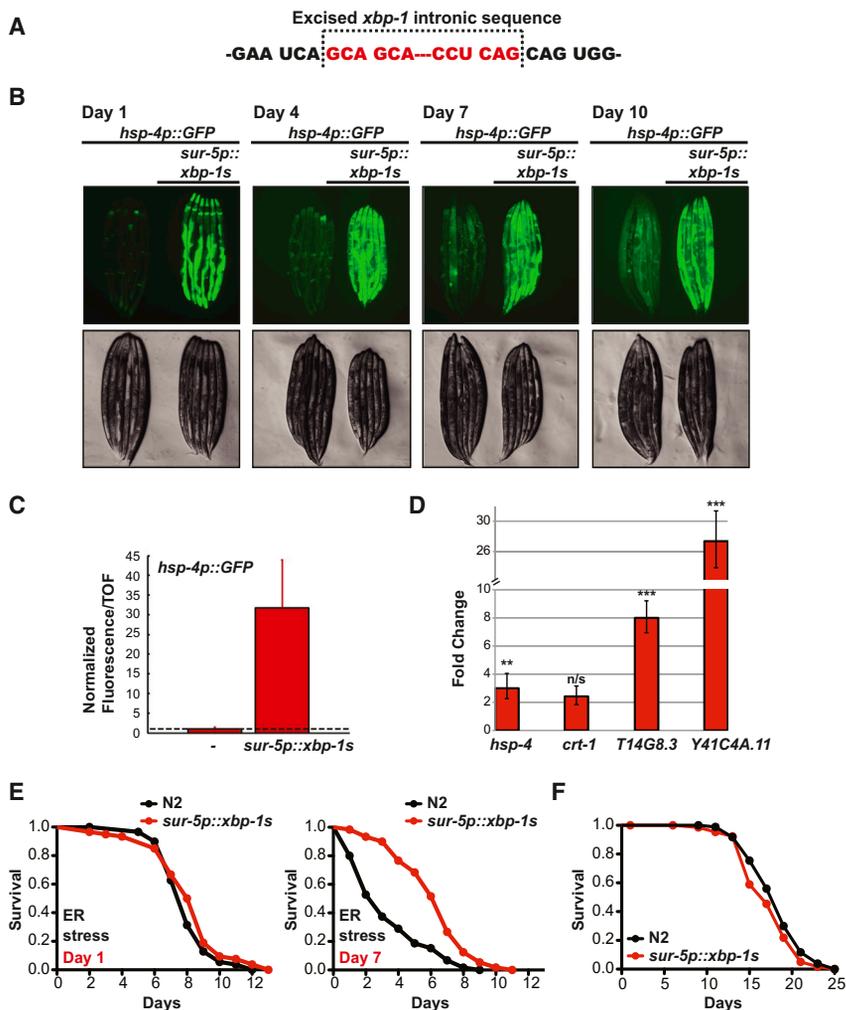
### Expression of *xbp-1s* Constitutively Activates the UPR<sup>ER</sup> Late in Life

Because *xbp-1* splicing and expression of UPR<sup>ER</sup> targets are lost with age, we hypothesized that the expression of a constitutively active, spliced form of *xbp-1* might be sufficient to induce UPR<sup>ER</sup> activation past the age at which stress-induced splicing of endogenous *xbp-1* ceases. Activation of *C. elegans xbp-1* is achieved by IRE-1-mediated removal of a 23 nucleotide intron, allowing the translation of an active transcription factor (Calfon

(D) Schematic describing the measurement of age-dependent ER stress resistance. Animals are transferred to plates containing tunicamycin at either day 1 or day 7 of adulthood.

(E) N2 (black) and *xbp-1(zc12)* (red) animals were transferred at day 1 and day 7 of adulthood to plates containing 50 µg/ml tunicamycin, and survival monitored (N2 day 1, median lifespan 7 days; *xbp-1(zc12)* day 1, median lifespan 5 days;  $p < 0.0001$ . N2 day 7, median lifespan 4 days; *xbp-1(zc12)* day 7, median lifespan 4 days;  $p = 0.9211$ . See Table S1).

See also Figure S1 and Table S2.



**Figure 2. Expression of *xbp-1s* under the *sur-5p* Promoter Constitutively Activates the UPR<sup>ER</sup> and Prevents a Decline in ER Stress Resistance**

(A) Schematic of the exon/intron boundaries of the 23 bp intron removed from the *xbp-1* sequence to activate the transcription factor.

(B) Fluorescent micrographs of *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms expressing an integrated *sur-5p::xbp-1s* transgene at days 1, 4, 7, and 10 of adulthood.

(C) A COPAS Biosort was used to measure length (TOF) and GFP fluorescence of *hsp-4p::GFP* (–) and *hsp-4p::GFP; sur-5p::xbp-1s* (*sur-5p::xbp-1s*) animals at day 1 of adulthood. Mean GFP fluorescence/TOF and standard deviation of each genotype were calculated and normalized to control *hsp-4p::GFP* animals.

(D) Transcript levels of UPR targets in *sur-5p::xbp-1s* animals at day 1 of adulthood were measured by quantitative RT-PCR. Results are shown relative to transcript levels in N2 worms, with error bars indicating mean  $\pm$  SD. A Student's *t* test was used to assess significance: \*\*\**p* < 0.005, \*\**p* < 0.01, *n/s* = not significant.

(E) N2 (black) and *sur-5p::xbp-1s* (red) animals were transferred at day 1 and day 7 of adulthood to plates containing 50  $\mu$ g/ml tunicamycin, and survival monitored (*sur-5p::xbp-1s* day 1, median lifespan 7 days; N2 day 1, median lifespan 7 days; *p* = 0.0078. *sur-5p::xbp-1s* day 7, median lifespan 7 days; N2 day 7, median lifespan 3 days; *p* < 0.0001. See Table S1).

(F) Survival of *sur-5p::xbp-1s* (red) and N2 (black) worms on *E. coli* OP50 at 20°C (*sur-5p::xbp-1s*: median lifespan 17 days; N2: black; median lifespan 19 days; *p* = 0.0925. See Table S3).

See also Figure S2 and Table S2.

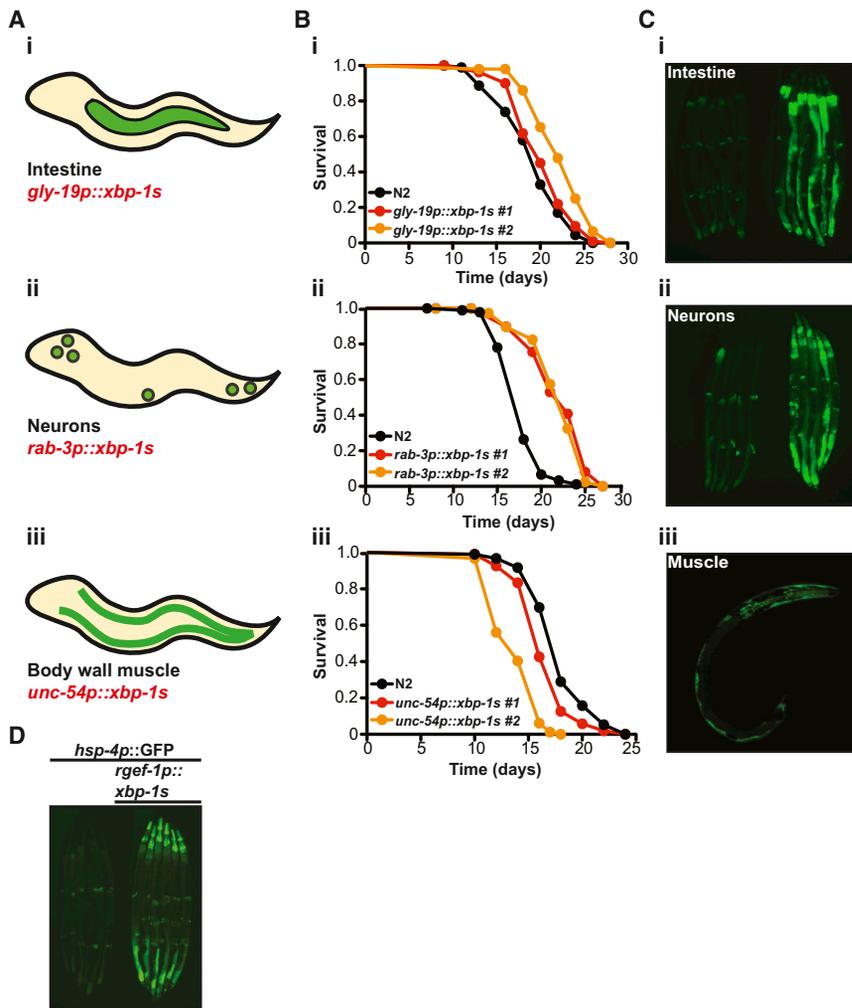
et al., 2002). We amplified *xbp-1* from *C. elegans* complementary DNA (cDNA) and removed this intronic sequence to create a cDNA sequence identical to that created by IRE-1-mediated splicing, *xbp-1s* (Figure 2A). We then expressed this gene in *C. elegans* under the ubiquitously expressed *sur-5p* promoter (Gu et al., 1998). Expression of *sur-5p::xbp-1s* in an *hsp-4p::GFP* background led to robust GFP expression throughout the animal at all ages, even those at which stress-induced splicing of *xbp-1* and target upregulation are no longer observed in wild-type animals (Figures 1C, 2B, and 2C). Transcript levels of UPR<sup>ER</sup> targets were also elevated in *sur-5p::xbp-1s* animals (Figure 2D).

