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Four glial cells regulate ER stress resistance and longevity via neuropeptide signaling in *C. elegans*

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Abstract

The ability of the nervous system to sense cellular stress and coordinate protein homeostasis is essential for organismal health. Unfortunately, stress responses that mitigate disturbances in proteostasis, such as the unfolded protein response of the endoplasmic reticulum (UPR^{ER}), become defunct with age. In this work, we expressed the constitutively active UPR^{ER} transcription factor, XBP-1s, in a subset of astrocyte-like glia, which extended the life span in *Caenorhabditis elegans*. Glial XBP-1s initiated a robust cell nonautonomous activation of the UPR^{ER} in distal cells and rendered animals more resistant to protein aggregation and chronic ER stress. Mutants deficient in neuropeptide processing and secretion suppressed glial cell nonautonomous induction of the UPR^{ER} and life-span extension. Thus, astrocyte-like glial cells play a role in regulating organismal ER stress resistance and longevity.

During aging, there is an organism-wide loss of protein homeostasis, exacerbated by the inability to mount an effective unfolded protein response of the endoplasmic reticulum (UPR^{ER}), which likely contributes to tissue damage and increased susceptibility to disease (1-3). The age-dependent decline in the ability to induce the UPR^{ER} can be prevented by the selective overexpression of constitutively active *xbp-1s* in neurons. Neuronal XBP-1s leads to cell nonautonomous activation of the UPR^{ER} in distal intestinal cells, which is sufficient

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Author contributions: A.E.F. conceived the study, generated *C. elegans* strains, performed experiments (life spans, microscopy, COPAS biosorting, and data analysis), and wrote the manuscript. M.G.M. performed life spans, microscopy, tunicamycin ER stress assays, and worm crosses and provided intellectual input. S.U.T. performed life spans, worm crosses, and COPAS biosorting and prepared artwork for Fig. 1 and fig. S13. R.B.-Z. analyzed RNA-seq data, generated figures, and provided intellectual input. J.D. helped generate and analyze strains, prepared artwork in fig. S7, and provided intellectual input. H.K.G. performed backcrosses and promoter characterization and provided intellectual input. N.K. assisted in generating strains and performed crosses and life spans. S.M. performed life spans and assisted with crosses and preparation of samples for RNA-seq. A.D. provided invaluable feedback throughout the project and toward the manuscript. All authors reviewed and edited the manuscript.

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Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials. The RNA-seq dataset supporting the conclusions of this article is available in the National Center for Biotechnology Information Sequence Read Archive repository, under accession number BioProject PRJNA589459. Further information and requests for reagents may be directed to dillinlabmaterials@berkeley.edu and will be fulfilled by A.D.

SUPPLEMENTARY MATERIALS

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to confer ER stress resistance and prolong life span (2). To date, cell non-autonomous stress signaling has been ascribed only to neurons (2, 4-7). However, glial cells— the gatekeepers and guardians of the central nervous system—may also play a role in regulating organismal stress resistance and longevity (8).

To determine whether glia play a role in regulating protein homeostasis and longevity, we generated *Caenorhabditis elegans* strains overexpressing *xbp-1s* under a glial-specific promoter, *ptr-10*, which is expressed in most glia except amphid sheath glia (fig. S1A) (9,10). Animals expressing *xbp-1s* in most glia (*ptr-10p::xbp-1s*) exhibited a marked increase in survival compared with control (N2) animals (Fig. 1A). To identify which glial cells were mediating XBP-1s-dependent longevity, we expressed *xbp-1s* within select subtypes of the 56 *C. elegans* glial cells (11). Expression of *xbp-1s* specifically in two amphid and two phasmid sheath glia (AMsh and PHsh) using the *fig-1* promoter did not extend life span beyond that of control animals (Fig. 1B and fig. S1B) (12). However, expression of *xbp-1s* in the four cephalic astrocyte-like sheath glia (CEPsh) using the *hlh-17* promoter resulted in an extension of life span (Fig. 1C and fig. S1C) (13,14).

