Homeodomain-interacting protein kinase maintains neuronal homeostasis during normal *Caenorhabditis elegans* aging and systemically regulates longevity from serotonergic and GABAergic neurons

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Abstract

Aging and the age-associated decline of the proteome is determined in part through neuronal control of evolutionarily conserved transcriptional effectors, which safeguard homeostasis under fluctuating metabolic and stress conditions by regulating an expansive proteostatic network. We have discovered the *Caenorhabditis elegans* homeodomain-interacting protein kinase (HPK-1) acts as a key transcriptional effector to preserve neuronal integrity, function, and proteostasis during aging. Loss of *hpk-1* results in drastic dysregulation in expression of neuronal genes, including genes associated with neuronal aging. During normal aging *hpk-1* expression increases throughout the nervous system more broadly than any other kinase. Within the aging nervous system, *hpk-1* induction overlaps with key longevity transcription factors, which suggests *hpk-1* expression mitigates natural age-associated physiological decline. Consistently, pan-neuronal overexpression of *hpk-1* extends longevity, preserves proteostasis both within and outside of the nervous system, and improves stress resistance. Neuronal HPK-1 improves proteostasis through kinase activity. HPK-1 functions cell non-autonomously within serotonergic and GABAergic neurons to improve proteostasis in distal tissues by specifically regulating distinct components of the proteostatic network. Increased serotonergic HPK-1 enhances the heat shock response and survival to acute stress. In contrast, GABAergic HPK-1 induces basal autophagy and extends longevity, which requires *mxl-2* (MLX), *hlh-30* (TFEB), and *daf-16* (FOXO). Our work establishes *hpk-1* as a key neuronal transcriptional regulator critical for preservation of neuronal function during aging. Further, these data provide novel insight as to how the nervous system partitions acute and chronic adaptive response pathways to delay aging by maintaining organismal homeostasis.

Keywords: Neuronal cell-nonautonomous control, gene expression, proteostasis, longevity, aging, stress response, autophagy, homeodomain interacting protein kinase

Introduction

The gradual decline of function within the proteome (proteostasis) is a characteristic of aging, which precipitates the onset and progression of a growing number of age-associated diseases (Balch et al., 2008; Gidalevitz et al., 2006; Gidalevitz et al., 2010; Kikis et al., 2010; Morimoto, 2011, 2019; Sala et al., 2017; Taylor and Dillin, 2011): Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS) are neurodegenerative diseases driven by genetic alterations that typically predispose a mutant protein isoform to aggregate and have toxic gain of function properties on the neuronal proteome. Outside of the nervous system age-associated proteostatic decline also leads to disease, for example: the onset of diabetes mellitus (Jaisson and Gillery, 2014); thus the decline in proteostasis during normal aging is not limited to the nervous system. A growing number of studies suggest that aging is not simply the result of the stochastic accumulation of damage, but is determined through genetics and coordinated mechanisms across tissues and cell types (Labbadia and Morimoto, 2015; Lazaro-Pena et al., 2022; Lopez-Otin et al., 2013). Proteostatic decline corresponds with the age-associated breakdown of a large proteostasis network (PN), which integrates stress-responsive control of protein folding, degradation, and translation in response to myriad cell intrinsic and non-autonomous signals. And yet, while major components of the proteostatic network have been identified, it is still poorly understood how multi-cellular organisms maintain systemic proteostasis within and across tissues to delay aging.

The relatively simple metazoan animal *Caenorhabditis elegans* (*C. elegans*) is a premier model system to learn how proteostasis is coordinated across cell- and tissue-

74 types in response to myriad signals. Discoveries of cell non-autonomous signaling first
75 made in *C. elegans* have evolutionarily conserved components, and revealed that the
76 nervous system acts as a key cell non-autonomous regulator of organismal proteostasis
77 and longevity (reviewed in (Miller et al., 2020)). For example, a pair of thermosensory
78 neurons systemically regulate the heat shock response (HSR) in a serotonin-dependent
79 manner (Prahlad et al., 2008; Tatum et al., 2015). In contrast, a second regulatory
80 component from the GABAergic and cholinergic systems normally limits muscle cell
81 proteostasis (Garcia et al., 2007; Silva et al., 2013).

82 The first discovery of a putative link between the transcriptional co-factor
83 homeodomain-interacting protein kinase (HPK-1) and longevity was from our genome-
84 wide RNAi screen that identified 103 genes that were essential for decreased insulin-like
85 signaling (ILS) to extend longevity (Samuelson et al., 2007). In *C. elegans, hpk-1*
86 preserves proteostasis, stress response and organismal longevity (Berber et al., 2016;
87 Das et al., 2017; Samuelson et al., 2007). Additionally, we have previously shown that
88 HPK-1 extends longevity through distinct genetic pathways defined by HSF-1, and the
89 target of rapamycin complex 1 (TORC1) (Das et al., 2017). Activation by either metabolic
90 or genotoxic stressors has been observed from yeast to mammals, suggesting that this
91 family of transcriptional cofactors arose early in evolution to couple metabolic and stress
92 signaling (Hofmann et al., 2003; Kim et al., 2006; Lee et al., 2008; Shojima et al., 2012).

93 In general, mammalian HIPK family members regulate the activity of transcription
94 factors (TFs), chromatin modifiers, signaling molecules and scaffolding proteins in
95 response to cellular stress (Calzado et al., 2007; de la Vega et al., 2012; Song and Lee,
96 2003a, b). For example, nutrient stress, such as glucose deprivation can activate Hipk1

97 and Hipk2 (Ecsedy et al., 2003; Garufi et al., 2013; Song and Lee, 2003b). Conversely,
98 hyperglycemia triggers HIPK2 degradation via the proteasome (Baldari et al., 2017).
99 We sought to identify the tissue- and cell-types in which the HPK-1 transcriptional
100 circuits act to extend longevity, increase resistance to acute proteotoxic stress, and which
101 PN components are regulated across tissues. We have discovered that HPK-1 functions
102 as a key regulator of the proteostatic response, originating in the nervous system of *C.*
103 *elegans*. We find that *hpk-1* is the most broadly upregulated kinase during normal *C.*
104 *elegans* aging, predominantly within the nervous system, and acts to preserves neuronal
105 function and proteostasis within the nervous system. Further, HPK-1 kinase activity within
106 the nervous system initiates cell non-autonomous signals that trigger protective peripheral
107 responses to maintain organismal homeostasis. Responses can qualitatively differ
108 depending on the neuronal type from which they arise: serotonergic HPK-1 activity
109 increases thermotolerance and protects the proteome from acute stress by enhancing the
110 HSR, without increasing lifespan or altering basal autophagy. In contrast, GABAergic
111 HPK-1 activity induces autophagy to extend longevity, without altering thermotolerance.
112 HPK-1 overexpression is sufficient to induce autophagy gene expression, and
113 autophagosome formation requires *hlh-30* (TFEB), *mxl-2* (MLX), and *daf-16* (FOXO)
114 transcription factors. We posit that serotonergic HPK-1 signaling responds to acute
115 proteotoxic stress, whereas GABAergic HPK-1 responds to chronic metabolic changes
116 mediated through target of rapamycin complex 1 (TORC1) (Das et al., 2017). Yet, each
117 of these distinct adaptive responses is sufficient to activate the PN and improve
118 proteostasis outside of the nervous system. Our findings reveal HPK-1 as a key neuronal

119 regulator in coordinating adaptive metabolic and stress response pathways across

120 tissues to delay aging throughout the animal.

121 **Results**

122 **HPK-1 acts from neuronal tissue to promote longevity and health span.**

123 To precisely determine where HPK-1 functions in the regulation of longevity, we

124 overexpressed *hpk-1* within neuronal (*rab-3p*), muscle (*myo-3p*), intestine (*ges-1p*) or

125 hypodermal (*dpy-7p*) tissues to identify where activity was sufficient to increase lifespan.

126 Increased neuronal expression of *hpk-1* was sufficient to increase lifespan by 17%, which

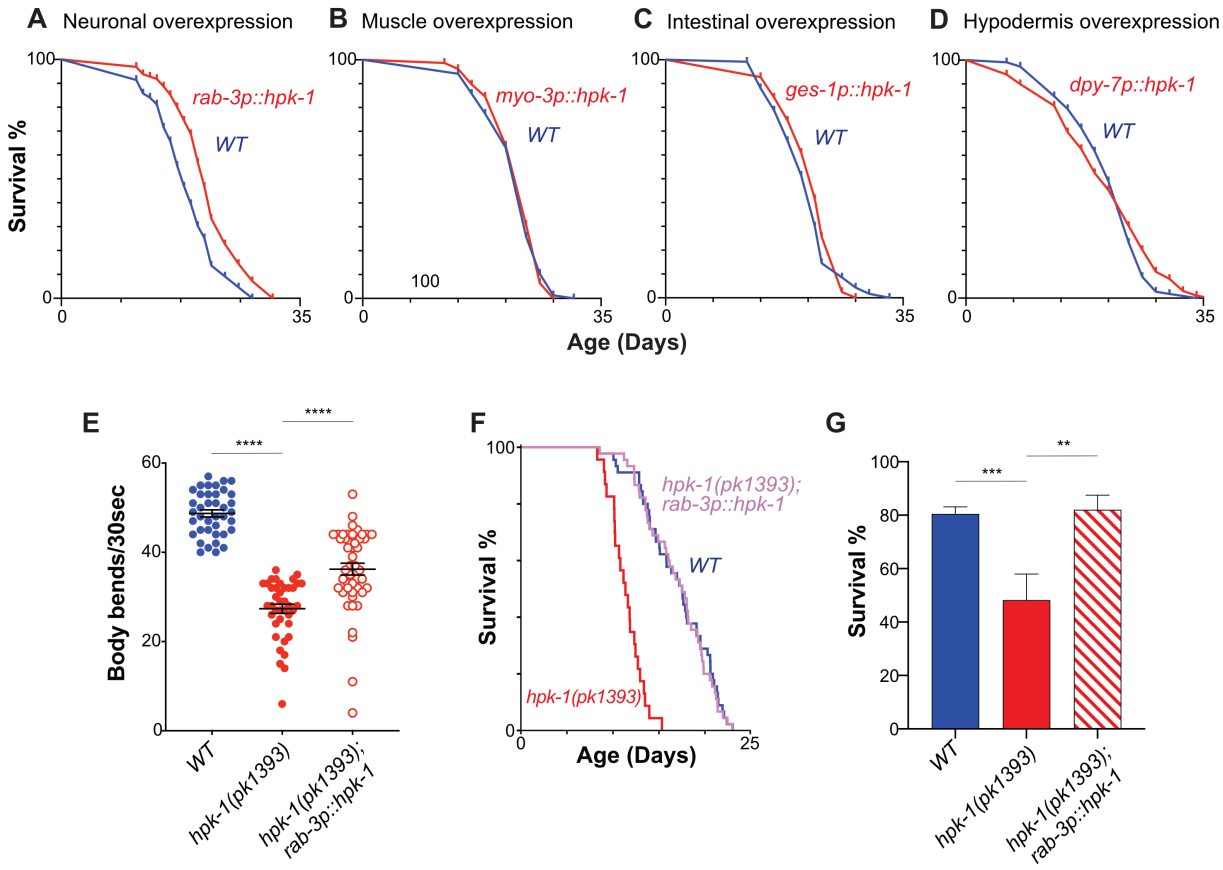


Figure 1. Neuronal HPK-1 extends longevity and promotes healthspan.
Lifespan curves of animals overexpressing *hpk-1* (red line) in the nervous system **(A)**, muscle **(B)**, intestine **(C)** and hypodermis **(D)** compared with control non-transgenic siblings (blue line). (n≥175, log-rank test, $P < 0.0001$ for A, n≥78 for B, n≥82 for C and n≥105 for D). Lifespan graphs are representative of two biological replicates. **(E)** Frequency of body bends of *wild type*, *rab-3p::hpk-1* and *hpk-1(pk1393); rab-3p::hpk-1* day 2 adult animals (n≥48). **(F)** Lifespan curves of wild-type (blue), *hpk-1(pk1393)* (red), and *hpk-1(pk1393); rab-3p::hpk-1* animals (pink). **(G)** Survival of day 1 adult animals subjected to heat shock treatment. Graph represents one of the two individual trials (n≥66). *T* test analysis with $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{***}P < 0.0001$. See *Supplementary Files 1, 2 and 3* for details and additional trials.

127 is comparable to constitutive overexpression of *hpk-1* throughout the soma (*Figure 1A*)
128 (Das et al., 2017). In contrast, transgenic animals overexpressing *hpk-1* in either
129 muscular, intestinal, or hypodermal cells had no significant change in lifespan (*Figure 1B-*
130 *D*). These results were somewhat unexpected, as we previously found that loss of *hpk-1*
131 solely within either neuronal, intestinal, or hypodermal cells is sufficient to shorten lifespan
132 (Das et al., 2017). *hpk-1* is broadly expressed during embryogenesis and larval
133 development, but HPK-1 protein is only expressed within the nervous system in adult
134 animals (Berber et al., 2013; Berber et al., 2016; Das et al., 2017; Raich et al., 2003).
135 Furthermore, *hpk-1* and orthologs are known to have roles in development, differentiation
136 and cell fate-specification (Berber et al., 2013; Blaquiere and Verheyen, 2017; Calzado
137 et al., 2007; Hattangadi et al., 2010; Rinaldo et al., 2007; Rinaldo et al., 2008; Steinmetz
138 et al., 2018). Therefore, we conclude that HPK-1 functions primarily within the nervous
139 system to regulate aging.

140 We performed a locomotion assay to test whether neuronal *hpk-1* could rescue an
141 age-associated decline in movement, a readout of *C. elegans* healthspan (Berber et al.,
142 2016; Das et al., 2017; Herndon et al., 2002; Huang et al., 2004; Samuelson et al., 2007).
143 As expected, *hpk-1(pk1393)* null mutants displayed a reduced frequency of body bends
144 at day 2 of adulthood (*Figure 1E*). Increased expression of *hpk-1* in neurons was sufficient
145 to partially mitigate the locomotion defect of *hpk-1* null mutants in response to active
146 stimuli. Next, we tested whether restoring *hpk-1* exclusively within the nervous system
147 would prevent the premature age-associated decline in movement of *hpk-1* null mutant
148 animals. Indeed, restoring *hpk-1* neuronal expression rescued the premature age-
149 associated decline in movement of *hpk-1* null mutant animals (*Video 1*). Thus, HPK-1

150 activity within the nervous system is sufficient to improve healthspan. Lastly, neuronal
151 *hpk-1* rescue was sufficient to restore a wild-type lifespan in *hpk-1* null mutant animals
152 (*Figure 1F*). It should be noted this same transgene increases wild-type lifespan (*Figure*
153 *1A*), which suggests HPK-1 retains essential functions for adult healthspan and lifespan
154 outside of the nervous system; we posit these functions are related to development as
155 HPK-1 is no longer expressed outside of the nervous system in adult animals (Das et al.,
156 2017).