If the constitutive expression of *xbp-1s* circumvents an age-associated loss of XBP-1 activation, we would predict that this would lead to increased resistance to ER stress at older ages. *sur-5p::xbp-1s* worms had resistance to ER stress similar to that of wild-type when transferred to plates containing tunicamycin at day 1 of adulthood (Figure 2E). However, when transferred to stress conditions at day 7 of adulthood, *sur-5p::xbp-1s* animals lived substantially longer. This difference was not caused by a reduced uptake of tunicamycin in the

food, as pharyngeal pumping rates were similar in both genotypes (Figure S2E). This effect was also specific, as resistance to paraquat was unaffected by *sur-5p::xbp-1s* expression (Figure S2F). However, and surprisingly, longevity of *sur-5p::xbp-1s* animals in the absence of ER stress was not increased; these animals exhibited a lifespan slightly shorter than that of N2 (Figure 2F).

#### Tissue-Specific Expression of *xbp-1s* Extends Lifespan

Ubiquitous expression of *xbp-1s* did not extend lifespan, despite increasing ER stress resistance. This suggested that *xbp-1s* expression might have positive effects on some tissues, while having negative effects on others. To test this hypothesis, we asked how lifespan was affected if we expressed *xbp-1s* under tissue-specific promoters (Figure 3A). Neuronal *xbp-1s* transgenic animals had a surprising and significant extension of longevity. Expression in the intestine also resulted in a modest increase in longevity, whereas expression in the muscle cells shortened lifespan (Figure 3B). We observed in these animals no defect in muscle cell structure or function that could directly explain their reduced longevity (Figures S3A and S3B).



### Neuronal *xbp-1s* Activates the UPR<sup>ER</sup> Cell Nonautonomously

We then used the *hsp-4p::GFP* UPR<sup>ER</sup> reporter to examine in which tissues activation of the UPR<sup>ER</sup> occurred in each of these tissue-specific *xbp-1s* expression strains. As expected, *gly-19p::xbp-1s* animals had increased GFP fluorescence exclusively in their intestine, and *unc-54p::xbp-1s* animals had GFP expression exclusively in their body-wall muscle cells (Figure 3C). We found that muscle-specific UPR<sup>ER</sup> activation was strongest in larvae, as is typical for promoters driving muscle expression in *C. elegans* (Honda and Epstein, 1990). Animals showing the highest degree of GFP fluorescence at larval stages tended to arrest before development to adulthood (data not shown). Surprisingly, worms expressing *xbp-1s* under the pan-neuronal *rab-3p* promoter had GFP expression not only in neuronal cell bodies and processes but also in the intestine, particularly the posterior intestine and the anterior two intestinal cells (Figure 3C). We confirmed the intestinal induction of *hsp-4p::GFP* using an additional neuronal-specific promoter, *rgef-1p* (Figure 3D) (Brignull et al., 2006). In contrast, animals expressing RFP under the same promoter (*rab-3p::*

### Figure 3. Neuronal and Intestinal Expression of *xbp-1s* Increases Lifespan, and Neuronal *xbp-1s* Can Induce Cell-Nonautonomous Activation of the UPR<sup>ER</sup>

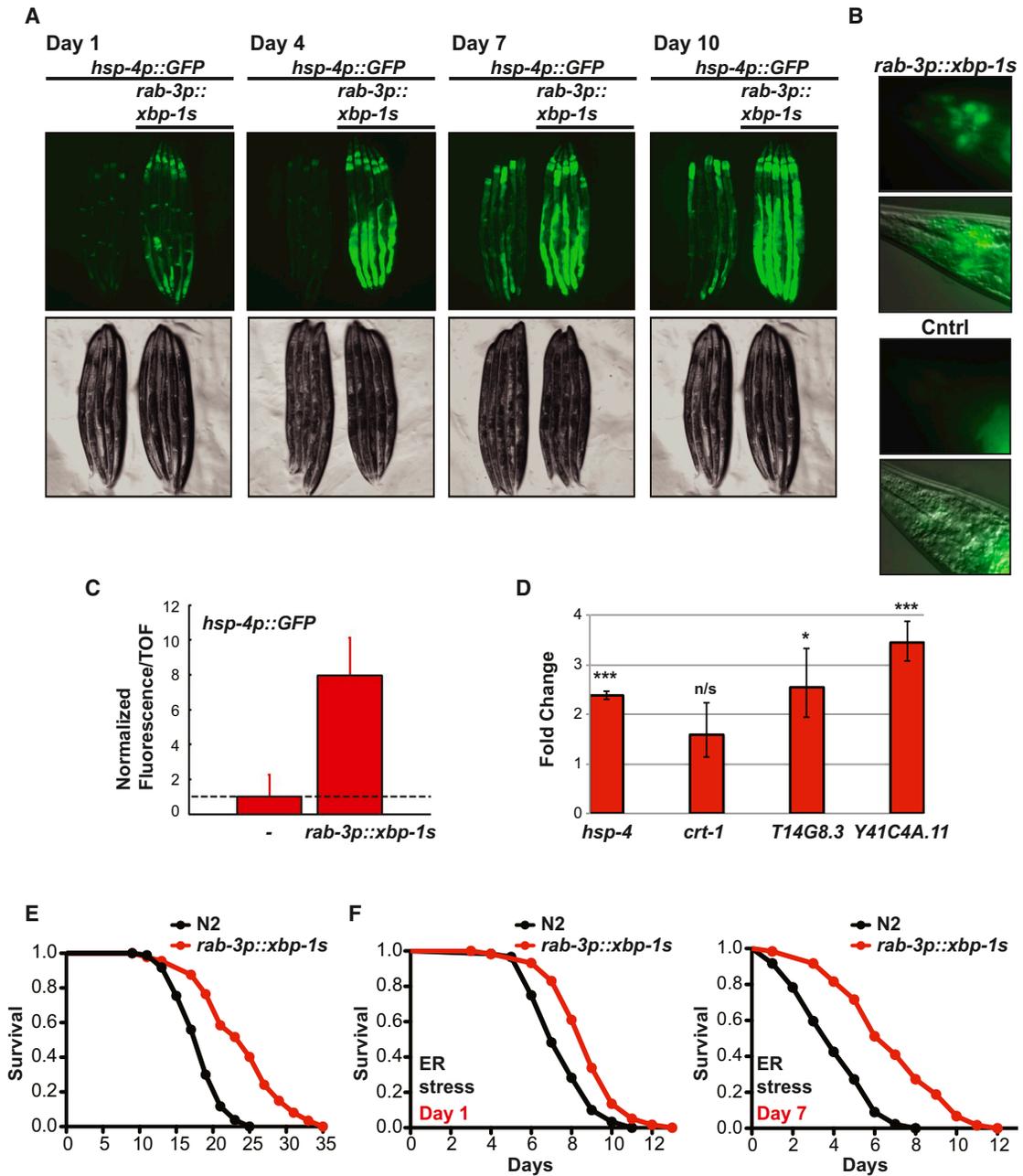
(A) Schematic of a tissue-specific *xbp-1s* expression system driving *xbp-1s* expression in neurons, intestine, and body-wall muscle cells. (B) Survival of N2 (black) and transgenic strains on *E. coli* OP50 at 20°C: (i) *gly-19p::xbp-1s* (line 1 [red], median lifespan 22 days;  $p = 0.0013$ ; line 2 [orange], median lifespan 24 days;  $p < 0.0001$ ), (ii) *rab-3p::xbp-1s* (line 1 [red], median lifespan 22 days;  $p < 0.0001$ ; line 2 [orange], median lifespan 22 days;  $p < 0.0001$ ), and (iii) *unc-54p::xbp-1s* (line 1 [red], median lifespan 16 days;  $p = 0.0003$ ; line 2 [orange], median lifespan 14 days;  $p < 0.0001$ ). See Table S3. (C) Fluorescent micrographs of *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms expressing (i) *gly-19p::xbp-1s*, (ii) *rab-3p::xbp-1s*, and (iii) *unc-54p::xbp-1s* transgenes at day 1 of adulthood (i and ii) and L2/L3 (iii). (D) Fluorescent micrograph of *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms expressing *rgef-1p::xbp-1s*. See also Figure S3.