We hypothesized that glial *xbp-1s* was inducing a beneficial UPR^{ER}, leading to life-span extension. To characterize the localization and extent of UPR^{ER} activation, we generated animals expressing *xbp-1s* in glia with the UPR^{ER} reporter strain, *hsp-4p::GFP* (15). At day 1 of adulthood, expression of *xbp-1s* in most glia (*ptr-10p::xbp1s*) or all glia (*mir-228p::xbp-1s*) induced *hsp-4::GFP* in glial cells and in the distal intestine (fig. S2, A to C). Animals overexpressing *xbp-1s* in AMsh and PHsh glia (*fig-1p::xbp1s*) exhibited robust *hsp-4::GFP* induction in *fig-1*–expressing glial cells and in the distal intestine. This expression pattern was distinct from that of *fig-1p::tdTomato* reporter animals, in which tdTomato fluorescence was restricted to AMsh and PHsh glia (fig. S1B and fig. S3, A and B). Animals overexpressing *xbp-1s* in the four CEPsh glia (*hlh-17p::xbp-1s*) showed induction of *hsp-4p::GFP* in the CEPsh glia and in the distal intestine and pharynx (Fig. 1, D and E). Notably, green fluorescent protein (GFP) expression was limited to CEPsh glial cells in *hlh-17p::GFP* reporter animals (fig. S1C) (9, 10, 13, 14, 16-18). These data suggest that *xbp-1s* expression in glial cells can induce cell nonautonomous UPR^{ER} in distal intestinal cells and that CEPsh glia have a unique role in regulating *xbp-1s*–mediated longevity.

To elucidate how CEPsh glia promote longevity via *xbp-1s*, we first tested whether life-span extension and cell nonautonomous activation of the UPR^{ER} from CEPsh glia was dependent on the known signaling components of the UPR^{ER} branches, PERK, ATF6, and XBP1, encoded by *pek-1, atf-6*, and *xbp-1*, respectively, in *C. elegans*. No difference was observed in hsp-4::GFP induction with *pek-1* or *atf-6* RNA interference (RNAi)–mediated knockdown in *hlh-17p::xbp-1s* animals (Fig. 2A and fig. S4). However, knockdown of *xbp-1* reduced GFP fluorescence of *hlh-17p::xbp-1s; hsp-4p::GFP* animals and abolished the life-span extension of *hlh-17p::xbp-1s* animals (Fig. 2, A and B, and fig. S4). Whole-worm RNA sequencing (RNA-seq) of *hlh-17p::xbp-1s* animals revealed 115 differentially expressed genes (adjusted *P* value <0.05), including a significant increase in *xbp-1s*–dependent transcripts (Fig. 2, C and D, and table S2) (19). Gene ontology analysis showed enrichment of genes involved in the immune response, stress response, and, as expected, response to ER stress (table S3).

We hypothesized that the increased activation of the UPR^{ER} in *hlh-17p::xbp-1s* animals would render these animals more resistant to age-dependent protein aggregation and chronic ER stress. Expression of *xbp-1s* in CEPsh glia notably reduced aggregation of yellow fluorescent protein (YFP)–tagged, Huntington-like polyglutamine protein in the intestine (with age) compared with controls (Fig. 3A). Furthermore, animals expressing *xbp-1s* in CEPsh glia exhibited an increase in survival when chronically exposed to tunicamycin, a chemical inducer of ER stress (Fig. 3B). Perturbing CEPsh glial development, using a partially penetrant reconstituted caspase (recCasp), abrogated the ER stress resistance of *hlh-17p::xbp-1s* animals grown on tunicamycin-containing plates and decreased the median life span of *hlh-17p::xbp-1s* animals grown on control plates (Fig. 3B and fig. S5, A and B). Moreover, distal UPR^{ER} was reduced in *hlh-17p::xbp-1s* animals harboring *hlh-17::recCasp* (Fig. 3C). Consistent with these findings, *hsp-4p::GFP* induction was suppressed in *hlh-17p::xbp-1s* animals harboring a loss-of-function mutation in *vab-3*, a *Pax6/7*-related gene required for CEPsh glial cell development (fig. S5, C and D) (10). In contrast to other model organisms, ablation of glial cells does not lead to neuronal cell death in *C. elegans* (20).