157 We next asked whether neuronal *hpk-1* expression would be sufficient to rescue
158 the ability of animals to survive acute stress. We and others previously found that in the
159 absence of *hpk-1*, animals are vulnerable to thermal stress (Berber et al., 2016; Das et
160 al., 2017). Restoring *hpk-1* expression solely within the nervous system was sufficient to
161 rescue the reduced thermotolerance of *hpk-1* null mutant animals (*Figure 1G*).
162 Collectively, these findings are consistent with the notion that *hpk-1* primarily acts within
163 the nervous system to regulate aging and stress resistance.

164 ***hpk-1* expression increases broadly during normal aging.**

165 We sought to determine whether *hpk-1* expression changes during normal aging.
166 *hpk-1* mRNA expression increases in wild-type animals during normal aging between day
167 2 and 10 or adulthood (*Figure 2A*). Adult *C. elegans* are composed of 959 somatic cells,
168 of which 302 are neuronal; all somatic cells are all post-mitotic and arise from an invariant
169 lineage. A recent comprehensive single-cell analysis of age-associated changes in gene
170 expression throughout the *C. elegans* identified 211 unique "cell-clusters" within the aging
171 soma: groups of cells within a lineage or tissue defined by gene expression profiles (Roux
172 et al., 2022). The "*C. elegans* Aging Atlas" analysis identified 124 neuronal cell clusters

173 and obtained age-associated changes in gene expression for 122, with high concordance
174 of overall gene expression between neuron clusters to a previous project, which mapped
175 the complete gene expression profiles of the *C. elegans* nervous system in late larval
176 animals (The CeNGEN project (Taylor et al., 2021)). The *C. elegans* Aging Atlas provides
177 the means to investigate age-associated changes in gene expression at an
178 unprecedented level of resolution.

179 Using the *C. elegans* Aging Atlas dataset, in which differentially expressed genes
180 were identified within each cell cluster in old versus young animals, we find that *hpk-1*
181 expression increases during aging within 48 cell clusters: broadly within the nervous
182 system and in eleven non-neuronal cell clusters (*Figure 2B--figure supplement 1*, see
183 Materials and methods). With aging, neuronal expression of *hpk-1* increased within
184 interneurons, motor neurons, sensory neurons, and all neuronal sub-types (cholinergic,
185 dopaminergic, GABAergic, serotonergic, and glutamatergic). We found no cell-clusters in
186 which *hpk-1* is downregulated during normal aging. It is well known that many longevity-
187 associated TFs increase lifespan when overexpressed and shorten lifespan when lost
188 (reviewed in (Denzel et al., 2019)), which infers these TFs might be upregulated, or
189 become chronically activated, during normal aging. A major finding of the *C. elegans*
190 Aging Atlas study was that many longevity-associated TFs are upregulated during normal
191 aging in more than 200 cell-type clusters (Roux et al., 2022). Increased expression of
192 longevity-associated TFs is thought to reflect an age-associated adaptive response to
193 dysregulation of homeostatic mechanisms and accumulating damage (Roux et al., 2022).
194 We posit *hpk-1* neuronal expression increases during normal aging in wild-type animals
195 to mitigate accumulating damage and cellular dysfunction.

196 **During normal aging, *hpk-1* and longevity-associated TFs are upregulated in**
197 **shared subsets of neurons.**

198 We next assessed whether any TFs showed patterns of spatial upregulation similar
199 to the age-associated increase in expression of *hpk-1*, and then segregated those cell
200 types into neuronal and non-neuronal subgroups. Seventy-one TFs were differentially
201 upregulated with *hpk-1* during aging within three or more neuron clusters, thirty within
202 non-neuronal clusters, with an overlap of twenty-one TFs between neurons and non-
203 neurons (*Figure 2B--figure supplement 2* and *Supplementary File 5*). Overall, twenty-six
204 of these TFs have previously been implicated in longevity. There are ten TFs broadly
205 upregulated with aging in the nervous system that overlap with *hpk-1* (significant
206 differential expression in 9 or more neuronal clusters), six of which have previously been
207 linked to longevity: *daf-16* (FOXO, overlaps in 19/37 neuronal clusters), *hlh-30* (TFEB,
208 18/37), *dpy-22* (mediator complex subunit 12L, 15/37), *gei-3* (10/37), *skn-1* (NRF2,
209 10/37), and *hif-1* (hypoxia inducible factor 1, 10/37) (*Figure 2C*) (An and Blackwell, 2003;
210 Chen et al., 2009; Fawcett et al., 2015; Kenyon et al., 1993; Lapierre et al., 2013; Lin et
211 al., 1997; Ogg et al., 1997; Roux et al., 2022; Suriyalaksh et al., 2022; Tacutu et al., 2012;
212 Zhang et al., 2009). Conversely, 12 TFs exhibited an inverse correspondence, and were
213 largely downregulated with aging in the same neurons where *hpk-1* is normally
214 upregulated (significant differential expression in 9 or more neuronal clusters) (*Figure*
215 *2D*); only two of which have previously been implicated in longevity: *xbp-1* (X-box binding
216 protein 1) a key regulator of the endoplasmic reticulum unfolded protein response (ER-
217 UPR), and *mdl-1* (MAX dimerization protein) (Johnson et al., 2014; Nakamura et al., 2016;
218 Riesen et al., 2014). Of note, *mdl-1* is a Myc-family member with opposing roles in

longevity from *mxl-2* (Mlx) (Johnson et al., 2014), and *mxl-2* is required for *hpk-1* to extend longevity. (Das et al., 2017).

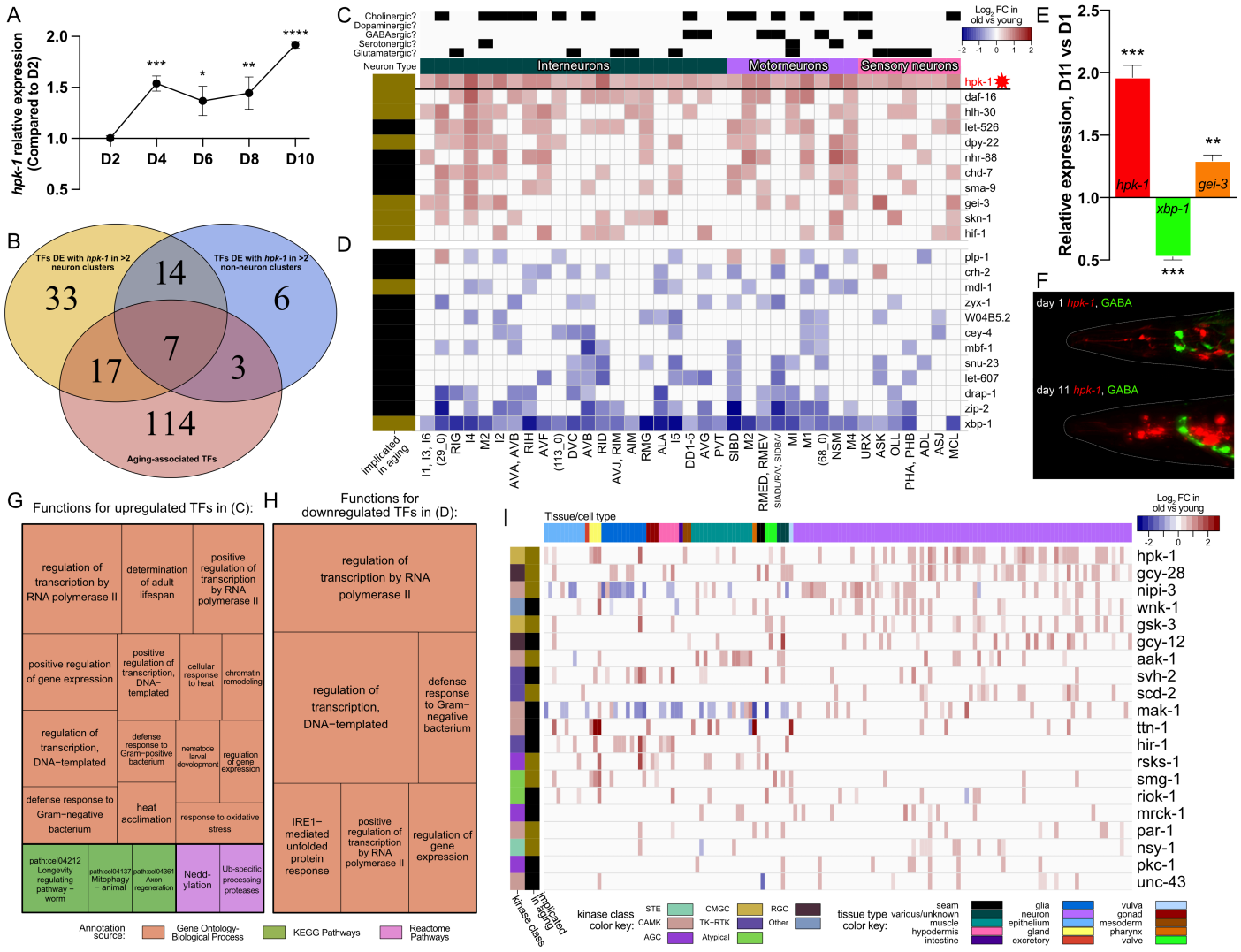


Figure 2. During normal aging *hpk-1* is the most broadly upregulated kinase and overlaps with key longevity-associated transcription factors within the nervous system.
(A) Relative expression of *hpk-1* mRNA during normal aging, as measured by RT-qPCR. *T*-test analysis with **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. **(B)** Venn diagram depicting transcription factors (TFs) with significant age-associated differential expression with *hpk-1* within three or more of the 36 neuronal and/or 11 non-neuronal cell clusters (yellow and blue, respectively), along with TFs that have previously been implicated in *C. elegans* longevity (red). **(C, D)** Heat map of neuronal cell clusters in which *hpk-1* expression increases during normal aging along with most broadly co-expressed TFs (≥ 9 cell-clusters). Positive (red) and negative (blue) fold change with aging of a given TF are shown in (C) and (D), respectively, grouped by average across the indicated clusters. Neuronal types, subtypes, cell-cluster or individual neuronal pair, as well as whether a TF has previously been implicated in aging is indicated. Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022) and significant differences in expression with aging were determined by filtering differential expression results from a comparison of old vs young animals, based on adjusted p-values <0.05 and a $\log_2$ foldchange magnitude of at least 0.5 (between days 8,11,15 and days 1,3,5). **(E)** Relative expression of *hpk-1, xbp-1, and gei-3* mRNA of day 11 adult animals, compared to day 1 and as measured by RT-qPCR. *T* test analysis with ***P* < 0.01 and ****P* < 0.001. **(F)** Representative images of *hpk-1* expression within the nervous system of day 1 and 11 adult animals (*hpk-1p::mCherry; unc-47p::GFP*). See *Figure 7F* and Figure 7- *figure supplement 1A-C* for representative images of whole animals. (n≥5). **(G, H)** Functions and pathways associated with two or more of the TFs listed in (C) and (D), respectively. **(I)** *hpk-1* is differentially expressed with age in more cell clusters than any other kinase, and mostly in neurons. Heatmap shows $\log_2$ foldchange in old vs young animals for kinases with differential expression in ≥10 cell clusters. Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022) and filtered for significant age-associated expression changes as described in (C, D). See *Supplementary Files 4* for additional RT-qPCR details, and *5* and *6* for dataset.

221 We tested whether we could verify changes in age-associated gene expression for
222 several candidate longevity-associated transcription factors identified in the Aging Atlas
223 study. Age-synchronized wild-type animals at either day 1 or 11 of adulthood were
224 harvested and mRNA levels of *hpk-1, xbp-1* and *gei-3* were assessed. As expected, both
225 *hpk-1* and *gei-1* were significantly induced in older animals, and conversely *xbp-1*
226 expression was decreased (*Figure 2E*)**.** We conclude that we can recapitulate results
227 obtained from the Aging Atlas study and confirm that longevity-associated TFs are
228 significantly differentially expressed during normal aging. Next we determined whether
229 *hpk-1* expression is significantly induced within the *C. elegans* nervous system during
230 normal aging; we assessed age-associated changes neuronal *hpk-1* mRNA levels *in vivo*
231 (*hpk-1p::mCherry*). As expected, we find that *hpk-1* is significantly upregulated within
232 isolated *C. elegans* neurons during normal aging (*Figure 2F).*

233 In non-neuronal clusters we found a much smaller subset of TFs with
234 corresponding positive age-associated fold-changes. Only six of these TFs have been
235 implicated in longevity: *fkh-9* (FOXG), *sea-2*, *zfh-2* (ZFHX3) (Walter et al., 2011), *xbp-1,*
236 *mdl-1,* and *hlh-6* (achaete-scute family bHLH transcription factor 3) (*Figure 2--figure*
237 *supplement 1*).

238 Within neuronal cell-clusters, *hpk-1* and the aforementioned longevity-associated
239 TFs are broadly expressed throughout the nervous system by late larval development,
240 with the exception of the nematode specific gene *gei-3*, for which overlap is restricted to
241 a subset of neurons (*Figure 2--figure supplement 3*). TFs with age-associated increases
242 in expression in clusters overlapping with *hpk-1* are associated with positive regulation of
243 adult lifespan, heat and oxidative stress response, bacterial innate immunity, chromatin

244 remodeling, development, mitophagy, axon regeneration, neddylation, and ubiquitination
245 (*Figure 2C*). TFs with decreased expression in aging in *hpk-1*-upregulated neuronal cell
246 clusters have been implicated in the ER unfolded protein response and the innate immune
247 response (*Figure 2D*). Interestingly, when assessed at the level of individual time points
248 rather than "old" and "young" groups, we find that the age-associated up-regulation of
249 *hpk-1* and expression changes of longevity-associated TFs does not necessarily occur in
250 a tightly-linked manner suggestive of a single upstream regulatory process (see Appendix
251 1). Rather, we posit *hpk-1* is induced within the nervous system along with key longevity
252 transcription factors during normal aging in response to accumulating stressors and
253 collapsing homeostatic mechanisms to mitigate the physiological effects of aging.