*tdTomato*) showed RFP fluorescence only in the neurons (Nonet et al., 1997) (Figure S4A).

Intrigued by the ability of neuronal *xbp-1s* to drive cell-nonautonomous UPR<sup>ER</sup> activation, we derived integrated *rab-3p::xbp-1s* transgenic lines. Neuronal *xbp-1s* transgenic animals had a 32% increase in longevity, one of the longest caused by transgene expression in the worm (Henderson and Johnson, 2001; Hsu et al., 2003; Carrano et al., 2009; Bishop and Guarente, 2007; Viswanathan and Guarente, 2011). We then crossed these into an *hsp-4p::GFP* background. Intestinal as well as neuronal UPR<sup>ER</sup> activation was evident from larval stages throughout adulthood (Figures 4A–4C). Transcript levels of UPR<sup>ER</sup> targets were also increased in *rab-3p::xbp-1s* animals (Figure 4D). The extended longevity of *rab-3p::xbp-1s* worms (Figure 4E) could not be explained by toxicity of *xbp-1s* and functional ablation of specific neurons previously shown to affect lifespan (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004), as both the dye-filling capacity of the sensory neurons and chemotaxis behavior in these worms were normal (Figures S4B and S4C).

### Neuronal *xbp-1s* Rescues ER Stress Resistance

We asked whether expression of *xbp-1s* in the nervous system was sufficient to rescue ER stress resistance in older animals. We found that *rab-3p::xbp-1s* expression increased resistance to tunicamycin to a small but significant degree at day 1 of adulthood and to a much greater extent at day 7 (Figure 4F). Again, pharyngeal pumping was comparable at both ages, and



**Figure 4. Expression of *xbp-1s* in Neurons Activates the UPR<sup>ER</sup> in the Intestine and Prevents a Decline in Stress Resistance**

(A) Fluorescent micrographs of *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms expressing an integrated *rab-3p::xbp-1s* transgene at days 1, 4, 7, and 10 of adulthood. (B) Tail region of *hsp-4p::GFP* UPR<sup>ER</sup> reporter worms expressing an integrated *rab-3p::xbp-1s* transgene showing neurons at 63 $\times$  magnification. (C) Length (TOF) and GFP fluorescence of *hsp-4p::GFP* (-) and *hsp-4p::GFP*; *rab-3p::xbp-1s* (*rab-3p::xbp-1s*) animals were measured using a COPAS Biosort at day 1 of adulthood. Mean GFP fluorescence/TOF and standard deviation of each genotype were calculated and normalized to control *hsp-4p::GFP* animals. (D) Transcript levels of UPR targets in *rab-3p::xbp-1s* animals were measured at day 1 of adulthood by quantitative RT-PCR. Results are shown relative to transcript levels in N2 worms, with error bars indicating mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , \* $p < 0.05$ , n/s = not significant. (E) Survival of *rab-3p::xbp-1s* (red) and N2 (black) worms on *E. coli* OP50 at 20 $^{\circ}$ C (*rab-3p::xbp-1s*: median lifespan 25 days; N2: median lifespan 19 days;  $p < 0.0001$ . See Table S3). (F) *rab-3p::xbp-1s* (red) and N2 (black) animals were transferred at day 1 and day 7 of adulthood to plates containing 50  $\mu$ g/ml tunicamycin, and survival monitored (*rab-3p::xbp-1s* day 1, median lifespan 9 days; N2 day 1, median lifespan 7 days;  $p = 0.0078$ ; *rab-3p::xbp-1s* day 7, median lifespan 7 days; N2 day 7, median lifespan 4 days;  $p < 0.0001$ . See Table S1). See also Figure S4 and Table S2.

paraquat resistance was unaffected (Figures S4D and S4E). Expression of *xbp-1s* in neurons is therefore sufficient to increase resistance to ER stress in older animals and to increase lifespan substantially.

### Expression of *xbp-1s* Specifically Activates the UPR<sup>ER</sup>

We then asked whether cell-nonautonomous stress-response activation by *xbp-1s* was specific to the UPR<sup>ER</sup>. We found that neither the *hsp-6p::GFP* UPR<sup>mt</sup> nor the *hsp-16.2p::GFP* HSR reporter, nor transcriptional targets of either response, were activated by the neuronal expression of *xbp-1s* (Figures 5A–5E). In addition, RNA interference (RNAi) against *ubl-5*, essential for longevity in mitochondrial mutant animals in which the UPR<sup>mt</sup> is activated (Durieux et al., 2011), did not abolish lifespan extension in *rab-3p::xbp-1s* expressors (Figure S5A). Therefore the expression of *xbp-1s* in neurons specifically activates the UPR<sup>ER</sup> distally and promotes lifespan extension by a mechanism distinct from the activation of the UPR<sup>mt</sup> or HSR.

We also analyzed the role of other regulators of lifespan in *rab-3p::xbp-1s* animals. RNAi against *pha-4*, the FOXA transcription factor required for longevity in *C. elegans* models of dietary restriction (Panowski et al., 2007), did not shorten the lifespan of *rab-3p::xbp-1s* animals (Figure S5B). In contrast, RNAi against *daf-16*, the FOXO homolog required for the long lifespan of mutants with reduced insulin signaling (Kenyon et al., 1993), reduced longevity in this model (Figure S5C). However, *rab-3p::xbp-1s* expression further extended the long lifespan of animals with reduced levels of *daf-2*, the sole insulin receptor in *C. elegans* (Kimura et al., 1997) (Figure S5D). These results suggest that *rab-3p::xbp-1s* extends longevity by a mechanism distinct from previously described lifespan-extension pathways but partially requiring *daf-16*/FOXO.

### Cell-Nonautonomous Activation of the UPR<sup>ER</sup> Is Dependent upon Endogenous *xbp-1* and *ire-1*

The activation of *hsp-4p::GFP* expression upon ER stress is dependent on the presence of functional IRE-1 and XBP-1 (Calafon et al., 2002) (Figure S1A). We tested whether the cell-nonautonomous activation of the *hsp-4p* promoter observed in the intestine of *rab-3p::xbp-1s* animals was also dependent upon an intact UPR<sup>ER</sup>. We first expressed *rab-3p::xbp-1s* in *hsp-4p::GFP* animals that had an *ire-1(v33)* mutant background, with an 878 bp deletion extending from 199 bp upstream of the ATG start codon to bp 679 of the *ire-1* gene. We found that the *ire-1(v33)* mutation prevented UPR<sup>ER</sup> activation in the intestine of *rab-3p::xbp-1s* animals, whereas neuronal UPR<sup>ER</sup> activation was still significantly upregulated, suggesting that endogenous IRE-1 signaling is required for cell-nonautonomous UPR<sup>ER</sup> activation in distal cells (Figures 6A, 6B, S6A, and S6C). As an additional measure for the requirement of an intact UPR<sup>ER</sup> in distal cells, we fed worms *xbp-1* RNAi. Under these conditions, the UPR<sup>ER</sup> in neurons was significantly activated by neuronally expressed *xbp-1s*, whereas distal UPR<sup>ER</sup> induction by *rab-3p::xbp-1s* was entirely abolished, suggesting that endogenous *xbp-1* is required for perception of this signal (Figures 6C, 6D, S6B, and S6D). Taken together, loss of either *ire-1* or *xbp-1* blocks induction of the UPR<sup>ER</sup> in distal cells but not cell-autono-

mous UPR<sup>ER</sup> induction in the nervous system by neuronal expression of *xbp-1s*.