Next, we assessed whether overexpression of *xbp-1s* in CEPsh glia induces other stress responses known to affect protein homeostasis and longevity, such as the mitochondrial UPR (UPR^{MT}), the heat shock response (HSR), or reduced insulin and insulin-like growth factor 1 (IGF-1) signaling (5, 21, 22). We did not observe induction of the UPR^{MT} reporter, *hsp-6::GFP*, the HSR reporter, *hsp-16.2::GFP*,or the *sod-3p::GFP* reporter with *hlh-17p::xbp1s* expression. However, *hlh-17p::xbp-1s* animals were still capable of activating these responses (fig. S6). Taken together, these data indicate that expression of *xbp-1s* in CEPsh glia specifically induces the UPR^{ER}, which protects animals from age-dependent protein aggregation and chronic ER stress.

Previously, our laboratory had found that cell nonautonomous activation of the UPR^{ER} by neuronal *xbp-1s* is dependent on the release of small clear synaptic vesicles (SCVs) containing neurotransmitters (2). To determine if glial *xbp-1s* signals through a mechanism similar to that of neuronal *xbp-1s*, we generated *hlh-17p::xbp-1s*; *hsp-4p::GFP* animals containing an *unc-13* mutation, which are deficient in SCV exocytosis (23). Notably, cell nonautonomous signaling remained intact in *hlh-17p::xbp-1s* animals harboring either *unc-13(e51)* or *unc-13(s69)* mutations (Fig. 4A and fig. S7, A to D). Therefore, glia do not transmit UPR^{ER} to distal tissues via a SCV-dependent mechanism like neurons.

CEPsh glia reside nearly 300 μm from where we observed robust distal activation of the UPR^{ER}. Therefore, we hypothesized that this transcellular signaling mechanism is dependent on neuropeptides, which are packaged into dense core vesicles (DCVs); can be secreted from neurons, glia, or neuroendocrine cells; and can function as long-range signaling hormones. We crossed *hlh-17p::xbp-1s* animals with an *unc-31* loss-of-function mutant in which DCV exocytosis is disrupted. The *unc-31(e928)* mutation suppressed cell nonautonomous activation of the UPR^{ER}, with GFP fluorescence nearly equal to levels observed in *hsp-4::GFP* controls (Fig. 4, B and C, and fig. S7, E and F) (24). The *unc-31(e928)* mutation had no effect on cell autonomous activation of the UPR^{ER} in intestinal cells or neuronal cell nonautonomous activation of the UPR^{ER} (Fig. 4, D and E)

(2). Furthermore, we tested a loss-of-function mutation in the proprotein convertase, *egl-3*, which is deficient in neuropeptide processing, and found that induction of the cell nonautonomous UPR^{ER} by CEPsh glia was suppressed (Fig. 4, F and G, and fig. S8, A and B) (25). Blocking neuropeptide processing had no effect on cell autonomous *hsp-4p::GFP* induction in intestinal cells or cell nonautonomous activation of the UPR^{ER} in animals expressing neuronal *xbp-1s* (fig. S9, A to D, and Fig. 4, H and I). Thus, glial-mediated cell nonautonomous induction of the UPR^{ER} is dependent on neuropeptides, which is an entirely distinct mechanism to that initiated by neurons expressing *xbp-1s*.

As an additional measure of the separation between neuronal and glial induction of peripheral UPR^{ER}, we removed CEPsh glial cells in animals expressing *xbp-1s* solely in neurons, and cell nonautonomous activation of the UPR^{ER} remained intact (fig. S10). Thus, neuronal activation of the peripheral UPR^{ER} via *xbp-1s* is independent of CEPsh glia. Next, we investigated whether combinatorial *xbp-1s* overexpression in both neurons and CEPsh glia would result in an additive increase in activation of the UPR^{ER} and life-span extension. Animals overexpressing *xbp-1s* in both neurons and CEPsh glia induced *hsp-4p::GFP* and extended life span to a greater degree than animals expressing *xbp-1s* only within CEPsh glia or neurons (fig. S11, A and B, and Fig. 4J).

To identify the cell type responsible for secreting the peptides mediating cell nonautonomous UPR^{ER}, we expressed wild-type *unc-31(cDNA)* in either neurons or glia in *hlh-17p::xbp-1s*; *unc-31(e928)* animals. Neuronal *unc-31(cDNA)* did not restore activation of the UPR^{ER} in the intestine of *hlh-17p::xbp-1s*; *unc-31(e928)* animals (fig. S12, A and B). In contrast, expression of *unc-31(cDNA)* in CEPsh glia or *egl-3(cDNA)* in CEPsh glia or all glia led to an increase in activation of the UPR^{ER}, albeit a modest increase (fig. S12, C and D). These data suggest that the neuropeptides required for glial-mediated cell nonautonomous activation of the UPR^{ER} do not originate from neurons but are secreted, in part, by glial cells themselves.