254 ***hpk-1* is the most broadly upregulated kinase during normal aging**

255 We were surprised to find that *hpk-1* mRNA expression increased during aging, as
256 kinase regulation at the level of gene expression seemed atypical. Therefore, using the
257 *C. elegans* Aging Atlas dataset we assessed whether any of the 438 *C. elegans* kinases
258 (Zaru et al., 2017) are differentially expressed during aging: 115 kinases are upregulated
259 and conversely 120 are downregulated in at least one cell cluster, while 254 kinases do
260 not change expression (*Figure 2--figure supplement 4, Supplementary File 6*). *hpk-1* is
261 upregulated in more cell-clusters than any other kinase, while *hpk-1* is upregulated in 48,
262 the next most broadly upregulated kinase is *gcy-28* (natriuretic peptide receptor 1, 38 cell-
263 clusters) (Li and van der Kooy, 2018; Shinkai et al., 2011; Tsunozaki et al., 2008). Only
264 14 kinases are downregulated in 10 or more cell-clusters. Conversely, 20 kinases are
265 upregulated in ten or more cell-clusters (*Figure 2--figure supplement 4*). In addition to
266 *hpk-1* and *gcy-28*, five kinases previously linked to longevity are upregulated: *nipi-3* (a

267 Tribbles pseudokinase that regulates SKN-1 (Wu et al., 2021)), *aak-1* (AMP-activated
268 kinase catalytic subunit alpha-2) (Lee et al., 2009), *scd-2* (ALK receptor tyrosine kinase)
269 (Shen et al., 2007), *rsks-1* (ribosomal protein S6 kinase) (Hansen et al., 2007; Pan et al.,
270 2007), and *smg-1* (SMG1) (Masse et al., 2008).

271 We previously demonstrated that HPK-1 is an essential component of TORC1-
272 mediated longevity and autophagy (Das et al., 2017). Interestingly we find that only two
273 of 11 TOR-associated genes: *rsks-1* and *aak-1,* are upregulated during aging in three or
274 more cell-clusters (Blackwell et al., 2019; Hindupur et al., 2015; Pan et al., 2007). We find
275 *rsks-1* is exclusively upregulated outside of the nervous system and has poor overlap with
276 *hpk-1* (2/13). *aak-1* is primarily but not exclusively upregulated outside of the nervous
277 system during aging, with some overlap with *hpk-1*. In future studies it will be interesting
278 to dissect whether HPK-1 intersects with these components of TORC1 signaling within
279 and/or outside of the nervous system.

280 **HPK-1 preserves integrity of the *C. elegans* nervous system.**

281 Based on the neuronal HPK-1 capacity to promote longevity, we sought to
282 determine whether HPK-1 preserves the anatomy and physiology of the nervous system
283 during aging. To assess age-associated changes in neuroanatomy of ventral and dorsal
284 cord motor neurons (VD and DD), we used the *unc-25p::GFP* cell-specific reporter (*Figure*
285 *3A*) (Cinar et al., 2005; Kraemer et al., 2003). Aged populations of *hpk-1* mutant animals
286 displayed more than a 3-fold difference in VD axonal breaks and more than a 2-fold
287 difference in DD axonal breaks compared to wild type animals (*Figure 3A-B, D*). These
288 age-associated breaks could be rescued by neuronal expression of *hpk-1* (*Figure 3C*).
289 Importantly, young wild-type and *hpk-1* mutant animals did not show neuronal breaks

(*Figure 3B*). Thus, loss of *hpk-1* does not disrupt axon formation during development, rather HPK-1 preserves axonal integrity in aging motor neurons.

In *C. elegans,* locomotion is controlled by acetylcholine-releasing excitatory motor neurons and GABA (γ-aminobutyric acid)-releasing-inhibitory motor neurons (Jorgensen and Nonet, 1995; Richmond and Jorgensen, 1999). Thus, locomotion is a useful readout to assess neurotransmission. To examine the role of *hpk-1* in synaptic transmission, we performed a paralysis assay after acetylcholinesterase inhibition with aldicarb, where mutations that impair cholinergic signaling cause a

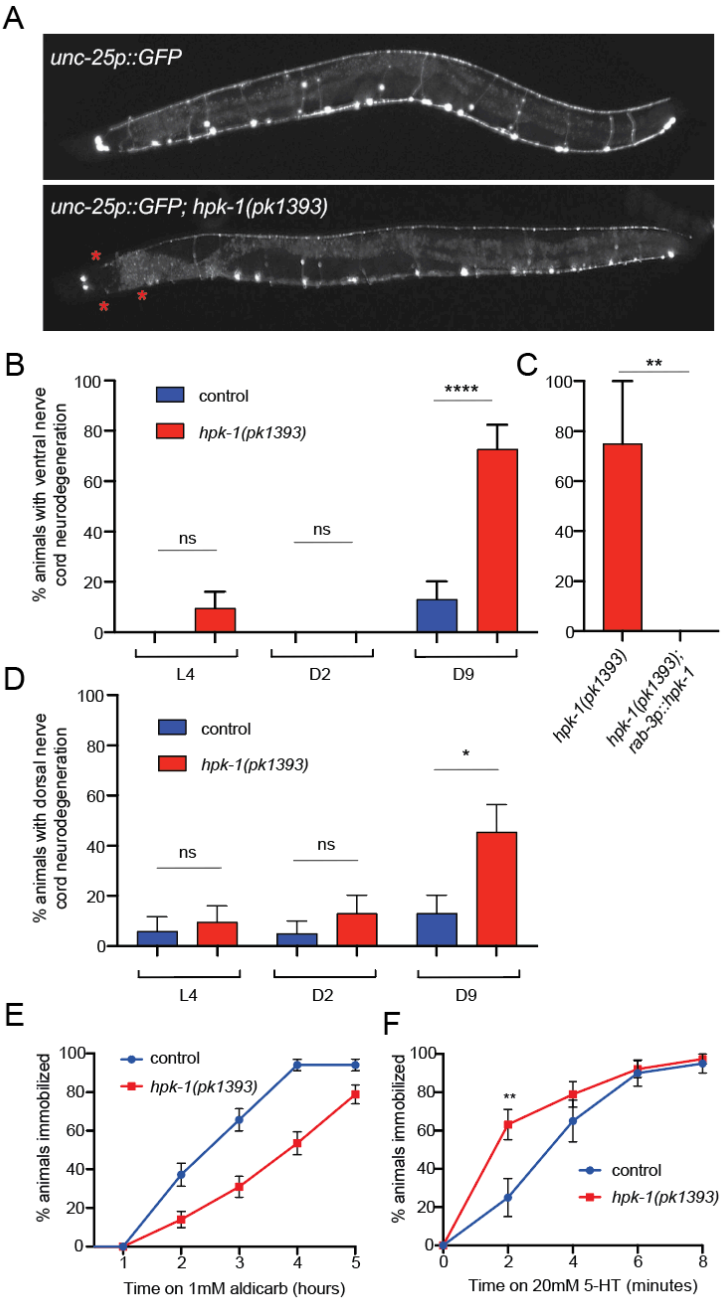


Figure 3. Neuronal HPK-1 maintains neuronal integrity during aging. **(A)** Fluorescent micrographs of control and *hpk-1(pk1393)* 9 day old animals expressing GFP in GABAergic DD and VD motor neurons. Asterisks (*) indicate axonal breaks in the motor neurons. **(B)** Percentage of wild type and *hpk-1* mutant animals with neurodegeneration in VD motor neurons (n≥23). **(C)** Percentage of *hpk-1* rescue animals with neurodegeneration in VD motor neurons (n≥10). **(D)** Percentage of wild type and *hpk-1* mutant animals with neurodegeneration in DD motor neurons (n≥20). Percentage of immobilized animals after exposure to aldicarb (n≥67) **(D)** and to 20mM 5-HT (n≥38) **(E)**. *T* test analysis with $^{**}P < 0.01$ and $^{***}P < 0.001$. See *Supplementary Files 7, 8 and 9* for details and additional trials.

313 reduction in rate of paralysis (Mahoney et al., 2006; Oh and Kim, 2017). Therefore,
314 mutants with lower synaptic transmission undergo reduced paralysis (i.e., animals are
315 resistant), while mutants with higher synaptic transmission exhibit greater sensitivity.
316 Interestingly, *hpk-1* mutants showed increased aldicarb resistance by L4/YA (*Figure 3E*),
317 suggesting that loss of *hpk-1* reduces cholinergic synaptic transmission and that HPK-1
318 normally preserves neuronal function.

319 *C. elegans* serotonin (5-HT) signaling in conjunction with dopamine (DA)
320 modulates locomotion; animals treated with exogenous 5-HT exhibit paralysis over time
321 (Hardaker et al., 2001; Omura et al., 2012). In a paralysis assay with exogenous 5-HT,
322 we observed that late L4/YA *hpk-1* mutant animals paralyzed sooner: at 2 minutes of 5-
323 HT treatment, 63% of mutants were immobilized compared to 25% of wild type animals
324 immobilized (*Figure 3F*). Based on the aforementioned findings, we conclude that HPK-
325 1 prevents an age-associated decline in axonal and synaptic integrity.

326 **Loss of *hpk-1* results in neuronal dysregulation of neuronal gene expression**

327 We sought to gain deeper insight into how loss of *hpk-1* negatively impacted the
328 *C. elegans* nervous system. To identify early transcriptional dysregulation events that
329 occur due to the absence of *hpk-1*, we conducted bulk RNA-sequencing of day 2 adult
330 wild-type and *hpk-1(pk1393)* null mutant animals, an age at which axonal breaks have
331 yet to occur (*Figure 3B, D*), but after neuronal function has begun to decline (*Figure 3E,*
332 *F*). Differential expression analysis identified 2201 total genes with significant expression
333 changes with loss of *hpk-1*, 1853 upregulated and 348 downregulated (adjusted p-value
334 < 0.05 and $\log_2$ foldchange magnitude ≥ 1) (*Figure 4A* and *Supplementary File 10*).
335 Functional enrichment with over-representation analysis was performed for the up- and

down-regulated genes separately (GOSeq, adjusted p-value < 0.01). Among down-regulated genes, we find 25 genes implicated in the innate immune response, as well as TGF-beta and WNT signaling (five ubiquitin ligase complex component genes), glycosphingolipid metabolism, and surfactant metabolism. (*Figure 4B*). By contrast, more than 50 GO terms and pathways were enriched for the upregulated genes. The most significantly enriched single term reveals a substantial number of cuticle and collagen associated genes as dysregulated (*Figure 4C*). Upregulation of some collagens and ECM-remodeling processes have been associated with longevity in *C. elegans* (Ewald et

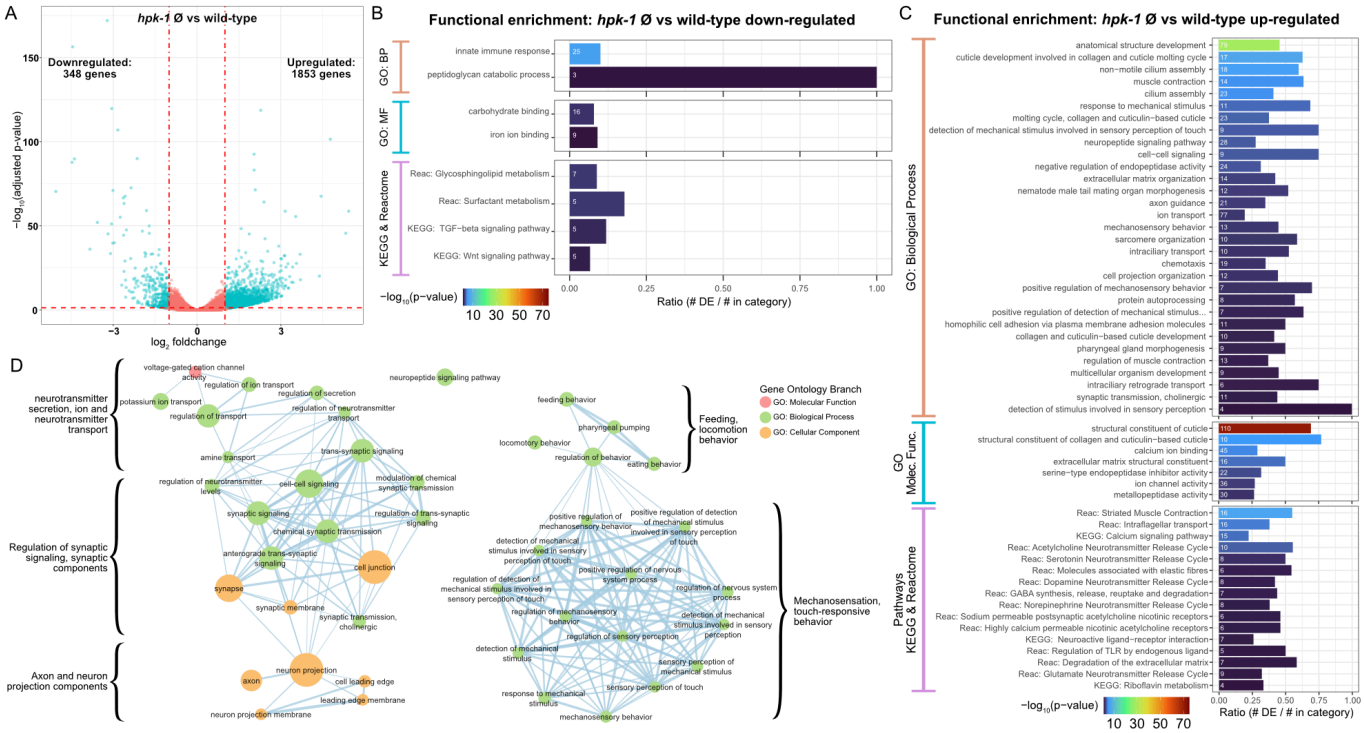


Figure 4. Loss of *hpk-1* results in broad dysregulation of neuronal gene expression.
(A) Differential expression analysis for RNA-Seq of day 2 adult *hpk-1* null mutant animals (n=3) compared to N2 wild-type animals (n=3) identified 2,201 genes with significantly altered expression with loss of *hpk-1* (adjusted p-value < 0.05 and $|\log_2$ foldchange$| \geq 1$), 84% of which were upregulated in the mutants. The volcano plot illustrates the criteria applied for selecting genes with significant and substantial expression changes, shown as blue dots: vertical dashed lines are the foldchange threshold and the horizontal dashed line is the p-value threshold. **(B, C)** Enrichment for functions and pathways associated with the 348 downregulated genes **(B)** or the 1853 upregulated genes **(C)** in *hpk-1* animals yields innate immune response as the most significantly over-represented term for downregulated genes, but reveals a large number of significant associations to pathways and processed for the upregulated genes- many of which are associated with neurons and neuronal signaling. Each bar represents a functional term or pathway from the Gene Ontology (GO), KEGG, or Reactome databases, as indicated. Bar colors show the $-\log_{10}$ transformed enrichment p-value. The number in each bar is the size of the overlap between the set of differentially expressed genes and the genes in the term or pathway. The x-axis indicates the fraction of the total genes in the term or pathway that were differentially expressed. All results shown have significant enrichment after adjustment for multiple testing. **(D)** Functional enrichment for 283 genes upregulated with *hpk-1* loss that are specific to, or enriched in, *C. elegans* neurons shows broad dysregulation of functions associated with neurons including neurotransmitter transport and release, cell-cell junctions, axons, neuropeptides, and sensory processes, among others. 880 genes uniquely expressed or significantly enriched in neurons were derived from bulk tissue-specific RNA-Seq profiling results in (Kaletsky et al., 2018) and 32% of these are significantly upregulated in *hpk-1* null animals. See *Supplementary Files 10 and 11* for dataset and additional analysis.