### Cell-Nonautonomous Activation of the UPR<sup>ER</sup> Is Required for Extended Longevity

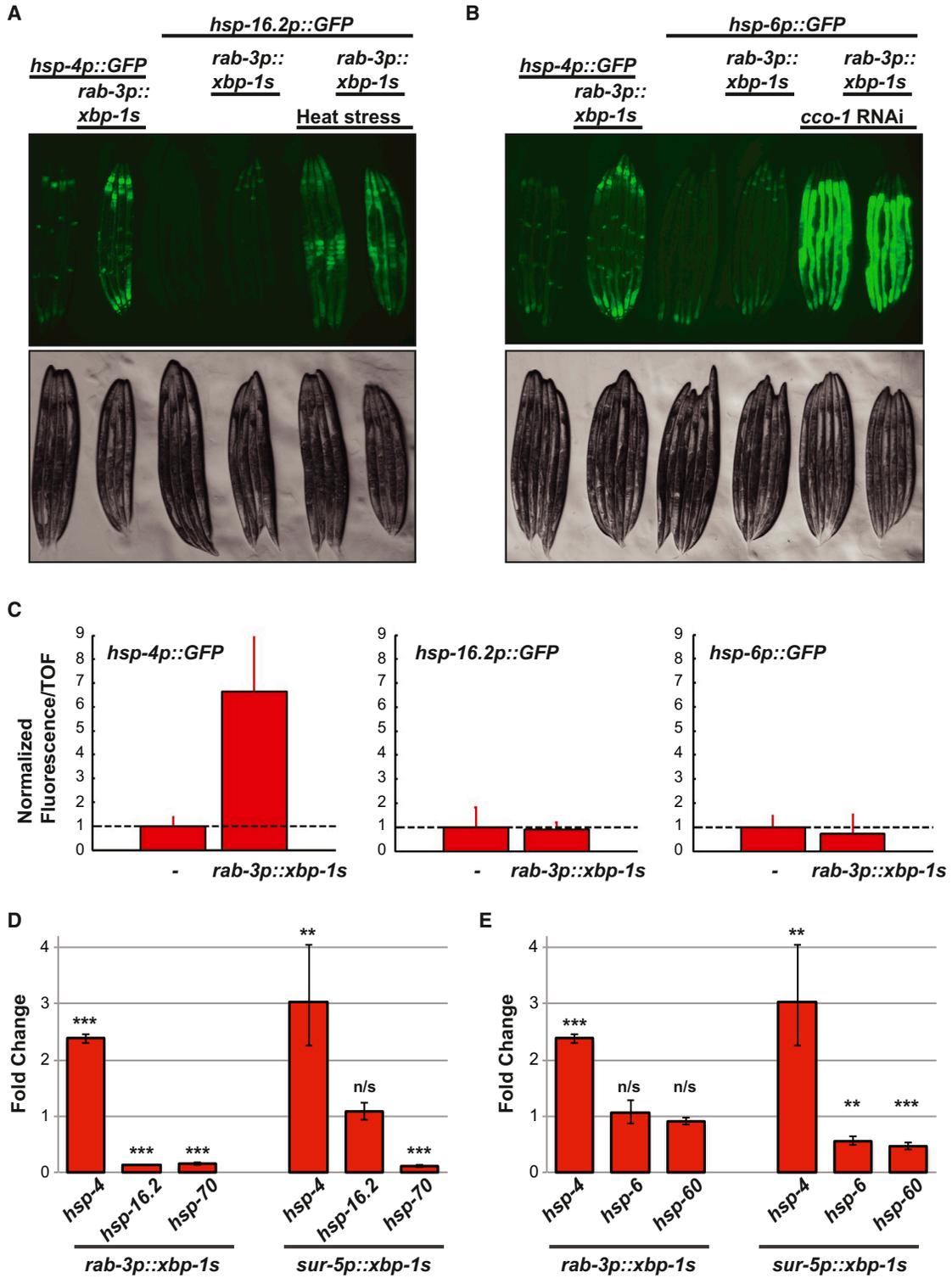
As animals expressing *xbp-1s* in their nervous systems are exceptionally long-lived, we next asked whether the communication of UPR<sup>ER</sup> activation between the nervous system and distal cells was required for increased longevity of these animals. We grew *rab-3p::xbp-1s* animals upon bacteria producing *xbp-1* RNAi to abolish non-neuronal UPR<sup>ER</sup> activation and asked whether this cell-nonautonomous activation was required for the extended longevity of *rab-3p::xbp-1s* worms. *rab-3p::xbp-1s* animals cultured on *xbp-1* RNAi bacteria had a wild-type lifespan, whereas *rab-3p::xbp-1s* animals on control RNAi were substantially long-lived (Figure 6E). The cell-nonautonomous activation of the UPR<sup>ER</sup> is therefore required for lifespan extension caused by neuronal UPR<sup>ER</sup> activation. However, as *xbp-1* RNAi also knocked down *xbp-1* in tissues other than the intestine, the specific tissues contributing to this lifespan suppression remain unknown.

### Cell-Nonautonomous Activation of the UPR<sup>ER</sup> Requires *unc-13*

We hypothesized that the ability of neuronal *xbp-1s* to activate the UPR<sup>ER</sup> in a distal non-neuronal tissue suggested the existence of a neuroendocrine signaling pathway through which neurons are able to influence the UPR<sup>ER</sup> status of distant cells. To test this, we crossed *rab-3p::xbp-1s*; *hsp-4p::GFP* animals with strains deficient in the release of neuronal signaling molecules. Specifically, we utilized the *unc-13(e450)* mutant strain, which is defective in the release of SCVs, and the *unc-31(e928)* mutant strain, which has a defect in the Ca<sup>2+</sup>-mediated release of dense core vesicles (DCVs) (Figure 7A) (Madison et al., 2005; Speese et al., 2007). When crossed with *unc-13(e450)*, cell-nonautonomous UPR<sup>ER</sup> activation in the intestine of *rab-3p::xbp-1s*; *hsp-4p::GFP* animals was reduced, but autonomous activation of the UPR<sup>ER</sup> in the nervous system remained intact (Figures 7B and S6E). *unc-13(e450)* animals could activate the UPR<sup>ER</sup> in the intestine upon expression of *gly-19::xbp-1s*, indicating that the ability to activate the UPR<sup>ER</sup> cell autonomously in an *unc-13(e450)* mutant background was not lost (Figures S6F and S6G). In contrast to a loss of *unc-13*, when the *rab-3p::xbp-1s* strain was crossed with *unc-31(e928)*, we found very little, if any, effect on distal UPR<sup>ER</sup> activation or activation in the nervous system (Figures 7B and S6E). Taken together, the release of SCVs from *xbp-1s*-expressing neurons by an *unc-13*-mediated mechanism is required for cell-nonautonomous activation of the UPR<sup>ER</sup> in distal tissues. The *unc-13* dependence suggests that the signaling molecule mediating cell-nonautonomous activation of the UPR<sup>ER</sup> may be released from SCVs and is therefore most likely a small-molecule neurotransmitter (Figure 7A).