Lastly, we sought to determine whether neuropeptide signaling was mediating longevity in *hlh-17p::xbp-1s* animals. Loss-of-function *egl-3* mutants are inherently long-lived because of reduced insulin and IGF-1 signaling (26). However, we did not observe an additive increase in survival of *hlh-17p::xbp-1s* animals harboring the *egl-3(ok979)* mutation, suggesting that lifespan extension of *hlh-17p::xbp-1s* animals requires neuropeptides (Fig. 4K).

Previously, cell nonautonomous stress signaling from the brain to the periphery has been ascribed only to neurons. However, our data identify a subtype of astrocyte-like glial cells that coordinate systemic protein homeostasis and aging via neuropeptide signaling—a distinct mechanism from that initiated by neuronal XBP-1s (fig. S13). This suggests there is regional and functional specificity of glial cells to control physiology and aging that evolved as early as the nematode. We speculate that, depending on the physiological cue received by the nervous system, either neurons or glia can signal via XBP-1s to peripheral tissues to coordinate organismal protein homeostasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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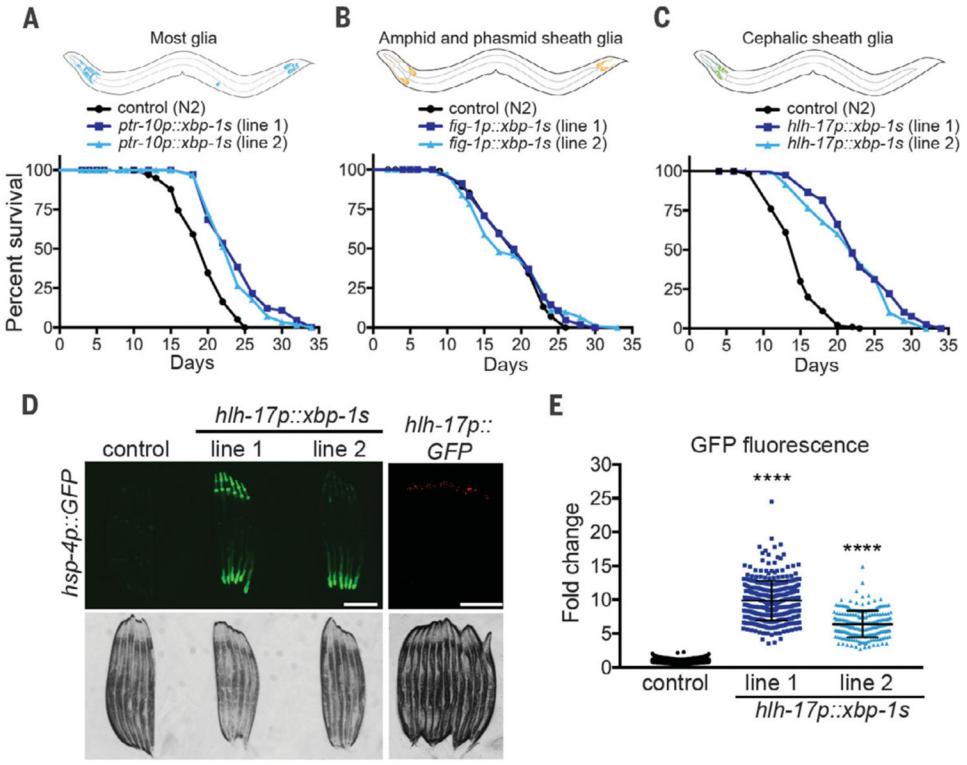

Fig. 1. Glial *xbp-1s* extends life span and induces cell nonautonomous UPR^{ER}.