344 al., 2015; Rahimi et al., 2022). Alternatively, aberrant upregulation could lead to stiffening
345 of the cuticle, a hallmark of aged *C. elegans* and some progeric mutants (Ewald et al.,
346 2015; Rahimi et al., 2022).

347 A striking number of other functions significantly enriched in the *hpk-1* upregulated
348 genes from across GO, KEGG, and Reactome are related to neuronal function and
349 signaling, including: neuropeptide and neurotransmitter pathways, mechanosensation,
350 chemotaxis, and axon guidance, among others (*Figure 4C*). To explore further, we looked
351 at the *hpk-1* null upregulated genes in the context of a curated compendium of genes with
352 functions that are important for the nervous system (Hobert, 2013), as well as genes
353 identified as uniquely expressed in neurons or significantly enriched in neurons from
354 tissue-specific bulk RNA-Seq (Kaletsky et al., 2018).

355 Among 1496 genes known, or expected through homology, to function in the
356 nervous system, 264 are upregulated with loss of *hpk-1*, representing a much larger
357 overlap than expected by chance (hypergeometric test p-value < 0.0001) (*Supplementary*
358 *File 11*). Genes with higher fold-change of expression in *hpk-1* mutants were associated
359 with ciliated sensory neurons (motor proteins, sensory cilia transport), calcium binding,
360 and neuropeptides. We find that 55 of these neuron-associated genes are normally
361 upregulated with age in wild-type animals in at least one cell cluster in the *C. elegans*
362 Aging Atlas, 87 are downregulated with age, with the remaining having mixed or no
363 significant age-associated changes. This suggests that while loss of *hpk-1* results in
364 expression of some genes associated with aging, the bulk of differentially expressed
365 genes at day 2 of adulthood are consistent with dysregulation of neuronal gene

366 expression. Refined genetic perturbation within the nervous system coupled with single
367 cell RNA-sequencing over time would be required to fully reveal the impact of HPK-1
368 function on age-associated changes in gene expression.

369 We next investigated how many of these genes are predominantly- or exclusively-
370 expressed in neurons, based on a set of 880 neuron-enriched and neuron-specific genes
371 (Kaletsky et al., 2018). We found that 15% of all *hpk-1* upregulated genes are enriched
372 or specific to neurons in wild-type animals (283 genes, hypergeometric test p-value <
373 0.0001) whereas only five *hpk-1* down-regulated genes normally exhibit such neuron-
374 restricted expression. To determine what functions are over-represented among the
375 upregulated neuronally expressed genes, we ran Gene Ontology enrichment analysis.
376 Representing these results as a network, and clustering terms which have overlapping
377 gene associations, we identified some major themes: neurotransmitter and ion transport,
378 synaptic components and signaling, axon and neuron projection components, feeding
379 and locomotion behavior, mechanosensation and touch-responsive behavior are all
380 upregulated in the absence of *hpk-1* (*Figure 4D*). Among all genes upregulated in the
381 *hpk-1* null mutants, the largest group correspond to genes related to neuropeptide
382 encoding, processing and receptors. Neuropeptides are short sequences of amino acids
383 important for neuromodulation of animal behavior, broadly expressed in sensory, motor
384 and interneurons. Of the 120 neuropeptide-encoding genes in the *C. elegans* genome,
385 40 (33%) are upregulated in the *hpk-1* null mutants. Nine of these are insulin-related (*ins*),
386 which suggest increased *hpk-1* expression during normal aging or *hpk-1* overexpression
387 may positively affect longevity through decreased *ins* neuropeptide expression. We
388 surmise that many of the neuronal genes that are induced in the absence of *hpk-1* might

389 be compensatory responses to maintain neuronal function. We conclude loss of *hpk-1*
390 upregulates expression of a number of neuronal genes including those involved in:
391 neuropeptide signaling, neurotransmission, release of synaptic vesicles, calcium
392 homeostasis, and function of sensory neurons (Appendix 2).

393 **Neuronal HPK-1 regulates proteostasis cell-intrinsically and cell non-**
394 **autonomously via neurotransmission.**

395 We hypothesized that HPK-1 maintains neuronal function by fortifying the neural
396 proteome. We measured polyglutamine toxic aggregate formation in neuronal cells (*rgef-*
397 *1p::Q40::YFP*) of animals overexpressing *hpk-1* pan-neuronally (*rab-3p*). These animals
398 showed a decreased number of fluorescent foci within the nerve ring (*Figure 5Ai - iii, B*).
399 To assess whether this was associated with improved neuronal function, we assessed
400 alterations in locomotion capacity (body bends) after placement in liquid. Increased
401 neuronal expression of *hpk-1* significantly increased the average number of body bends
402 (17.7 ± 1 vs 12.5 ± 1.2 bends/30sec), suggesting that neuronal expression of *hpk-1*
403 improves proteostasis in the nervous system and mitigates locomotion defects associated
404 with the decline in proteostasis (*Figure 5C*).

405 We next tested whether HPK-1 was necessary to preserve proteostasis within
406 neurons. We crossed the *rgef-1::Q40::YFP* reporter with neuronal enhanced RNAi
407 background (*sid-1(pk3321);unc-119p::sid-1*) and assessed whether neuronal inactivation
408 of *hpk-1* hastened age-associated proteostatic decline. Animals with neuronal *hpk-1*
409 inactivation had a significant increase in foci number within neurons and a decreased
410 number of body bends in liquid (19.7 ± 0.8 vs 25.5 ± 1.1 bends/30sec) (*Figure 5Aiv, D,*
411 *E*). Thus, HPK-1 functions cell intrinsically to maintain neuronal proteostasis.

412 We next assessed whether neuronal HPK-1 activates a cell non-autonomous
413 signal to regulate proteostasis in distal tissues. We used a proteostasis reporter
414 expressed exclusively in muscle tissue (*unc-54p::Q35::YFP*); animals display an age-
415 associated accumulation of fluorescent foci, which are proteotoxic aggregates, and
416 become paralyzed as muscle proteome function collapses (Lazaro-Pena et al., 2021;
417 Morley et al., 2002). Animals with increased neuronal *hpk-1* expression had significantly
418 decreased fluorescent foci in muscle tissue (*Figure 5Fi-ii*). For instance, at day 3 of
419 adulthood, animals overexpressing neuronal HPK-1 had an average of 21 ± 0.9 polyQ
420 aggregates, while control animals had an average of 26 ± 1.1 polyQ aggregates (*Figure*
421 *5G*). In *C. elegans*, the age-related progressive proteotoxic stress of muscle polyQ
422 expression is pathological and results in paralysis. Of note, increased *hpk-1* expression
423 in neurons was sufficient to reduce paralysis; at day 13 of adulthood only 61% ± 0.10 of
424 neuronal *hpk-1* overexpressing animals were paralyzed compared to 81% ± 0.08 of
425 paralyzed control animals (*Figure 5H*). Thus, increased neuronal HPK-1 activity is
426 sufficient to improve proteostasis cell non-autonomously.

427 We confirmed that the kinase activity is essential for HPK-1 to improve
428 proteostasis. We find the kinase domain of HPK-1 is highly conserved from yeast to
429 mammals, both at the amino acid level and predicted tertiary structure, based on multiple
430 sequence alignments, AlphaFold structure prediction, and visualization with PyMol
431 (*Figure 5--figure supplement 1*) (DeLano, 2002; Jumper et al., 2021; Varadi et al., 2022).
432 We mutated two highly conserved residues within the kinase domain, K176A and D272N,

433 which are known to be essential for the kinase activity of HPK-1 orthologs (He et al.,

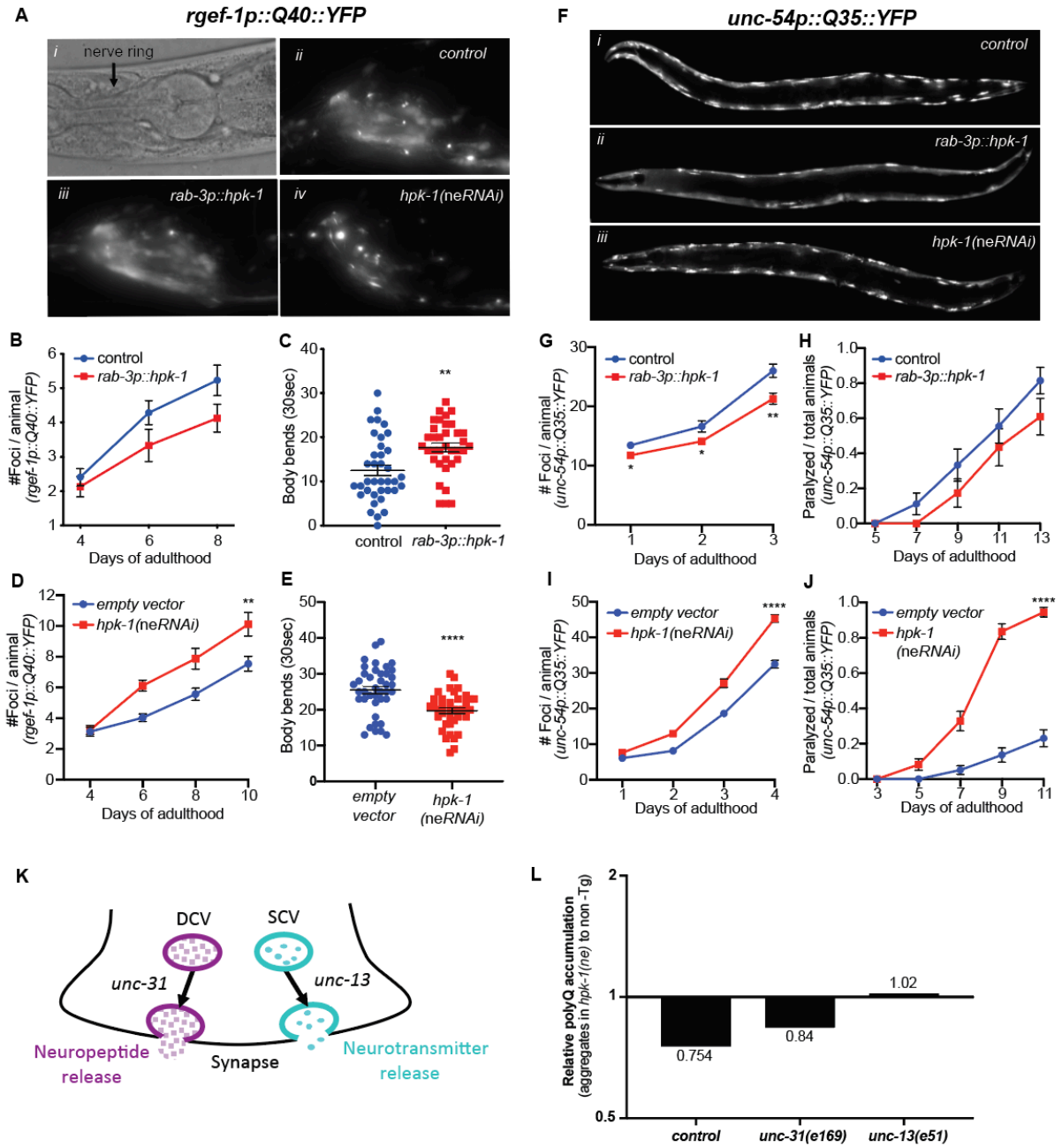


Figure 5. Neuronal HPK-1 prevents the decline in proteostasis through neurotransmitter release.
(A) Nomarski (*i*) and fluorescent (*ii-iv*) micrographs of animals expressing polyglutamine fluorescent reporter within the nervous system. (**B** and **C**) Quantification of foci in the nerve ring (B) and body bend frequency (C) (n≥21 for B and n≥36 for C). (**D** and **E**) Quantification of foci in the nerve ring (D) and frequency of body bends (E) in neuronal enhanced RNAi animals (n≥19 for D and n≥39 for E). **(F)** Fluorescent micrographs of animals expressing the polyglutamine fluorescent reporter in muscle. (**G** and **H**) Quantification of foci in muscle (G) and paralysis rate (H). Graphs are representative of 5 individual transgenic lines (n≥18 for G and n≥23 for H). (**I** and **J**) Quantification of foci in muscle (I) and paralysis rate (J) in neuronal enhanced RNAi animals (n≥22 for I and n≥73 for J). **(K)** Neuropeptides and neurotransmitters are essential for neuronal signaling; vesicle release depends on *unc-31* and *unc-13*, respectively. (**L**) Quantification of relative *polyQ* fluorescent foci in muscle cells after *rab-3p::hpk-1* overexpression in control, *unc-31(e169)* and *unc-13(e51)* animals; see Figure 5- *figure supplement 2* for absolute values and statistical analysis. *T* test analysis with $^{**}P < 0.01$ and $^{***}P < 0.001$. See *Supplementary Files 12, 2, and 13* for details and additional trials.