### *unc-13* Is Required for Lifespan Extension and Increased Stress Resistance by Neuronal *xbp-1s* Expression

Because *unc-13*-mediated vesicle release was required for cell-nonautonomous UPR<sup>ER</sup> activation, we asked whether

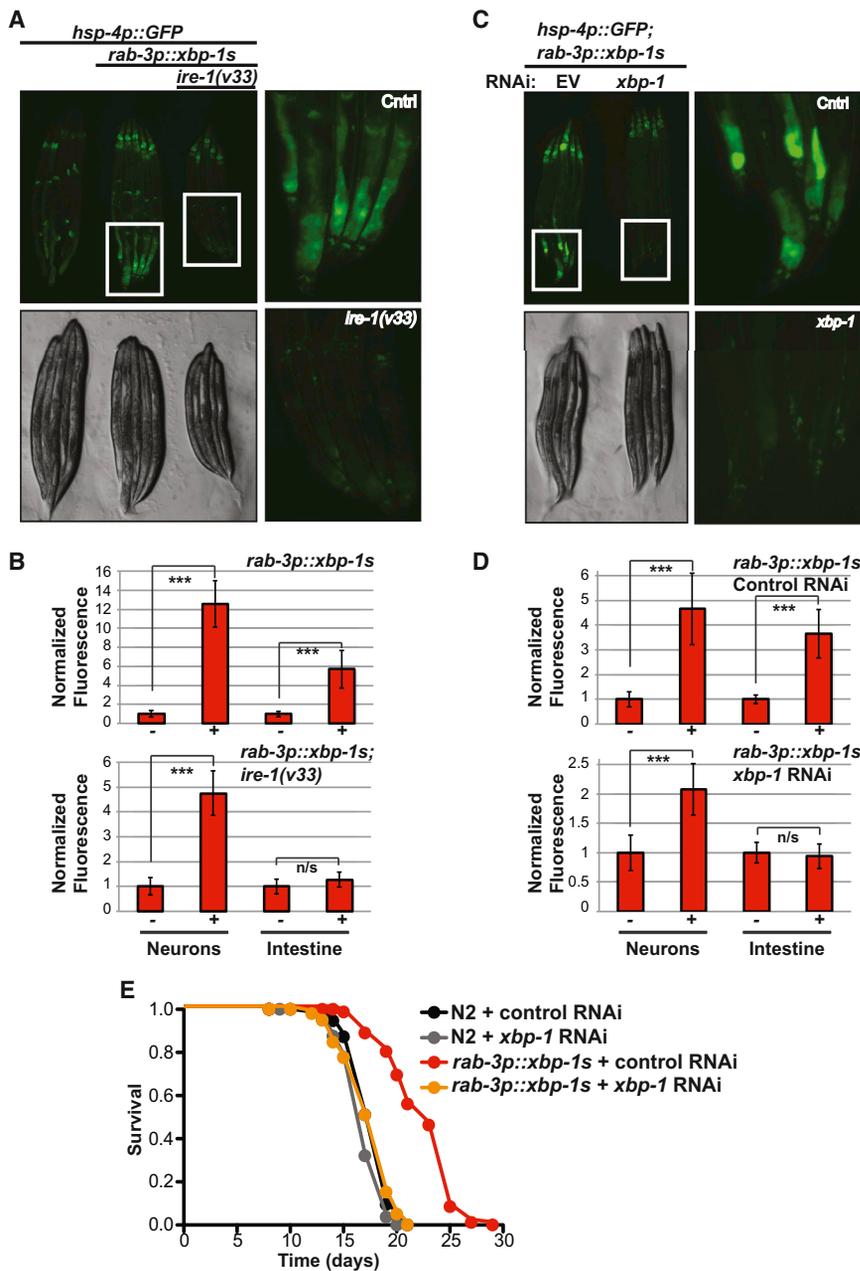


**Figure 5. Neuronal *xbp-1s* Specifically Activates the UPR<sup>ER</sup> Cell Nonautonomously**

(A) Fluorescent micrograph of *hsp-4p::GFP* UPR<sup>ER</sup> and *hsp-16.2p::GFP* HSR reporter worms with and without the *rab-3p::xbp-1s* transgene at day 1 of adulthood. Fluorescence in the pharyngeal area of *rab-3p::xbp-1s* animals is due to the *myo-2p::tdTomato* injection marker. As controls, separate populations of *hsp-16.2p::GFP* and *hsp-16.2p::GFP*; *rab-3p::xbp-1s* worms were incubated at 34°C for 6 hr.

(B) Fluorescent micrograph of *hsp-4p::GFP* UPR<sup>ER</sup> and *hsp-6p::GFP* UPR<sup>mt</sup> reporter worms, with and without the *rab-3p::xbp-1s* transgene at day 1 of adulthood. Fluorescence in the pharyngeal area of *rab-3p::xbp-1s* animals is due to the *myo-2p::tdTomato* injection marker. As controls, separate populations of *hsp-6p::GFP* and *hsp-6p::GFP*; *rab-3p::xbp-1s* animals were treated with *cco-1* RNAi from hatch.

(legend continued on next page)



**Figure 6. Cell-Nonautonomous Activation of the UPR<sup>ER</sup> Requires *ire-1* and *xbp-1***

(A) Fluorescent micrograph of *hsp-4p::GFP* and *hsp-4p::GFP; ire-1(v33)* worms expressing *rab-3p::xbp-1s* at day 1 of adulthood.

(B) Using a COPAS Biosort, fluorescence in the nerve ring and intestine were approximated by measuring GFP fluorescence in equidistant sections through the proximal 25% (nerve ring) and distal 75% (intestine) of *hsp-4p::GFP* (–) and *hsp-4p::GFP; rab-3p::xbp-1s* or *hsp-4p::GFP; rab-3p::xbp-1s; ire-1(v33)* (+) worms. Total fluorescence in each region was divided by number of sections to give average fluorescence per section, which was then averaged among animals and normalized to control *hsp-4p::GFP* worms. Error bars indicate mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , n/s = not significant. See Figure S6C.

(C) Fluorescent micrograph of *hsp-4p::GFP; rab-3p::xbp-1s* worms grown on empty vector and *xbp-1* RNAi.

(D) Fluorescence in the nerve ring and intestine were approximated as above in *hsp-4p::GFP* animals on control empty vector RNAi (–) and *hsp-4p::GFP; rab-3p::xbp-1s* animals on control or *xbp-1* RNAi (+). Error bars indicate mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , n/s = not significant. See Figure S6D.

(E) Survival of *rab-3p::xbp-1s* and N2 worms grown on empty vector control or *xbp-1* RNAi (*rab-3p::xbp-1s* on *xbp-1* [red], median lifespan 18 days; *rab-3p::xbp-1s* on empty vector [orange], median lifespan 22 days;  $p < 0.0001$ . See Table S3).

See also Figure S6.

stress resistance in a *unc-13(e450)* mutant background, strongly suggesting that communication between neurons and intestine through release of SCVs is essential for increased lifespan and stress resistance downstream of neuronal *xbp-1s* expression (Figures 7C and 7D). However, it should be noted that *unc-13(e450)* itself increases lifespan, complicating the interpretation of longevity data, and that as the muta-

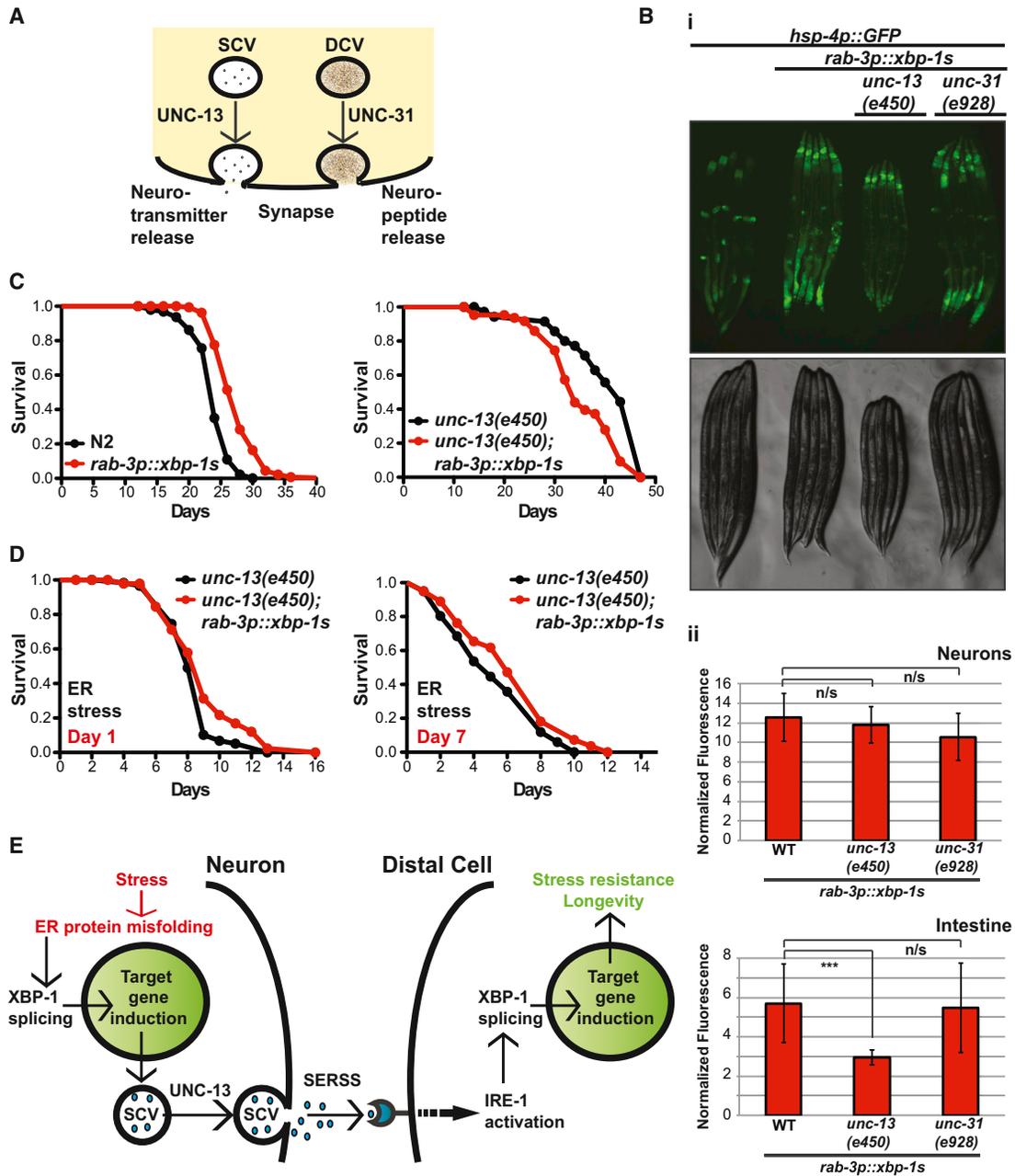
tion was not specific to neurons, loss of *unc-13* activity in other tissues may have contributed to this loss of longevity and stress resistance.