(**A**) Survival of animals expressing *xbp-1s* in most glia [*ptr-10p::xbp-1s*, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (**B**) Survival of animals expressing *xbp-1s* in four amphid and phasmid sheath glia [*fig-1p::xbp-1s*, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (**C**) Survival of animals expressing *xbp-1s* in four cephalic sheath glia [*hlh-17p::xbp-1s*, line 1 (dark blue), line 2 (light blue)] compared with control N2 animals (black). (**D** and **E**) Fluorescent micrograph (D) and quantification (E) of UPR^{ER} reporter worms (*hsp-4p::GFP*) expressing *hlh-17p::xbp-1s* (left). *hlh-17p::GFP* reporter worms, pseudo-colored red (right), are shown. Data in (D) are representative of $n > 10$. Scale bars, 250 μm. Quantification of *hsp-4p::GFP* fluorescence using COPAS biosorter was normalized to time of flight (length) and extinction (thickness) of animals. Results are shown relative to *hsp-4p::GFP* alone (control) with error bars representing means ± SD. One-way analysis of variance (ANOVA) Tukey's post hoc test, $n = 2$, ****$P < 0.0001$. Life spans are representative of $n = 3$. See table S1 for life-span statistics.

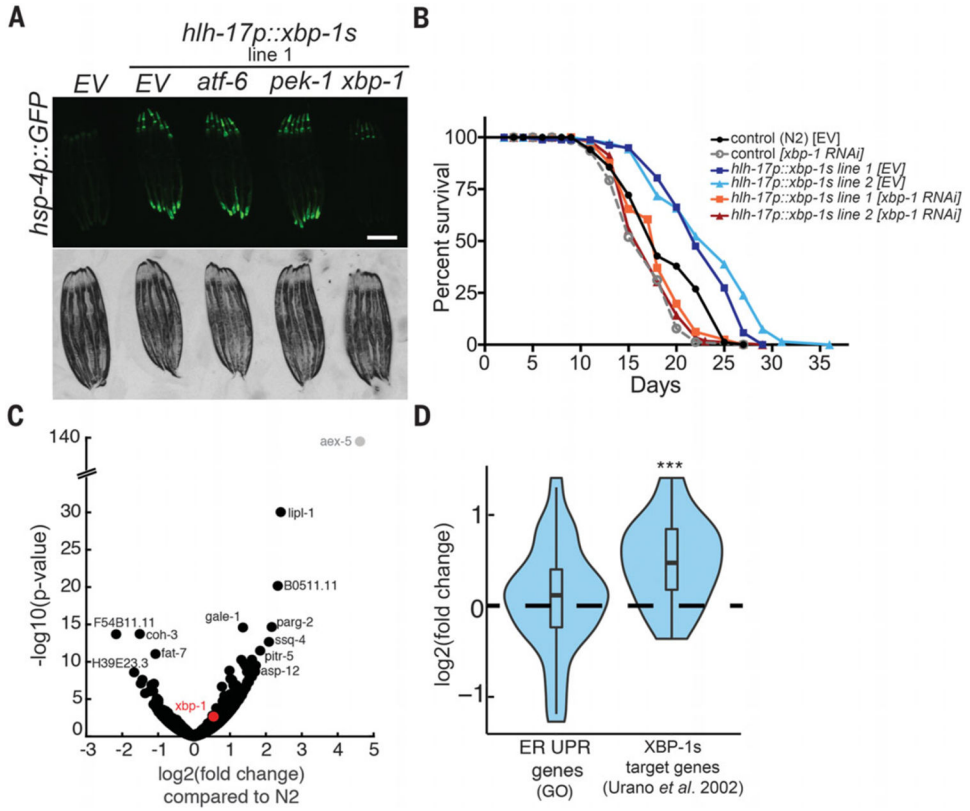

Fig. 2. Cell nonautonomous induction of the UPR^{ER} is dependent on *xbp-1*, but not *atf-6* or *pek-1*.

(**A**) Fluorescent micrographs of day 1 *hsp-4p::GFP; hlh-17p::xbp1-s* animals grown on control empty vector (EV), *atf-6, pek-1*, or *xbp-1* RNAi from hatch. Scale bar, 250 μm; n = 3. (**B**) Survival of control (N2) and *hlh-17p::xbp-1s* animals grown on EV control RNAi or RNAi-targeting *xbp-1*. See table S1 for life-span statistics; n = 2. (**C**) Volcano plot of whole-animal transcriptional profiling from *hlh-17p::xbp-1s* animals compared with wild type (N2). *xbp-1* is highlighted in red. Note that *aex-5* (gray) was detected as highly overexpressed because of a small *aex-5* promoter and exon fragment present in the 3′ untranslated region in the backbone plasmid used for all constructs. All *aex-5* reads aligned to this short fragment. (**D**) The UPR^{ER} is activated in animals expressing *xbp-1s* in CEPsh glia compared with N2, shown by fold change of two gene groups: UPR^{ER} (GO: 0030968 and 1900103) or *xbp-1* targets (19). The line inside the box represents the median change of the gene group. ***P < 0.001. GO enrichment analysis for genes with a fold change P value <0.05 for terms with a false discovery rate Q value <0.05 can be found in table S3.