434 2010), and tested whether these kinase-dead mutant versions could improve proteostasis
435 when neuronally overexpressed. In contrast to wild-type HPK-1 (*Figure 5G, H*), neither
436 mutant had any effect on either polyglutamine foci accumulation (*Figure 5--figure*
437 *supplement 1C, D*) or paralysis (*Figure 5--figure supplement 1E, F*) in muscle cells (*unc-*
438 *54p::Q35::YFP*); thus, increased *hpk-1* expression improves proteostasis through kinase
439 activity.

440 We tested whether neuronal HPK-1 is required for maintaining proteostasis in
441 distal muscle cells. Neuronal inactivation of *hpk-1* (*sid-1(pk3321); unc-119p::sid-1; unc-*
442 *54p::Q35::YFP; hpk-1(RNAi)*) was sufficient to hasten the collapse of proteostasis,
443 significantly increasing the number of polyQ aggregates (45 $\pm$ 1 vs 32 $\pm$ 1.1 at day 4) and
444 paralysis rate (84% $\pm$ 0.04 vs 14% $\pm$ 0.04 at day 9) (*Figure 5Fiii, I, J*). Thus, HPK-1 function
445 within the nervous system impacts proteostasis in distal muscle tissue, which implies that
446 HPK-1 coordinates organismal health through cell non-autonomous mechanisms.

447 To identify the signaling mechanism through which HPK-1 regulates distal
448 proteostasis, we tested whether either neuropeptide or neurotransmitter transmission are
449 essential for cell non-autonomous regulation of proteostasis (using *unc-31* and *unc-13*
450 mutant animals, respectively *Figure 5K*). Neuronal expression of *hpk-1* in *unc-31* mutants
451 improved proteostasis in the absence of neuropeptide transmission and dense core
452 vesicles, as increased neuronal *hpk-1* expression still delayed the age-associated
453 accumulation of aggregates within muscle cells. In contrast, in the absence of
454 neurotransmitter function, neuronal expression of *hpk-1* in *unc-13* mutants failed to
455 improve proteostasis in muscle tissue (*Figure 5L--figure supplement 2*). We conclude that

456 HPK-1 mediated longevity-promoting cell non-autonomous signaling occurs through
457 neurotransmission.

458 **Neuronal HPK-1 induces molecular chaperones and autophagy in distal tissues.**

459 We sought to identify the components of the PN that are cell non-autonomously
460 regulated via HPK-1 activity in the nervous system. We tested whether increasing levels
461 of neuronal *hpk-1* alters the expression of molecular chaperones (*hsp-16.2p::GFP*).
462 Increased neuronal expression of *hpk-1* in wild type animals did not alter the basal
463 expression of molecular chaperones (*Figure 6A*). In contrast, after heat shock, increased
464 neuronal *hpk-1* expression resulted in hyper-induction of *hsp-16.2p::GFP* expression,

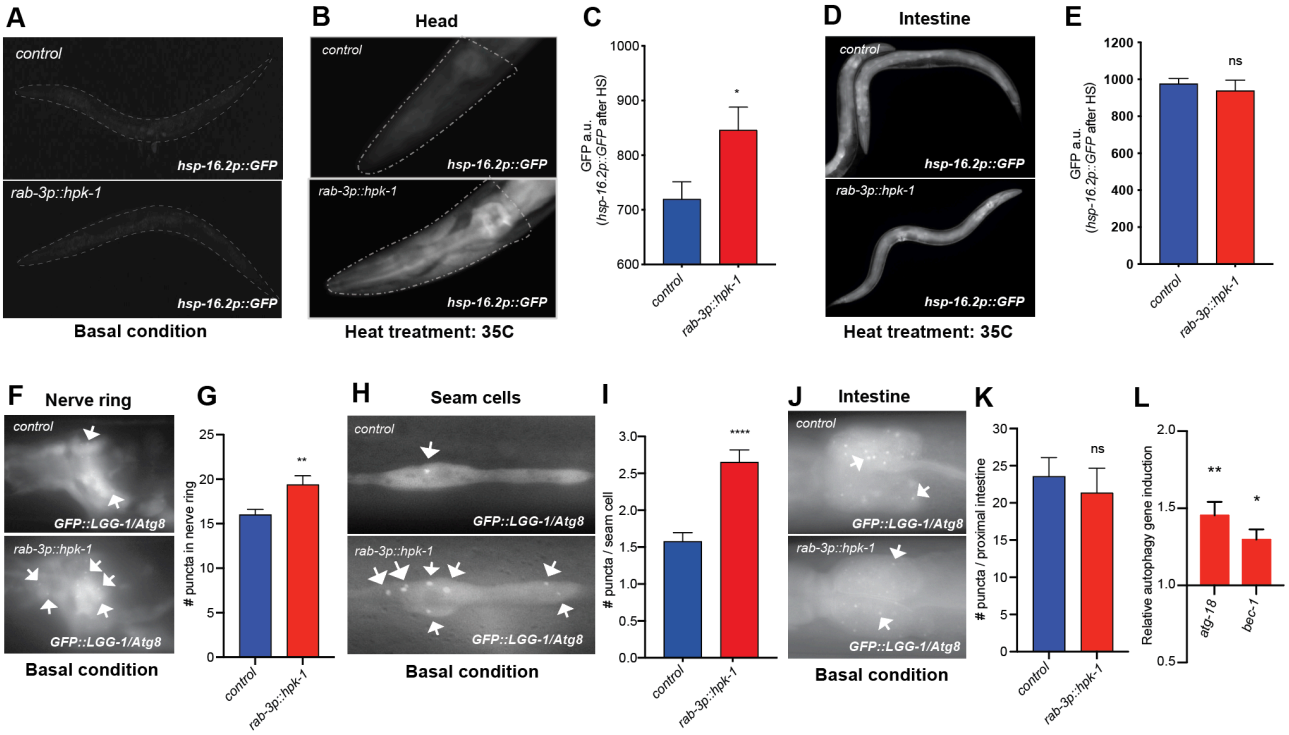


Figure 6. Neuronal HPK-1 induces the proteostatic network in distal tissues.
(**A**) Fluorescent micrographs of animals expressing *hsp-16.2p::GFP* in basal conditions. (**B** and **C**) Fluorescent micrographs (B) and fluorescent density quantification (C) of control and *rab-3p::hpk-1* day 1 adult animals expressing *hsp-16.2p::GFP* after heat shock (35°C) for 1 hour at (n≥15). (**D** and **E**) Fluorescent micrographs and densitometry quantification of the intestinal fluorescence of *hsp-16.2p::GFP* after heat shock (n≥24). (**F** and **G**) Fluorescent micrographs and quantification of fluorescent puncta in the nerve ring of control and *rab-3p::hpk-1* L4 animals expressing *rgef-1p::GFP::LGG-1/Atg8* (n≥21). (**H** and **I**) Fluorescent micrographs (H) and quantification (I) of puncta in seam cells of control and *rab-3p::hpk-1* animals expressing *lgg-1p::GFP::LGG-1/Atg8* (n≥43). (**J** and **K**) Fluorescent micrographs (J) and quantification (K) of puncta in proximal intestinal cells of control and *rab-3p::hpk-1* animals expressing the *lgg-1p::GFP::LGG-1/Atg8* autophagosome reporter (n≥17). (**L**) Expression of autophagy genes *atg-18* and *bec-1* via RT-qPCR in *rab-3p::HPK-1* animals compared to non-transgenic controls. *T*-test analysis with **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. See *Supplementary Files 4, 14 and 15* for details and additional trials.

465 particularly in the pharynx and head muscles, but surprisingly not within intestinal cells
466 (*Figure 6B-E*). We conclude that while *hpk-1* is required for the broad induction of
467 molecular chaperones (Das et al., 2017), neuronal HPK-1 primes inducibility of the HSR
468 within specific tissues, rather than in a systemic manner throughout the organism.

469 We next tested whether increased neuronal HPK-1 activity is sufficient to induce
470 autophagy. Using two LGG-1/Atg8 fluorescent reporter strains to visualize
471 autophagosome levels in neurons (*rgef-1p::GFP::LGG-1),* hypodermal seam cells and
472 intestine (*lgg-1p::GFP::LGG-1)* (Kumsta et al., 2017), we found that increased neuronal
473 *hpk-1* expression significantly induces autophagy in neurons and hypodermal seam cell
474 (*Figure 6F-I*), but not in the intestine (*Figure 6J, K*). Sustained autophagy and
475 transcriptional activation of autophagy genes are important components for somatic
476 maintenance and organismal longevity (Lapierre et al., 2015). Consistently *hpk-1* is
477 required for the induction of autophagy gene expression induced by TORC1 inactivation
478 (Das et al., 2017). We tested whether neuronal overexpression of *hpk-1* is sufficient to
479 induce autophagy gene expression, and found that increased neuronal overexpression
480 induces expression of *atg-18* (WD repeat domain, phosphoinositide interacting 2) and
481 *bec-1* (Beclin 1) (*Figure 6L*). Thus, increased HPK-1 activity within the nervous system is
482 sufficient to induce basal levels of autophagy both cell-intrinsically and non-
483 autonomously, which we posit occurs through increased autophagy gene expression to
484 extend longevity.

485 **GABAergic and Serotonergic HPK-1 differentially regulate longevity and acute**
486 **stress signaling.**

487 *hpk-1* is broadly expressed within the *C. elegans* adult nervous system (Das et al.,
488 2017), but the neuronal cell-types responsible for extending longevity are unknown. We
489 overexpressed *hpk-1* in different subtypes of neurons and assessed changes in muscle
490 proteostasis (*unc-54p::Q35::YFP*). Increased *hpk-1* expression in glutamatergic or
491 dopaminergic neurons was not sufficient to decrease the formation of fluorescent foci or
492 reduce paralysis (*Figure 7--figure supplement 1*). While it is possible that *hpk-1*
493 overexpression in these neuronal subtypes failed to reach a threshold effect, these results
494 suggest that HPK-1 does not act within these neuronal subtypes to cell non-autonomously
495 regulate proteostasis. In contrast, increased expression of *hpk-1* in either serotonergic

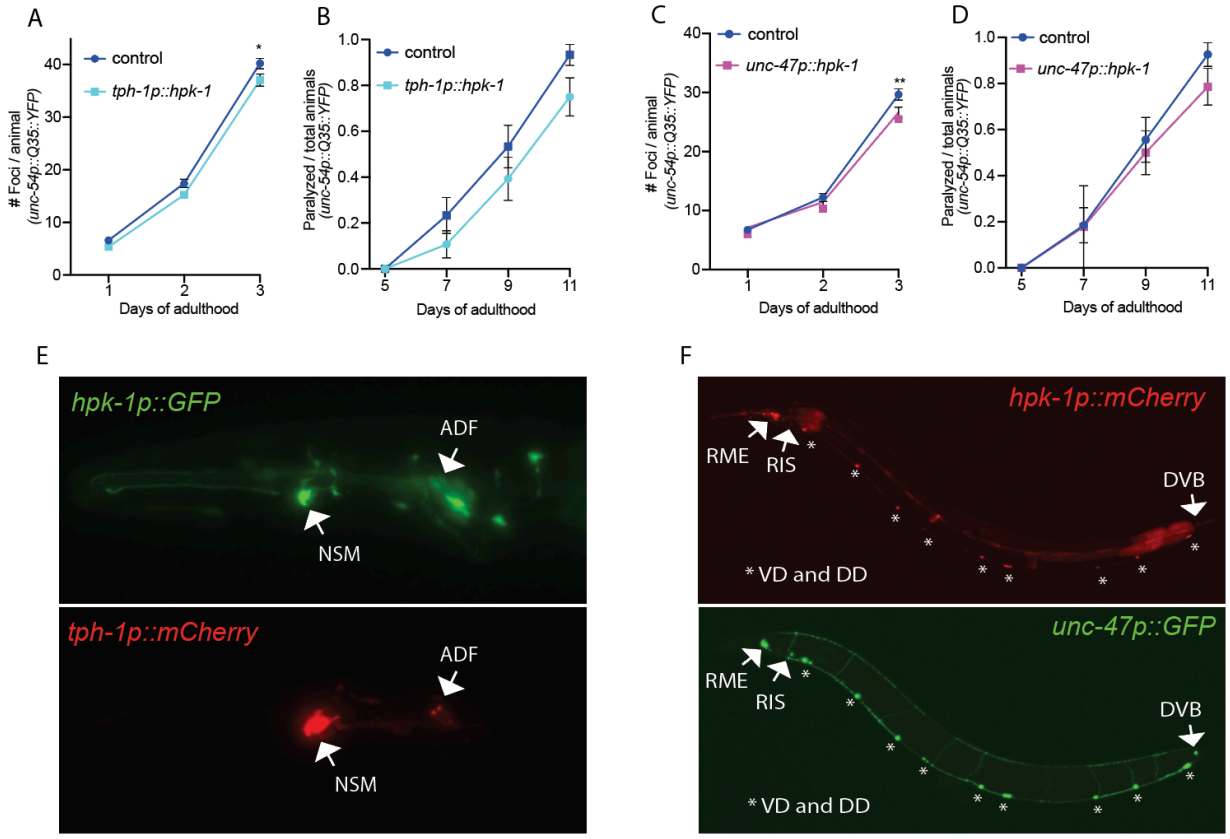


Figure 7. Expression of HPK-1 in serotonergic and GABAergic prevents the decline in proteostasis in muscle tissue.
(A and **B)** Quantification of foci (A) and paralysis rate (B) of animals expressing *hpk-1* in serotonergic neurons (n≥17 for A and n≥28 for B). **(C** and **D)** Quantification of foci (C) and paralysis rate (D) of animals expressing *hpk-1* in GABAergic neurons (n≥19 for C and n≥27 for D). **(E)** Representative fluorescent micrographs of *hpk-1* expression in serotonergic neurons. **(F)** Representative fluorescent micrographs of *hpk-1* expression in GABAergic neurons. *T* test analysis performed to each time point with **P* < 0.05 and ***P* < 0.01. See *Supplemental Files 12 and 13* for details and additional trials.