(C) A COPAS Biosort was used to measure length (TOF) and GFP fluorescence of *hsp-4p::GFP*, *hsp-6p::GFP*, and *hsp-16.2p::GFP* animals, with and without the *rab-3p::xbp-1s* transgene, at day 1 of adulthood. Mean GFP fluorescence/TOF and standard deviation of each genotype were calculated, with *rab-3p::xbp-1s* animals normalized to reporter controls.

(D) Transcript levels of HSR targets in *rab-3p::xbp-1s* and *sur-5p::xbp-1s* animals were measured by quantitative RT-PCR. Results are shown relative to transcript levels in N2 worms, with error bars indicating mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , \*\* $p < 0.01$ , n/s = not significant.

(E) Transcript levels of UPR<sup>mt</sup> targets in *rab-3p::xbp-1s* and *sur-5p::xbp-1s* animals were measured by quantitative RT-PCR. Results are shown relative to transcript levels in N2 worms, with error bars indicating mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , \*\* $p < 0.01$ , n/s = not significant.

See also Figure S5.



**Figure 7. Cell-Nonautonomous Activation of the UPR<sup>ER</sup> Requires *unc-13***

(A) Schematic of the roles of UNC-13 and UNC-31 in neurosecretory vesicle release. UNC-13 is required for the release of SCVs containing small-molecule neurotransmitters. UNC-31 is involved in the release of DCVs primarily containing neuropeptides.

(B) (i) Fluorescent micrograph of *hsp-4p::GFP*, *hsp-4p::GFP*; *rab-3p::xbp-1s*, *hsp-4p::GFP*; *rab-3p::xbp-1s*; *unc-13(e450)*, and *hsp-4p::GFP*; *rab-3p::xbp-1s*; *unc-31(e928)* worms at day 1 of adulthood.

(ii) Using a COPAS Biosort, fluorescence in the nerve ring and intestine of *hsp-4p::GFP*; *rab-3p::xbp-1s* animals in a wild-type (WT), *unc-13(e450)*, or *unc-31(e928)* background was approximated by measuring GFP fluorescence in equidistant sections through the proximal 25% (nerve ring) and distal 75% (intestine) of worms. Total fluorescence in each region was divided by number of sections to give average fluorescence per section, which was then averaged among animals and normalized to *hsp-4p::GFP* controls. Error bars indicate mean  $\pm$  SD. A Student's t test was used to assess significance: \*\*\* $p < 0.005$ , n/s = not significant. See Figure S6E.

(C) Survival of (left panel) *rab-3p::xbp-1s* and N2 and (right panel) *rab-3p::xbp-1s*; *unc-13(e450)* and *unc-13(e450)* animals was assessed at 15°C, following a single treatment with FUDR at  $t = 0$  (left panel: *rab-3p::xbp-1s* [red], median lifespan 28 days; N2 [black], median lifespan 24 days;  $p < 0.0001$ ; right panel: *rab-3p::xbp-1s*; *unc-13(e450)* [red], median lifespan 34 days; *unc-13(e450)* [black], median lifespan 43 days;  $p < 0.0001$ . See Table S3).

(D) (Left panel) *rab-3p::xbp-1s* and N2 and (right panel) *rab-3p::xbp-1s*; *unc-13(e450)* and *unc-13(e450)* animals were transferred at day 1 and day 7 of adulthood to plates containing 50  $\mu$ g/ml tunicamycin, and survival monitored (left panel: *rab-3p::xbp-1s*; *unc-13(e450)* day 1 [red], median lifespan 9 days; *unc-13(e450)* day 1

(legend continued on next page)

Intrigued to identify the molecular mediator originating from SCVs, we treated *hsp-4p::GFP* and *hsp-4p::GFP; rab-3p::xbp-1s; unc-13(e450)* animals with a range of small-molecule neurotransmitters and biogenic amines known to be secreted in an *unc-13*-mediated manner. Neurotransmitters and amines included (at concentrations previously demonstrated to be physiologically effective) acetylcholine, levamisole, glutamate, GABA, octopamine, tyramine, dopamine, and serotonin (Lewis et al., 1980; McIntire et al., 1993; Dent et al., 1997; Wragg et al., 2007; Schafer and Kenyon, 1995). However, none of these molecules consistently induced activation of the UPR<sup>ER</sup>, suggesting that they are unlikely to act as mediators of the cell-nonautonomous UPR<sup>ER</sup> response (Figures S7A and S7B). The identity of this signaling molecule remains to be defined.

We therefore propose a model whereby the detection of ER stress and activation of the IRE-1/XBP-1 UPR<sup>ER</sup>-signaling module in neurons leads to the release of one or more as-yet-uncharacterized small-molecule neurotransmitters from SCVs, leading to activation of the IRE-1/XBP-1 branch of the UPR<sup>ER</sup> in the intestine, with beneficial effects on ER stress resistance and longevity (Figure 7F).

## DISCUSSION

Cellular processes face an inevitable decline with age, exacerbated by a lack of evolutionary imperative to maintain proteostasis beyond early adulthood. Because many of the diseases associated with ER dysfunction are age onset and because prior evidence has suggested that the UPR<sup>ER</sup> becomes attenuated in older organisms, we hypothesized that a restoration of the UPR<sup>ER</sup> might have a protective effect on the viability of older animals.

Consistent with this hypothesis, we report that activation of *xbp-1* is lost in an age-dependent manner, leading to a decrease in ER stress resistance. It is not clear why *xbp-1* splicing is lost with age given that both *ire-1* and *xbp-1* transcripts are present in older animals; possible explanations include a failure of the *xbp-1* transcript to localize to the ER or a reduced ability to activate IRE-1 at the protein level (Aragón et al., 2009; Korennykh et al., 2009). Furthermore, and significantly, the reintroduction of a single element that declines with age in this system, *xbp-1s*, is sufficient to restore ER stress resistance in aged populations. Neuronal *xbp-1s* expression activates the UPR<sup>ER</sup> in distal cells nonautonomously, in a manner dependent upon the presence of endogenous *ire-1* and *xbp-1* in the receiving cells. Intriguingly, we only find nonautonomous UPR<sup>ER</sup> activation between neurons and the intestine, and only in one direction, with expression of *xbp-1s* in the intestine having no apparent effect on UPR<sup>ER</sup> activation in neurons. It is interesting to note that in *C. elegans*, the neurons and intestine are the tissues most prone to UPR<sup>ER</sup> activation when animals are faced with

ER stress (Shim et al., 2004), and the intestine in particular is extremely sensitive to perturbations in ER proteostasis (Richardson et al., 2010, 2011). This communication of ER stress from the nervous system is required for extension of longevity by neuronal *xbp-1s*.