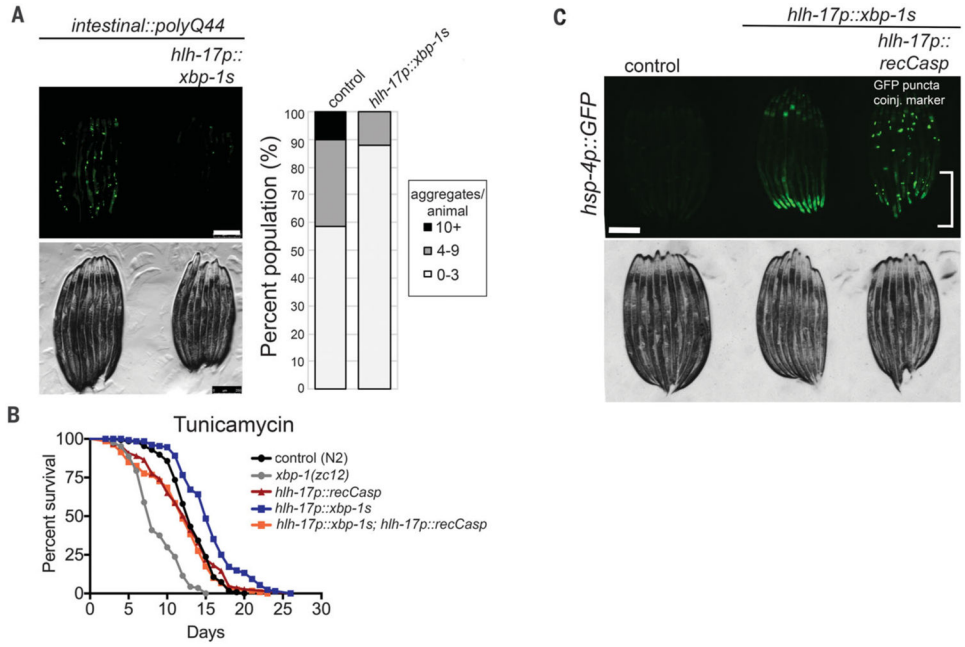

Fig. 3. Expression of *xbp-1s* in glial cells protects animals against protein aggregation and chronic ER stress.

(**A**) Fluorescent micrograph and quantification of age-dependent accumulation of polyQ44-YFP aggregates in control animals or animals expressing *hlh-17p::xbp-1s*. Control animals average 3.5 puncta per animal, compared with 1.3 in *hlh-17p::xbp-1s* animals ($P < 0.0001$). Scale bar, 250 μm; $n = 2$. (**B**) Survival of animals transferred to tunicamycin-containing plates at day 1 of adulthood. CEPsh glial ablation via *hlh-17p::recCasp* suppresses *hlh-17p::xbp-1s* ER stress resistance. $n = 2$. (**C**) Fluorescent micrograph of *hsp-4p::GFP* reporter worms expressing *hlh-17p::xbp-1s* and *hlh-17p::xbp-1s; hlh-17p::recCasp*. GFP puncta in *hlh-17p::recCasp* strain represent co-injection (coinj.) marker for *hlh-17p::recCasp* transgene, which is expressed in coelomocytes. White bracket marks distal intestine, where induction of cell nonautonomous UPR^{ER} is reduced in animals expressing *hlh-17p::recCasp*. Scale bar, 250 μm; $n = 3$.