496 (*tph-1p::hpk-1*) or GABAergic (*unc-47p::hpk-1*) neurons was sufficient to improve
497 proteostasis in muscle tissue (*Figure 7A-D*). Accordingly, we found that *hpk-1* is
498 expressed in serotonergic and GABAergic neurons, based on colocalization between
499 *hpk-1* and neuronal cell-type specific reporters (*Figure 7E, F--figure supplement 2*),
500 consistent with the notion that HPK-1 initiates pro-longevity signals from these neurons.

501 We sought to determine whether signals initiated from serotonergic and
502 GABAergic neurons induced similar or distinct components of the PN in distal tissues,
503 and assessed the overall consequence on organismal health. Since pan-neuronal
504 expression of *hpk-1* was sufficient to induce autophagy, improve thermotolerance, and
505 increase lifespan, we chose to dissect whether these three phenotypes were regulated
506 within distinct neuronal cell-types. Increased HPK-1 serotonergic signaling was sufficient
507 to improve thermotolerance, without altering lifespan or autophagy (*Figure 8A-D*). In
508 contrast, increased *hpk-1* expression in GABAergic neurons did not alter
509 thermotolerance, but induced autophagy and slightly but significantly increased lifespan
510 (*Figure 8E-H*).

511 To identify the mechanism through which increased neuronal HPK-1 activity
512 induces autophagy in distal tissues, we examined serotonergic neurons. As expected,
513 loss of *tph-1,* which encodes the *C. elegans* tryptophan hydroxylase 1 essential for
514 biosynthesis of serotonin (Sze et al., 2000), failed to block the induction of autophagy by
515 increased neuronal *hpk-1* expression (*Figure 8I*, *tph-1(mg280);rab-3p::hpk-1*). *unc-30*
516 encodes the ortholog of human paired like homeodomain 2 (PITX2) and is essential for
517 the proper differentiation of type-D inhibitory GABAergic motor neurons (Jin et al., 1994;
518 McIntire et al., 1993). Consistent with our previous result demonstrating that only

519 GABAergic expression of *hpk-1* promotes autophagy, loss of *unc-30* was sufficient to
520 completely abrogate the induction of autophagy in seam cells after increased pan-
521 neuronal expression of *hpk-1* (*Figure 8I*, *unc-30(ok613);rab-3p::hpk-1*). We conclude that
522 HPK-1 activity in serotonergic neurons promotes systemic thermotolerance, whereas

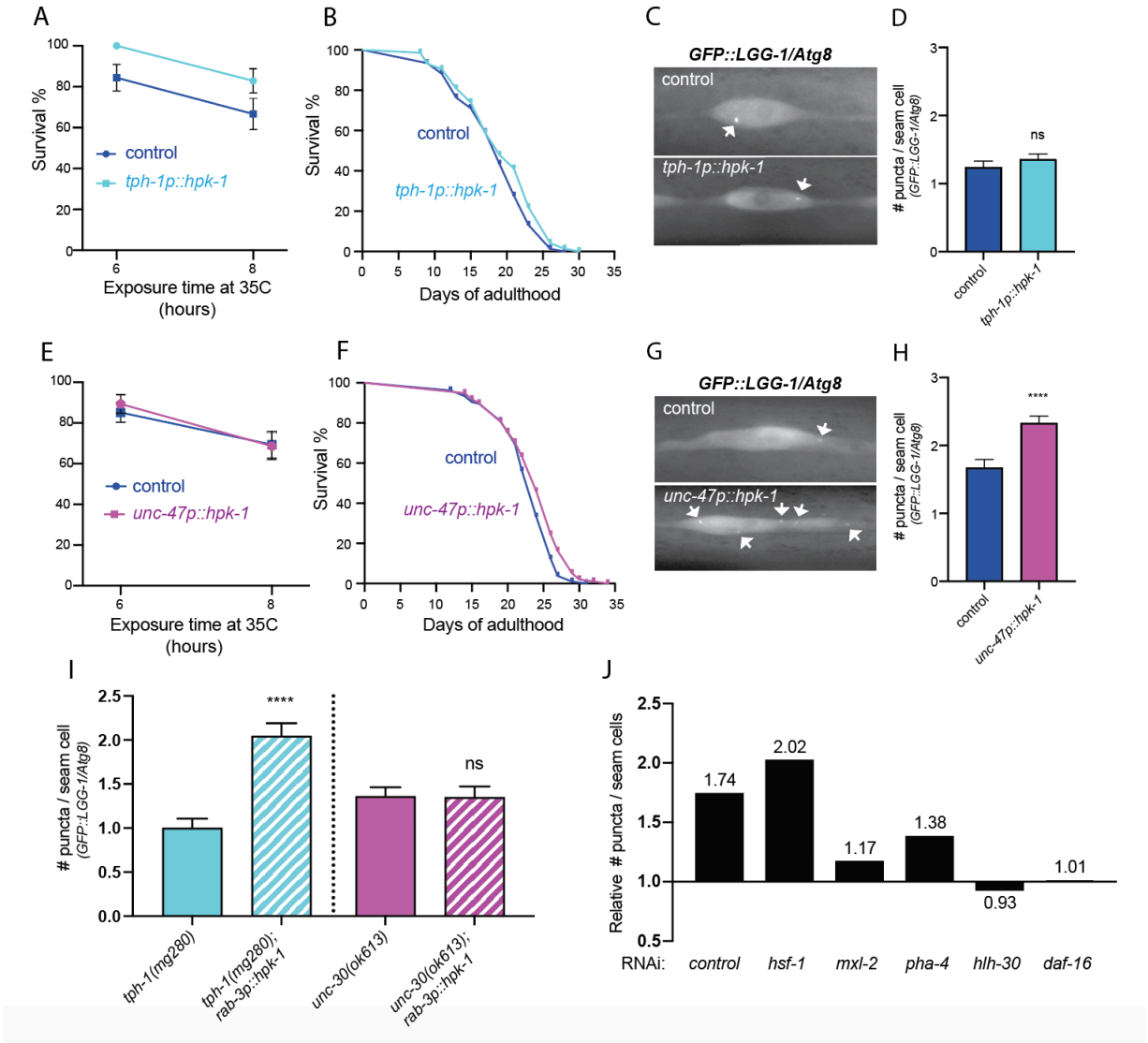


Figure 8. Serotonergic and GABAergic HPK-1 signaling activates distinct adaptive responses to regulate stress resistance and longevity.
(A) Survival of serotonergic HPK-1 expressing day 1 adult animals after heat shock (n≥32). **(B)** Lifespan of serotonergic HPK-1 expressing animals (n≥76). **(C** and **D)** Representative fluorescent micrographs (C) and quantification (D) of autophagosomes in hypodermal cells of serotonergic HPK-1 day 1 adult animals (n≥80). **(E)** Survival of GABAergic HPK-1 expressing day 1 adult animals after heat shock (n≥47). **(F)** Lifespan of GABAergic HPK-1 expressing animals (n≥119). **(G** and **H)** Representative fluorescent micrographs (G) and quantification of autophagosomes in hypodermal cells of GABAergic HPK-1 day 1 adult animals (n≥64). **(I)** Quantification of autophagosomes in hypodermal cells of mutant animals of serotonin and GABAergic signaling (*tph-1* and *unc-30*, respectively), expressing pan-neuronal *hpk-1* (day 1 adults, n≥ 67). **(J)** Relative change in number of puncta in seam cells of *unc-47p::hpk-1* animals expressing *lgg-1p::GFP::LGG-1/Atg8*, compared to non-transgenic controls after indicated gene inactivation (n≥28) for all conditions); see Figure 8- *figure supplement 1* for absolute values and statistical analysis. *T* test analysis with **P* < 0.05, ***P* < 0.01 and *****P* < 0.0001. See *Supplementary Files 1, 3, and 15* for details and additional trials.

523 HPK-1 in GABAergic neurons promotes autophagy. We surmise that the two neuronal
524 subtypes are likely to contribute synergistically to the extension of longevity.

525 HPK-1 regulates autophagy gene expression and across diverse species HIPK
526 family members directly regulate transcription factors (Sardina et al., 2023), therefore we
527 sought to identify transcription factors essential for HPK-1 overexpression within
528 GABAergic neurons to induce autophagy. *pha-4* (FOXA), *mxl-2* (MLX), *hlh-30* (TFEB),
529 *daf-16* (FOXO) and *hsf-1* are all required in specific contexts for the induction of
530 autophagy, and longevity (Hansen et al., 2008; Kumsta et al., 2017; Lapierre et al., 2013;
531 Lapierre et al., 2015; Nakamura et al., 2016; O'Rourke and Ruvkun, 2013). Furthermore,
532 we previously found that *hsf-1, mxl-2, or pha-4* are all necessary for the increased lifespan
533 conferred by *hpk-1* overexpression throughout the soma (Das et al., 2017). Using feeding
534 based RNAi, we found that *hpk-1* overexpression in GABAergic neurons still significantly
535 induced autophagosomes in seam cells after loss of *hsf-1* or *pha-4* (*Figure 8J*); thus HPK-
536 1 induction of autophagy is independent of *hsf-1* and *pha-4* and suggests the *pha-4*
537 requirement for HPK-1 mediated longevity is independent of autophagy gene regulation
538 (at least in hypodermal seam cells). In contrast, after inactivation of *mxl*-2, *hlh-30* or *daf-*
539 *16,* GABAergic overexpression of *hpk-1* failed to significantly increase autophagosomes
540 (*Figure 8J*); thus HPK-1 mediated induction of autophagy requires *mxl-2*, *hlh-30*, and *daf-*
541 *16*. We were puzzled to find that the average number of autophagosome in control
542 animals (i.e., lacking *hpk-1* overexpression) varied after gene inactivation (*Figure 8--*
543 *figure supplement 1A*). To rigorously test whether *hpk-1* overexpression required *hlh-30*
544 for the induction of autophagosomes, we crossed the *hlh-30* null mutation into animals
545 overexpressing *hpk-1* within GABAergic neurons (*unc-47p::hpk-1;hlh-30(tm1978);lgg-*

546 *1p::GFP::LGG-1*; we find that *hlh-30* is required for the induction of autophagosomes in
547 seam cells when *hpk-1* is overexpressed in GABAergic neurons (*Figure 8--figure*
548 *supplement 1B).*

549 We sought to fortify our previous findings that: 1) TORC1 negatively regulates *hpk-*
550 *1* under basal conditions; 2) *hpk-1* is essential for decreased TORC1 signaling to increase
551 longevity and induce autophagy; and 3) HPK-1-mediated longevity requires *mxl-2*, the
552 ortholog of mammalian Mlx transcription factor (Das et al., 2017). From these data, we
553 predicted that TOR-mediated longevity might require *mxl-2* via *hpk-1.* Indeed, we find that
554 inactivation of either *daf-15* (Raptor) or *let-363* (TOR), increased lifespan and required
555 *mxl-2*, as *mxl-2(tm1516)* null mutant animals failed to increase lifespan in the absence of
556 TORC1 (*Figure 8--figure supplement 2*). MXL-2 functions as a heterodimer with MML-1
557 (Pickett et al., 2007), which have similar roles in *C. elegans* longevity (Johnson et al.,
558 2014). Interestingly, one of the human homologs of MML-1, Carbohydrate Response
559 Element Binding Protein (ChREBP/MondoB/MLXIPL/WBSCR14), maps within a deleted
560 region in Williams-Beuren Syndrome patients, which among other symptoms causes
561 metabolic dysfunction, silent diabetes, and premature aging (Pober, 2010). This further
562 supports our model and is consistent with other studies linking the Myc-family of
563 transcription factors to longevity, autophagy, and TORC1 signaling (Johnson et al., 2014;
564 Nakamura et al., 2016; Samuelson et al., 2007). Collectively, we conclude that HPK-1
565 regulates serotonergic signals to improve survival in acute thermal stress, likely through
566 HSF-1, and initiates GABAergic signals to extend longevity through increased autophagy,
567 which is linked to decreased TORC1 signaling and regulation of the Myc TFs.

568 **Discussion**

569 It has been known that a core group of TFs integrates various types of metabolic
570 and stress signals; moreover, this group of TFs can extend longevity when
571 overexpressed, and conversely hasten aging when lost (reviewed in (Denzel et al.,
572 2019)), but whether the activity or expression of these longevity-associated transcriptional
573 regulators are activated by signals of increasing damage or dysfunction during normal
574 aging was unknown. Recently, single-cell RNA sequencing has revealed that these
575 longevity-associated TFs increase in expression during aging in wild-type animals across
576 multiple somatic tissues (Roux et al., 2022). This comprehensive empirical dataset
577 provides foundational support for a key theme in aging research: during aging in wild-type
578 animals, increasing damage and dysfunction is mitigated by the activation of stress
579 response programs. Our findings that: 1) *hpk-1* expression is upregulated during normal
580 aging in more neurons and non-neuronal cells than any other kinase, and 2) that *hpk-1* is
581 induced in cell-clusters that overlap with longevity-associated TFs, including *daf-16, hlh-*
582 *30, skn-1* and *hif-1*, implies that HPK-1 is a key transcriptional regulator that mitigates
583 aging. We posit this response is activated in the nervous system during normal aging in
584 wild-type animals to mitigate increasing stress and the breakdown of homeostatic
585 mechanisms within the nervous system. Consistently, the presence of human
586 neurodegenerative disorders, a severe form of neuronal proteotoxic stress, correlates
587 with activation of human *hpk-1* expression: for example, human HIPK2 is activated in
588 frontal cortex samples of patients with AD, in motor neurons of ALS patients, and in
589 mouse models of ALS, (Lee et al., 2016). Thus, our results suggest that overexpression
590 of *hpk-1* in the *C. elegans* nervous system may extend longevity by preemptively delaying
591 the accumulation of age-associated damage and dysfunction to the proteome (*Figure 9A*).