At this time, we do not yet understand the fundamental mechanisms by which this signal is generated or perceived. We do, however, find a dependency of nonautonomous signaling on *unc-13*, suggesting that one or more small molecules released from SCVs are required for the signal. UNC-13 function is also essential for increases in longevity and stress resistance by neuronal UPR<sup>ER</sup> activation. The fact that either knockdown of UPR<sup>ER</sup> signaling in distal cells or the abolishment of neuronal secretion through mutations in *unc-13*—very different interventions with different effects on physiology and longevity—can eliminate lifespan extension by neuronal *xbp-1s* expression suggests that cell-nonautonomous UPR<sup>ER</sup> activation plays a pivotal role in the long lifespan and stress resistance of these animals.

What, then, is the mechanism by which this increase in healthspan is achieved? It is possible that neuronal *xbp-1s* expression leads to the release of a signal that directly rejuvenates all tissues of the animal, activating the UPR<sup>ER</sup> in the intestine and potentially other responses in other tissues. Indeed, as longevity induced by intestinal *xbp-1s* expression does not extend lifespan to the extent of neuronal *xbp-1s* expression, the possibility remains that effects on other tissues contribute to this longevity. This hypothesis would be supported by evidence suggesting that *xbp-1s* delays phenotypes of age-related decline, such as tissue decay or necrosis, similarly in all tissues. Alternatively, however, our evidence strongly suggests a specific, pivotal role for the intestine in the lifespan extension seen in *rab-3p::xbp-1s* animals, implying that this longevity is due to increased protection from stress specifically in the intestine following the activation of the UPR<sup>ER</sup> in neurons. This is supported by the striking activation of the UPR<sup>ER</sup> in the intestine following *xbp-1s* expression in neurons, the increase in lifespan when *xbp-1s* is specifically expressed in the intestine, and the abrogation of lifespan extension in *rab-3p::xbp-1s* animals when intestinal UPR<sup>ER</sup> activation is blocked by loss of *unc-13* or *xbp-1*.

Our data therefore suggest a central role for the intestine in the maintenance of youthfulness, and indeed, this tissue has been previously implicated as a site in which insulin-signaling perturbations and stress-response activation extend longevity (Libina et al., 2003; Durieux et al., 2011). *xbp-1* and *ire-1* are required for the extension of lifespan by reduced IIS, and it has been proposed that XBP-1 and DAF-16 might function together to promote longevity (Henis-Korenblit et al., 2010). We have found that lifespan extension by *rab-3p::xbp-1s* is reduced, but not eliminated, upon knockdown of *daf-16*. It is therefore possible that these two transcription factors work together in

[black], median lifespan 8 days;  $p = 0.1295$ ; right panel: *rab-3p::xbp-1s; unc-13(e450)* day 7 [red], median lifespan 6 days; *unc-13(e450)* day 7 [black], median lifespan 5 days;  $p = 0.0908$ . See Table S1).

(E) Model for the communication of UPR<sup>ER</sup> activation between neurons and distal cells. ER stress in neurons induces splicing of *xbp-1* and activation of target gene transcription that leads to UNC-13-mediated release of a secreted ER stress signal (SERSS) from SCVs. This signal induces the UPR<sup>ER</sup> in distal intestinal cells through activation of endogenous IRE-1 and XBP-1, leading to target gene transcription and increased stress resistance and longevity.

See also Figure S7.

the cell-nonautonomous promotion of longevity. Coincidentally, *daf-16*, like *xbp-1s*, is required in the nervous system and intestinal cells to modulate longevity (Wolkow et al., 2000; Libina et al., 2003).

Our findings address a long-standing question—how the tissues of an organism age in a coordinate and seemingly orchestrated manner. It increasingly seems that cell-nonautonomous activation of stress responses, mediated by neurons, may be a common mechanism to coordinate stress resistance and aging in different tissues across an organism.

## EXPERIMENTAL PROCEDURES

### Strains

SJ4005 (*zcls4[hsp-4p::GFP]*), SJ17 (*xbp-1(zc12);zcls4[hsp-4p::GFP]*), SJ4100 (*zcls13[hsp-6p::GFP]*), CL2070 (*dvls[hsp-16.2::GFP]*), RE666 (*ire-1(v33)*), and N2 were obtained from the *Caenorhabditis* Genetics Center. Strain CB928 (*unc-31(e928)*) was a gift from the Jin lab (UCSD). Strain CB450 (*unc-13(e450)*) was a gift from the Chalasani lab (The Salk Institute). Strain AGD972 (*xbp-1(zc12)*) was derived by further out-crossing strain SJ17 four times to N2. To make strains expressing spliced *xbp-1*, *xbp-1* cDNA was amplified from N2 worms by PCR. A 23 nucleotide intron sequence was removed using the QuikChange site-directed mutagenesis kit (QIAGEN). The *xbp-1s* cDNA was inserted into sequence-verified vectors containing the *sur-5*, *gly-19*, *rab-3*, *unc-54*, and *rgef-1* promoters and the *unc-54* 3' untranslated region (UTR) sequence. DNA was injected into N2 worms at 25 ng/μl, with the exception of *gly-19p::xbp-1s*, which was injected at 75 ng/μl, in the presence of *myo2p::tdTomato* at 15 ng/μl. *sur-5p::xbp-1s* and *rab-3p::xbp-1s* extrachromosomal arrays were integrated and backcrossed to N2 seven and eight times, respectively. All transgenic lines were also crossed with strain SJ4005 (*zcls4[hsp-4p::GFP]*). The integrated *rab-3p::xbp-1s* line AGD927 was crossed with strains SJ4100 (*zcls13[hsp-6p::GFP]*), CL2070 (*dvls[hsp-16.2::GFP]*), RE666 (*ire-1(v33)*), CB928 (*unc-31(e928)*), and CB450 (*unc-13(e450)*).

### Induction of ER Stress

Eggs were isolated by bleaching and allowed to develop on *E. coli* OP50. At the indicated ages, animals were incubated for 4 hr at 20°C in a 25 ng/μl solution of tunicamycin in M9 buffer prepared from a 1 mg/ml stock solution in DMSO. Control animals were incubated in an equivalent dilution of DMSO in M9.

### Analysis of Fluorescence

For fluorescence microscopy, worms were anesthetized with 50 mM sodium azide at specified ages, and a Leica S6E dissecting microscope and a Zeiss Axio Observer Z1 used. Fluorimetry assays were performed on a TECAN infinite M1000 fluorescence plate reader. A COPAS Biosort (Union Biometrical) was also used to measure fluorescence of individual worms for quantification. Detailed methodologies for fluorescence protocols can be found in the Extended Experimental Procedures.

### qPCR

Total RNA was harvested from populations of 500 animals using Qiazol reagent (QIAGEN). RNA was purified on an RNeasy mini column (QIAGEN), and cDNA synthesized using the QuantiTect reverse transcription kit (QIAGEN). SybrGreen quantitative RT-PCR experiments were performed as described in the manual using an ABI Prism 7900HT (Applied Biosystems), and data analyzed using the comparative 2 $\Delta\Delta$ C<sub>t</sub> method. Experiments were repeated at least three times.

### ER Stress Survival Assays

Synchronized eggs were harvested by bleaching, and worms were grown on *E. coli* OP50 without tunicamycin until day 1 or day 7 of adulthood. Tunicamycin was added to NG plates at a final concentration of 50 μg/ml and a final DMSO concentration of 0.2%, and plates seeded with *E. coli* OP50 from an overnight culture for 24 hr before animals were transferred. Upon transfer to

tunicamycin-containing plates, viability was scored daily. All assays were performed with 60–100 animals at 20°C at least three times.

### Lifespan Analyses

Lifespan analyses were performed at 20°C, unless otherwise indicated, with the pre-fertile period of adulthood as  $t = 0$  and were repeated at least three times. Eighty to one hundred animals were used per condition and scored every day or every second day. Lifespans were performed on *E. coli* OP50, unless otherwise indicated, and animals were treated with 100 μg/ml FUDR at  $t = 0$  and again at day 5 of adulthood. All lifespans were also repeated without FUDR. Prism 5 and JMP 8 software were used for statistical analysis. In all cases,  $p$  values were calculated using the log-rank (Mantel-Cox) method.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cell.2013.05.042>.