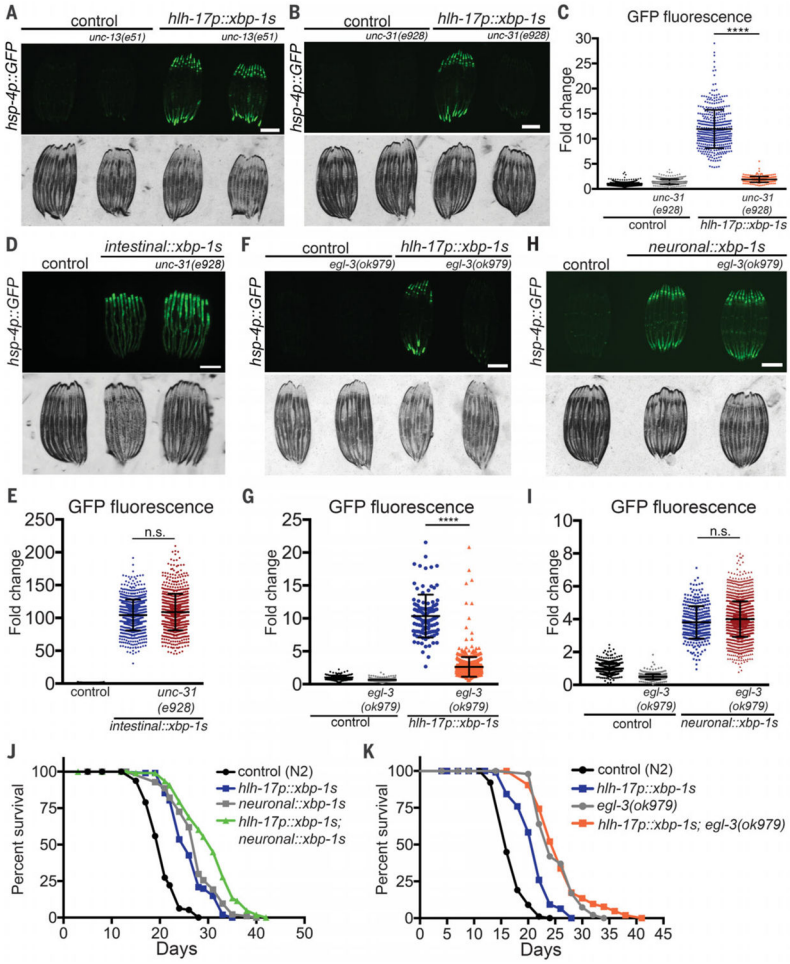

Fig. 4. Neuropeptides are required for glial cell nonautonomous activation of the UPR^{ER} and longevity.

(**A** and **B**) Fluorescent micrographs of control (*hsp-4p::GFP*) and *hsp-4p::GFP; hlh-17p::xpb-1s* (line 1) animals, with and without the *unc-13(e51)* or *unc-31(e928)* loss-of-function mutations, which render animals deficient in SCV or DCV release, respectively. Scale bars, 250 μm; $n = 3$. (**C**) COPAS quantification of animals in (B), $n = 3$. (**D** and **E**) Fluorescent micrographs (D) and COPAS quantification (E) of control and *hsp-4p::GFP* animals expressing intestinal *xbp-1s* (*vha-6p::xbp-1s*), with and without the *unc-31(e928)* mutation. Scale bar, 250 μm; $n = 2$. n.s., not significant. (**F** and **G**) Fluorescent micrographs (F) and COPAS quantification (G) of control and *hsp-4p::GFP; hlh-17p::xpb-1s* (line 1), with and without the *egl-3(ok979)* mutation, which renders animals unable to cleave pro-neuropeptides. Scale bar, 250 μm; $n = 3$. (**H** and **I**) Fluorescent micrographs (H) and COPAS quantification (I) of control and *hsp-4p::GFP* animals expressing *xbp-1s* in all neurons (*rgef-1p:: xbp-1s*), with and without the *egl-3(ok979)* mutation. Scale bar, 250 μm; $n = 2$. (**J**) Survival of control (N2) animals (black), *hlh-17p::xbp-1s* (dark blue), *neuronal(rgef-1p)::xbp-1s* (gray), and *hlh-17p::xbp-1s; neuronal(rgef-1p)::xbp-1s* (green). $n = 2$. (**K**) Survival of control (N2) animals (black), *hlh-17p::xbp-1s* (dark blue), *egl-3(ok979)* (gray), and *hlh-17p::xbp-1s; egl-3(ok979)* (orange). $n = 3$. See table S1 for life-span

statistics. COPAS results are shown relative to *hsp-4p::GFP* alone (control), with means ± SD. One-way ANOVA Tukey's post hoc test, ****$P < 0.0001$.