592 However, *hpk-1* homologs in more complex metazoans can also promote cell death, a
593 key homeostatic component of tissue regeneration, indicating that chronic activation of
594 HIPKs is not always beneficial (Calzado et al., 2007; Lee et al., 2016; Wang et al., 2020;
595 Zhang et al., 2020). Whether activated HIPKs will fortify proteostasis and extend
596 longevity, or promote cell death is likely to be dependent on many factors, including the
597 specific homolog, levels of expression, physiological context, genetic background, and
598 cell-type.

599 We find that *hpk-1* prevents age-associated decline of axonal and synaptic
600 integrity, which together preserve neuronal function during the aging process and thus
601 also promote longer healthspan. In support of this concept, adult *hipk2 -/-* mice display
602 normal neurogenesis but manifest delayed-onset severe psychomotor and behavioral
603 deficiencies, as well as degeneration of dopaminergic neurons, reminiscent of
604 Parkinson's Disease (Zhang et al., 2007). Loss of *hpk-1* results in dysregulation of
605 neuronal gene expression by early adulthood; some changes are consistent with neuronal
606 aging but in other cases we observe an inverse correlation. For example, one third of all
607 neuropeptides are induced in the absence of *hpk-1*; yet increased neuropeptide signaling
608 has been associated with extended *C. elegans* longevity and is considered protective in
609 AD (Chen et al., 2018; Hodge et al., 2022; Petrella et al., 2019). One possibility is in the
610 absence of *hpk-1*, attenuated inducibility of the proteostatic network accelerates age-
611 associated cellular dysfunction (*Figure 9A*), and upregulation of neuropeptide signaling
612 compensates in the nervous system for the loss of proteostatic response. This concept is
613 consistent with our finding that increasing neuronal HPK-1 activity does not require
614 neuropeptides to improve proteostasis in distal tissues. An alternative possibility for the

615 connection between protective effects of HPK-1 and neuropeptides in neurons is that
616 HPK-1 may reduce insulin signaling by suppressing the processing of *ins* peptides,
617 thereby decreasing ILS to activate DAF-16 (FOXO) transcriptional quality control
618 programs. For example, loss of *egl-3*, a neuropeptide processing gene, extends longevity
619 by inactivating processing of insulins (Hamilton et al., 2005), and *egl-3* is upregulated in
620 *hpk-1* loss of function.

621 Impairment of the proteostatic network after loss of *hpk-1* results in broad
622 upregulation of gene expression, which may be provide additional levels of compensatory
623 mechanisms to mitigate neuronal dysfunction. For example, loss of *hpk-1* induces
624 expression of intraflagellar transport (IFT) components in sensory cilia. Mutations within

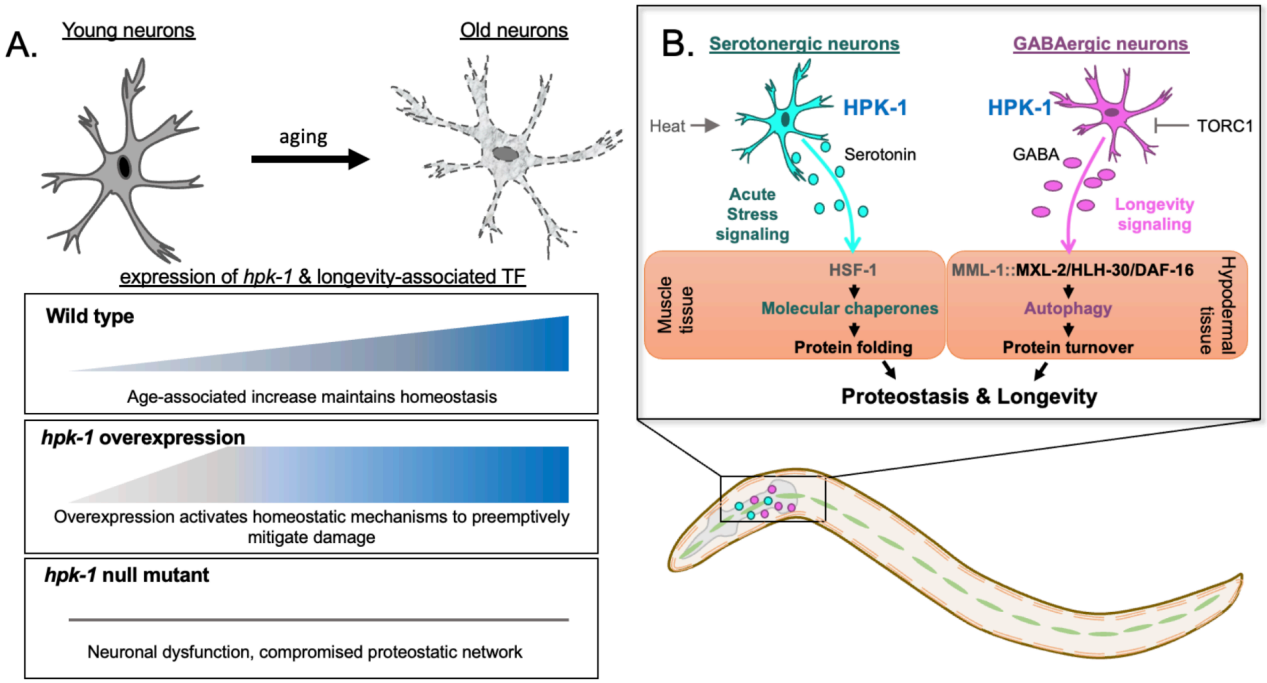


Figure 9. Differential regulation of the proteostatic network through HPK-1 activity in serotonergic and GABAergic neurons. **(A)** During normal aging *hpk-1* expression increases broadly throughout the nervous system in response to accumulating damage and dysfunction. Overexpression of *hpk-1* within the nervous system fortifies proteostasis by priming and preemptively activating mechanisms that delay the progression of aging. In the absence of *hpk-1* the proteostatic network is compromised, resulting in neuronal dysfunction and increased expression compensatory mechanisms to maintain homeostasis (see text for details). **(B)** HPK-1 activity in serotonergic and GABAergic neurons initiates distinct adaptive responses, either of which improve proteostasis in a cell non-autonomous manner. Serotonergic HPK-1 protects the proteome from acute heat stress, while GABAergic HPK-1 fortifies the proteome by regulating autophagy activity in response to metabolic stress, such as changes in TORC1 activity. The MXL-2, HLH-30 (TFEB), and DAF-16 (FOXO) transcription factors are all required for hypodermal induction of autophagy in response to increased HPK-1 activity in GABAergic neurons. Gray indicates predicted interactions based on our prior genetic analysis (Das et al., 2017). MML-1 and MXL-2 heterodimerize and encode the orthologs of the Myc-family of transcription factors Mondo A/ChREBP and Mlx, respectively.

625 sensory neurons can alter longevity (Alcedo and Kenyon, 2004; Apfeld and Kenyon,
626 1999), and decreased IFT during *C. elegans* aging impairs sensory perception and
627 metabolism (Zhang et al., 2021). Deficiencies in primary cilia impairs neurogenesis in AD
628 transgenic mice, resulting in accumulation of $A\beta_{42}$ and tau protein and disease
629 progression (Armato et al., 2013; Chakravarthy et al., 2012; Ma et al., 2022; Morelli et al.,
630 2017). However, our bulk RNA-seq analysis cannot decipher changes in gene expression
631 at the level of individual neurons after loss of *hpk-1. hpk-1* and orthologs are known to
632 have roles in development, differentiation and cell fate-specification (Berber et al., 2013;
633 Blaquiere and Verheyen, 2017; Calzado et al., 2007; Hattangadi et al., 2010; Rinaldo et
634 al., 2007; Rinaldo et al., 2008; Steinmetz et al., 2018), therefore we cannot preclude the
635 possibility that HPK-1 may function to broadly limit neuronal gene expression during the
636 course of normal development and differentiation, thereby specifying neuronal
637 transcriptional programs. Mis-differentiation of the nervous system in the absence of *hpk-*
638 *1* could result in both intracellular transcriptional noise and cell non-autonomous noise
639 across cell-types and tissues.

640 We show that HPK-1 expression in serotonergic neurons activates a cell non-
641 autonomous signal to prime induction of molecular chaperones in muscle cells, whereas
642 HPK-1 expression in GABAergic neurons signals induces autophagy in hypodermal
643 tissue (*Figure 9B*). Our findings place HPK-1 within a selective cohort of transcriptional
644 regulators within the *C. elegans* nervous system that are activated in response to
645 metabolic, environmental, and intrinsic cues; thereby initiating distinct cell non-
646 autonomous signals to regulate components of the PN in peripheral tissues (Douglas et
647 al., 2015; Durieux et al., 2011; Frakes et al., 2020; Imanikia et al., 2019; Prahlad et al.,

648 2008; Prahlad and Morimoto, 2011; Silva et al., 2013; Tatum et al., 2015; Taylor and
649 Dillin, 2013). Loss of serotonin production in *tph-1* mutant animals has a minimal impact
650 on lifespan at basal temperature (Murakami and Murakami, 2007). However,
651 simultaneous loss of both serotonin and dopamine biosynthesis shortens lifespan
652 (Murakami and Murakami, 2007). Serotonin biosynthesis is also required for several
653 conditions that extend longevity: animals treated with an atypical antidepressant serotonin
654 antagonist (mianserin) (Petrascheck et al., 2007), changes in food perception (Entchev
655 et al., 2015), and neuronal cell non-autonomous induction of the mitochondrial unfolded
656 protein response (Berendzen et al., 2016; Zhang et al., 2018). In contrast, loss of either
657 of the serotonin receptors *ser-1* or *ser-4* have opposing effects on lifespan, suggesting
658 antagonistic activity between serotonin receptors (Murakami and Murakami, 2007).

659 Surprisingly, we find that increased HPK-1 activity in serotonergic neurons does
660 not increase lifespan, but is sufficient to improve muscle proteostasis and amplify the
661 expression of molecular chaperones that maintain proteostasis within the cytosol and
662 nucleus. Interestingly, neuronal HSF-1 regulation of the HSR through thermosensory
663 circuits is also separable from lifespan (Douglas et al., 2015). Serotonin signaling and the
664 serotonin receptor *ser-1* are essential for the induction of the HSR (Prahlad et al., 2008;
665 Tatum et al., 2015). ADF serotoninergic neurons receive upstream stress signals from
666 the thermosensory circuit, consisting of the amphid sensory neurons (AFD) and the AIY
667 interneurons, which regulate HSF-1 activity in the germline in a serotonin-dependent
668 manner (Tatum et al., 2015). However, increased serotonergic HPK-1 activity is not
669 equivalent to altered of thermosensory signals: *hpk-1* improves proteostasis within body
670 wall muscles but not intestinal cells through neurotransmitters and independent from

671 dense core vesicles. In contrast, loss of the guanylyl cyclase (*gcy-8*) in AFD
672 thermosensory neurons or the LIM-homeobox transcription factor (*ttx-3*) in AIY
673 interneurons improves intestinal proteostasis through dense core vesicle neurosecretion
674 (i.e., *unc-31*). *hpk-1* is expressed in AFD, but only at very low levels in AIY neurons (data
675 not shown and (Taylor et al., 2021)), and *hpk-1* is essential for the induction of the HSR
676 within intestinal cells (Das et al., 2017). Whether *hpk-1* activity within thermosensory
677 neurons functions within the canonical thermosensory-serotonin circuit to regulate the
678 intestinal HSR or proteostasis is not mutually exclusive from our findings and will be
679 interesting to pursue in future studies. Our results suggest serotonergic HPK-1 is an
680 important regulator of the HSR, potentially functioning in parallel to the thermosensory-
681 serotonin circuit to restore proteostasis in response to acute thermal stress.

682 We posit that increased serotonergic HPK-1 activity further activates HSF-1 in
683 response to heat (*Figure 9B*). This would be consistent with known hyperphosphorylation
684 of HSF-1 and amplification of the HSR in response to increasingly severe heat stress
685 (reviewed in (Lazaro-Pena et al., 2022)). Interestingly, HPK-1 limits SUMOylation of HSF-
686 1, SUMO limits the heat shock response in both *C. elegans* and mammals, and severe
687 heat shock results in HSF1 deSUMOylation (Das et al., 2017; Hietakangas et al., 2003;
688 Hietakangas et al., 2006). To our surprise, while increased neuronal *hsf-1* expression
689 increases longevity (Douglas et al., 2015; Morley and Morimoto, 2004), we could find no
690 study that has identified specific neuronal cell-types in which increased HSF-1 activity is
691 sufficient to extend longevity or enhance the HSR. Whether or not HPK-1 regulates HSF-
692 1 within the nervous system and/or in peripheral muscle cells through cell non-
693 autonomous signals will be interesting to determine in future studies. HPK-1 co-localizes

694 with HSF-1 in neurons, HPK-1 protein is stabilized after heat shock, *hpk-1* is essential for
695 induction of the HSR, and *hsf-1* and *hpk-1* are mutually interdependent to extend
696 longevity (Das et al., 2017) (*Figure 9B*).

697 Increased HPK-1 activity in GABAergic neurons induces autophagy in peripheral
698 cells, improves proteostasis and longevity. Whether HPK-1 functions in GABAergic
699 neurons through alteration of GABA or another neurotransmitter is unknown, but proper
700 differentiation of GABAergic neurons is essential for neuronal HPK-1 activation of
701 autophagy in distal tissue. We posit HPK-1 cell non-autonomous signaling occurs via
702 antagonism of GABA signaling. Loss of GABA increases *C. elegans* lifespan (Chun et al.,
703 2015; Yuan et al., 2019). However, not all neurotransmitters regulate lifespan, as only
704 mutants for the GABA biosynthetic enzyme, *unc-25*, increase lifespan. This GABAergic-
705 mediated control in lifespan seems to be mediated by the *gbb-1* metabotropic $GABA_B$
706 receptor (Chun et al., 2015). Interestingly, GABA signaling inhibits autophagy and causes
707 oxidative stress in mice, which is mitigated via Tor1 inhibition (Lakhani et al., 2014). In
708 fact, we previously found that neuronal expression of HPK-1 is inhibited by TORC1 and
709 *hpk-1* is required for TORC1 inactivation to extend longevity and the induction of
710 autophagy gene expression (Das et al., 2017). Both TORC1 inactivation and *hpk-1*
711 overexpression requires the Myc-family member *mxl-2* to extend longevity (Das et al.,
712 2017; Johnson et al., 2014; Nakamura et al., 2016) (*Figure 8--figure supplement 2*).
713 Consistently, TORC1 also acts within neurons to regulate aging (Zhang et al., 2019); our
714 results suggest that GABAergic neurons might be the specific neuronal cell-type through
715 which alterations in TORC1 activity regulate longevity. Collectively, our results position

716 HPK-1 activity in GABAergic neurons as the likely integration point for cell non-
717 autonomous regulation of autophagy via TORC1 (*Figure 9B*).