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## REFERENCES

- Acosta-Alvarez, D., Zhou, Y., Blais, A., Tsikitis, M., Lents, N.H., Arias, C., Lennon, C.J., Kluger, Y., and Dynlacht, B.D. (2007). XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. *Mol. Cell* 27, 53–66.
- Akerfelt, M., Morimoto, R.I., and Sistonen, L. (2010). Heat shock factors: integrators of cell stress, development and lifespan. *Nat. Rev. Mol. Cell Biol.* 11, 545–555.
- Alcedo, J., and Kenyon, C. (2004). Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. *Neuron* 41, 45–55.
- Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* 402, 804–809.
- Aragón, T., van Anken, E., Pincus, D., Serafimova, I.M., Korennykh, A.V., Rubio, C.A., and Walter, P. (2009). Messenger RNA targeting to endoplasmic reticulum stress signalling sites. *Nature* 457, 736–740.
- Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. (2008). Adapting proteostasis for disease intervention. *Science* 319, 916–919.
- Ben-Zvi, A., Miller, E.A., and Morimoto, R.I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. USA* 106, 14914–14919.
- Bishop, N.A., and Guarente, L. (2007). Two neurons mediate diet-restriction-induced longevity in *C. elegans*. *Nature* 447, 545–549.
- Brignull, H.R., Moore, F.E., Tang, S.J., and Morimoto, R.I. (2006). Polyglutamine proteins at the pathogenic threshold display neuron-specific aggregation in a pan-neuronal *Caenorhabditis elegans* model. *J. Neurosci.* 26, 7597–7606.
- Calton, M., Zeng, H., Urano, F., Till, J.H., Hubbard, S.R., Harding, H.P., Clark, S.G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415, 92–96.

- Carrano, A.C., Liu, Z., Dillin, A., and Hunter, T. (2009). A conserved ubiquitination pathway determines longevity in response to diet restriction. *Nature* 460, 396–399.
- Caruso, M.E., Jenna, S., Bouche-careilh, M., Baillie, D.L., Boismenu, D., Halawani, D., Latterich, M., and Chevet, E. (2008). GTPase-mediated regulation of the unfolded protein response in *Caenorhabditis elegans* is dependent on the AAA+ ATPase CDC-48. *Mol. Cell. Biol.* 28, 4261–4274.
- Dent, J.A., Davis, M.W., and Avery, L. (1997). *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.* 16, 5867–5879.
- Dillin, A., Crawford, D.K., and Kenyon, C. (2002a). Timing requirements for insulin/IGF-1 signaling in *C. elegans*. *Science* 298, 830–834.
- Dillin, A., Hsu, A.-L., Arantes-Oliveira, N., Lehrer-Grauer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., and Kenyon, C. (2002b). Rates of behavior and aging specified by mitochondrial function during development. *Science* 298, 2398–2401.
- Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144, 79–91.
- Galbadage, T., and Hartman, P.S. (2008). Repeated temperature fluctuation extends the life span of *Caenorhabditis elegans* in a *daf-16*-dependent fashion. *Mech. Ageing Dev.* 129, 507–514.
- Gu, T., Orita, S., and Han, M. (1998). *Caenorhabditis elegans* SUR-5, a novel but conserved protein, negatively regulates LET-60 Ras activity during vulval induction. *Mol. Cell. Biol.* 18, 4556–4564.
- Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.-J., and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. *Aging Cell* 6, 95–110.
- Haynes, C.M., and Ron, D. (2010). The mitochondrial UPR - protecting organelle protein homeostasis. *J. Cell Sci.* 123, 3849–3855.
- Heifetz, A., Keenan, R.W., and Elbein, A.D. (1979). Mechanism of action of tunicamycin on the UDP-GlcNAc:dolichyl-phosphate Glc-Nac-1-phosphate transferase. *Biochemistry* 18, 2186–2192.
- Henderson, S.T., and Johnson, T.E. (2001). *daf-16* integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. *Curr. Biol.* 11, 1975–1980.
- Henis-Korenblit, S., Zhang, P., Hansen, M., McCormick, M., Lee, S.J., Cary, M., and Kenyon, C. (2010). Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. *Proc. Natl. Acad. Sci. USA* 107, 9730–9735.
- Honda, S., and Epstein, H.F. (1990). Modulation of muscle gene expression in *Caenorhabditis elegans*: differential levels of transcripts, mRNAs, and polypeptides for thick filament proteins during nematode development. *Proc. Natl. Acad. Sci. USA* 87, 876–880.
- Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300, 1142–1145.
- Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461–464.
- Kijima, Y., Ogunbunmi, E., and Fleischer, S. (1991). Drug action of thapsigargin on the Ca<sup>2+</sup> pump protein of sarcoplasmic reticulum. *J. Biol. Chem.* 266, 22912–22918.
- Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277, 942–946.
- Korennykh, A.V., Egea, P.F., Korostelev, A.A., Finer-Moore, J., Zhang, C., Shokat, K.M., Stroud, R.M., and Walter, P. (2009). The unfolded protein response signals through high-order assembly of Ire1. *Nature* 457, 687–693.
- Lewis, J.A., Wu, C.-H., Berg, H., and Levine, J.H. (1980). The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics* 95, 905–928.
- Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 115, 489–502.
- Lytton, J., Westlin, M., and Hanley, M.R. (1991). Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *J. Biol. Chem.* 266, 17067–17071.
- Madison, J.M., Nurrish, S., and Kaplan, J.M. (2005). UNC-13 interaction with syntaxin is required for synaptic transmission. *Curr. Biol.* 15, 2236–2242.
- McIntire, S.L., Jorgensen, E., and Horvitz, H.R. (1993). Genes required for GABA function in *Caenorhabditis elegans*. *Nature* 364, 334–337.
- Nonet, M.L., Staunton, J.E., Kilgard, M.P., Fergestad, T., Hartwig, E., Horvitz, H.R., Jorgensen, E.M., and Meyer, B.J. (1997). *Caenorhabditis elegans* rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. *J. Neurosci.* 17, 8061–8073.
- Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa mediates diet-restriction-induced longevity of *C. elegans*. *Nature* 447, 550–555.
- Richardson, C.E., Kooistra, T., and Kim, D.H. (2010). An essential role for XBP-1 in host protection against immune activation in *C. elegans*. *Nature* 463, 1092–1095.
- Richardson, C.E., Kinkel, S., and Kim, D.H. (2011). Physiological IRE-1-XBP-1 and PEK-1 signaling in *Caenorhabditis elegans* larval development and immunity. *PLoS Genet.* 7, e1002391.
- Ron, D., and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* 8, 519–529.
- Schafer, W.R., and Kenyon, C.J. (1995). A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. *Nature* 375, 73–78.
- Shen, X., Ellis, R.E., Sakaki, K., and Kaufman, R.J. (2005). Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in *C. elegans*. *PLoS Genet.* 1, e37.
- Shim, J., Umemura, T., Nothstein, E., and Rongo, C. (2004). The unfolded protein response regulates glutamate receptor export from the endoplasmic reticulum. *Mol. Biol. Cell* 15, 4818–4828.
- Sidrauski, C., and Walter, P. (1997). The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* 90, 1031–1039.
- Speese, S., Petrie, M., Schuske, K., Ailion, M., Ann, K., Iwasaki, K., Jorgensen, E.M., and Martin, T.F. (2007). UNC-31 (CAPS) is required for dense-core vesicle but not synaptic vesicle exocytosis in *Caenorhabditis elegans*. *J. Neurosci.* 27, 6150–6162.
- Viswanathan, M., and Guarente, L. (2011). Regulation of *Caenorhabditis elegans* lifespan by sir-2.1 transgenes. *Nature* 477, E1–E2.
- Wolkow, C.A., Kimura, K.D., Lee, M.S., and Ruvkun, G. (2000). Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. *Science* 290, 147–150.
- Wragg, R.T., Hapiak, V., Miller, S.B., Harris, G.P., Gray, J., Komuniecki, P.R., and Komuniecki, R.W. (2007). Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in *Caenorhabditis elegans* through two novel amine receptors. *J. Neurosci.* 27, 13402–13412.