718 A growing number of longevity-associated transcription factors have been linked
719 to autophagy, including *pha-4* (FOXA), *mxl-2* (MLX), *hlh-30* (TFEB), *daf-16* (FOXO) and
720 *hsf-1* (Hansen et al., 2008; Kumsta et al., 2017; Lapierre et al., 2013; Lapierre et al., 2015;
721 Nakamura et al., 2016; O'Rourke and Ruvkun, 2013). We find that *hlh-30, daf-16,* and
722 *hlh-30* are all required for the induction of autophagosomes when *hpk-1* is overexpressed
723 in GABAergic neurons. We posit these transcription factors respond within the distal
724 hypodermal seam cells in response to a neuronal signal initiated by HPK-1, as neurons
725 are largely refractory to RNAi. However, this does not preclude cell intrinsic neuronal
726 interactions; future refined spatial genetic analysis will be required to definitively decipher
727 the precise tissues of action. DAF-16 and HLH-30 have previously been shown to function
728 in combination to promote stress resistance and longevity (Lin et al., 2018). Analogously,
729 the MML-1::MXL-2 complex and HLH-30 activate each other and are downstream
730 effectors of longevity after TORC1 inhibition (*mml-1* encodes the MondoA/ChREBP
731 ortholog, an obligate interactor with MXL-2) (Nakamura et al., 2016). Based on our
732 discovery that all three of these longevity-associated transcription factors are essential
733 for GABAergic HPK-1 activity to induce autophagy, it is tempting to speculate HPK-1 acts
734 as a key mediator of pro-longevity signaling after TORC1 inhibition through coordinated
735 activation of MML-1::MXL-2, HLH-30, and DAF-16 (*Figure 9B*).

736 This work reveals a new level of specificity in the capacity of the nervous system
737 to initiate adaptive responses by triggering distinct components of the proteostatic
738 network across cell types. Results elucidating how neuronal cell-types generate

739 heterotypic signals to coordinate the maintenance of homeostasis are just beginning to
740 emerge. For example, a previous study discovered that activating non-autonomous
741 signaling of the endoplasmic reticulum unfolded protein response (ER-UPR) via the XBP-
742 1 transcription factor can be initiated from serotonergic neurons and dopaminergic
743 neurons; these signals are unique and converge to activate distinct branches of the ER-
744 UPR within the same distal tissues (intestinal cells) (Higuchi-Sanabria et al., 2020). In
745 contrast, our work demonstrates differential, yet specific, activation of cytosolic
746 components of the PN in separate peripheral tissues through neurotransmitters. Our work
747 suggests the nervous system partitions neuronal cell-types to coordinate the activation of
748 complementary proteostatic mechanisms across tissues, despite utilizing the same
749 transcriptional regulator within each neuronal cell-type. One possibility is that HPK-1
750 regulates specific adaptive responses based on the unique epigenetic states between
751 differentiated neuronal subtypes, which would be consistent with recent findings: during
752 development and differentiation, transcriptional rewiring of cells set chaperoning
753 capacities and alter usage of mechanisms that maintain protein quality control (Nisaa and
754 Ben-Zvi, 2021; Shemesh et al., 2021). Our work reveals HPK-1 as a novel transcriptional
755 regulator within the nervous system of metazoans, which integrates diverse stimuli to
756 coordinate specific adaptive responses that promote healthy aging.

757

758 ## Materials and Methods

Key Resources Table				
Reagent type (species) or resource	**Designation**	**Source or Reference**	**Identifiers**	**Additional Information**
genetic reagent (*C. elegans*)	N2	N2 (CGCM)		*Wild type*
genetic reagent (*C. elegans*)	AVS392	EK273		*hpk-1(pk1393) X*
genetic reagent (*C. elegans*)	AVS543	This paper	*artEx41*	*artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]*
genetic reagent (*C. elegans*)	AVS609	This paper	*artEx43*	*artEx43 [myo-3p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS614	This paper	*artEx48*	*artEx48 [dpy-7p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS627	This paper	*artEx52*	*artEx52 [ges-1p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS420	This paper	*artEx41*	*hpk-1(pk1393) X; artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]*
genetic reagent (*C. elegans*)	AVS602	CZ13799	*juIs76*	*juIs76 [unc-25p::GFP + lin-15(+)] II*
genetic reagent (*C. elegans*)	AVS608	This paper	*juIs76*	*hpk-1(pk1393); juIs76 [unc-25p::GFP + lin-15(+)] II*
genetic reagent (*C. elegans*)	AVS752	This paper	*artEx41*	*artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]; hpk-1(pk1393); juIs76 [unc-25p::GFP + lin-15(+)] II*
genetic reagent (*C. elegans*)	AVS832	This paper	*rmIs132; artEx58*	*rmIs132 [unc-54p::Q35::YFP] I; artEx58 [rab-3p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS833	This paper	*rmIs132; artEx59*	*rmIs132 [unc-54p::Q35::YFP] I; artEx59 [rab-3p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2*
genetic reagent (*C. elegans*)	AVS834	This paper	*rmIs132; artEx60*	*rmIs132 [unc-54p::Q35::YFP] I; artEx60 [rab-3p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 3*
genetic reagent (*C. elegans*)	AVS835	This paper	*rmIs132; artEx61*	*rmIs132 [unc-54p::Q35::YFP] I; artEx61 [rab-3p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 4*
genetic reagent (*C. elegans*)	AVS744	This paper	*rmIs132; artEx75*	*artEx75 (rab-3p::hpk-1(K176A) + pCFJ90 (myo-2p::mCherry); rmIs132 [Punc-54::Q35::YFP] I line 1*
genetic reagent (*C. elegans*)	AVS748	This paper	*rmIs132; artEx79*	*artEx79 (rab-3p::hpk-1(D272N) + pCFJ90 (myo-2p::mCherry); rmIs132 [Punc-54::Q35::YFP] I line 1*
genetic reagent (*C. elegans*)	AVS562	This paper	*rmIs132; artEx41*	*rmIs132 [unc-54p::Q35::YFP] I; artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)] line 5*
genetic reagent (*C. elegans*)	AVS563	This paper	*rmIs110; artEx41*	*rmIs110 [F25B3.3p::Q40::YFP]; artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS837	This paper	*rmIs110; artEx92*	*rmIs110 [F25B3.3p::Q40::YFP]; artEx92 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)] line 2*
genetic reagent (*C. elegans*)	AVS838	This paper	*rmIs110; artEx93*	*rmIs110 [F25B3.3p::Q40::YFP]; artEx93 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)] line 3*
genetic reagent (*C. elegans*)	AVS839	This paper	*rmIs110; artEx94*	*rmIs110 [F25B3.3p::Q40::YFP]; artEx94 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)] line 4*
genetic reagent (*C. elegans*)	AVS214	HC196		*sid-1(qt9) V*
genetic reagent (*C. elegans*)	AVS265	TU3401	*uIs69*	*sid-1(pk3321)V; uIs69 [unc-119p::sid-1; myo-2p::mCherry]V*
genetic reagent (*C. elegans*)	AVS540	This paper	*rmIs132; uIs69*	*rmIs132 [unc-54p::Q35::YFP] I; sid-1(pk3321)V; uIs69[unc-119p::sid-1; myo-2p::mCherry]V*
genetic reagent (*C. elegans*)	AVS541	This paper	*rmIs110; uIS69*	*rmIs110 [F25B3.3p::Q40::YFP]; sid-1(pk3321)V; uIs69[unc-119p::sid-1; myo-2p::mCherry]V*
genetic reagent (*C. elegans*)	AVS713	This paper	*rmIs132; artEx41*	*rmIs132 [unc-54p::Q35::YFP] I; artEx41(rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]; unc-31(e169)V*
genetic reagent (*C. elegans*)	AVS810	This paper	*rmIs132; artEx41*	*rmIs132 [unc-54p::Q35::YFP] I; artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]; unc-13(e51)I*

genetic reagent (*C. elegans*)	AVS84	TJ375	*gpIs1*	*gpIs1 [hsp-16.2p::GFP] IV*
genetic reagent (*C. elegans*)	AVS397	This paper	*gpIs1; artEx35*	*gpIs1 [hsp-16.2p::GFP]; artEx35 [sur-5p::hpk-1::CFP +pCFJ90 (myo-2p::mCherry)]*
genetic reagent (*C. elegans*)	AVS399	This paper	*gpIs1; artEx33*	*gpIs1 [hsp-16.2p::GFP]; artEx33 [rab-3p::hpk-1::CFP +pCFJ90 (myo-2p::mCherry)]*
genetic reagent (*C. elegans*)	AVS715	This paper	*artEx41; sqIs24*	*artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]; sqIs24 [rgef-1p::GFP::lgg-1 + unc-122p::RFP]*
genetic reagent (*C. elegans*)	AVS716	This paper	*artEx41; sqIs13*	*artEx41 [rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry)]; sqIs13 [lgg-1p::GFP::lgg-1 + odr-1p::RFP]*
genetic reagent (*C. elegans*)	AVS682	This paper	*rmIs132; artEx62*	*artEx62 [tph-1p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS683	This paper	*rmIs132; artEx63*	*artEx63 [tph-1p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS685	This paper	*rmIs132; artEx65*	*artEx65 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS686	This paper	*rmIs132; artEx66*	*artEx66 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS691	This paper	*rmIs132; artEx71*	*artEx71 [eat-4p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS692	This paper	*rmIs132; artEx72*	*artEx72 [eat-4p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS693	This paper	*rmIs132; artEx73*	*artEx73 [cat-2p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS694	This paper	*rmIs132; artEx74*	*artEx74 [cat-2p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2; rmIs132 [unc-54p::Q35::YFP] I*
genetic reagent (*C. elegans*)	AVS809	This paper	*sqIs13; artEx62*	*artEx62 [tph-1p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; sqIs13 [lgg-1p::GFP::lgg-1 + odr-1p::RFP]*
genetic reagent (*C. elegans*)	AVS794	This paper	*sqIs13; artEx65*	*artEx65 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; sqIs13 [lgg-1p::GFP::lgg-1 + odr-1p::RFP]*
genetic reagent (*C. elegans*)	AVS709	This paper	*artEx62*	*artEx62 [tph-1p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS710	This paper	*artEx65*	*artEx65 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1*
genetic reagent (*C. elegans*)	AVS888	This paper	*aetEx99*	*artEx99 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 2*
genetic reagent (*C. elegans*)	AVS816	This paper	*sqIs13; artEx41*	*artEx41(rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry); sqIs13 [lgg-1p::GFP::lgg-1 + unc-122p::RFP]; unc-30(ok613)*
genetic reagent (*C. elegans*)	AVS872	This paper	*sqIs13; artEx41*	*artEx41(rab-3p::hpk-1::CFP + pCFJ90 (myo-2p::mCherry); sqIs13 [lgg-1p::GFP::lgg-1 + unc-122p::RFP]; tph-1(mg280)*
genetic reagent (*C. elegans*)	AVS836	This paper	*artEx91*	*artEx91 [hpk-1p::GFP + tph-1p::mCherry] line 1*
genetic reagent (*C. elegans*)	AVS757	This paper	*artEx87*	*artEx87 [hpk-1p::mCherry + unc-47p::GFP] line 1*
genetic reagent (*C. elegans*)	AVS001	CB1370		*daf-2(e1370)*
genetic reagent (*C. elegans*)	AVS022	GR1309		*daf-2(e1370); daf-16(mgDf47)*
genetic reagent (*C. elegans*)	AVS495	CL6264	*uIs60*	*uIs60 [unc-119p::YFP + unc119p::sid-1]; eri-1(mg366)*
genetic reagent (*C. elegans*)	AVS817	This paper	*uIs60; artIs1*	*artIs1 [sur-5p::HPK-1::CFP + pCFJ90 (myo-2p::m-cherry)]; uIs60 [unc-119p::YFP + unc119p::sid-1]; eri-1(mg366)*
genetic reagent (*C. elegans*)	AVS488	This paper		*mxl-2(tm1516)*
genetic reagent (*C. elegans*)	AVS1000	This paper	*sqIs13; artEx65*	*artEx65 [unc-47p::hpk-1 + pCFJ90 (myo-2p::mCherry)] line 1; sqIs13 [lgg-1p::GFP::lgg-1 + odr-1p::RFP]; hlh-30(tm1978)*
Recombinant DNA reagent	pAVS1	This paper	plasmid	*rab-3p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS2	This paper	plasmid	*dyp-7p, hpk-1* cDNA, pPD95.75 backbone

Recombinant DNA reagent	pAVS3	This paper	plasmid	*myo-3p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS4	This paper	plasmid	*cat-2p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS5	This paper	plasmid	*eat-4p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS6	This paper	plasmid	*tph-1p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS7	This paper	plasmid	*unc-47p, hpk-1* cDNA, pPD95.75 backbone
Recombinant DNA reagent	pAVS8	This paper	plasmid	*rab-3p, hpk-1(K176A)*, derived from pAVS1
Recombinant DNA reagent	pAVS9	This paper	plasmid	*rab-3p, hpk-1(D272N)*, derived from pAVS1
Recombinant DNA reagent	pPD95.75	Addgene	Plasmid #1494	
Sequence-based reagent	*atg-18F*	This paper	PCR primer	5'-ACTTGAGAAAACGGAAGGTGTT
Sequence-based reagent	*atg-18R*	This paper	PCR primer	5'-TGATAGCATCGAACCATCCA
Sequence-based reagent	*cdc-42F*	This paper	PCR primer	5'-AGCCATTCTGGCCGCTCTCG
Sequence-based reagent	*cdc-42R*	This paper	PCR primer	5'-GCAACCGCTTCTCGTTTGGC
Sequence-based reagent	*bec-1F*	This paper	PCR primer	5'-TTTTGTTGAAAGAGCTCAAGGA
Sequence-based reagent	*bec-1R*	This paper	PCR primer	5'-CAACCAGTGAATCAGCATGAA
Sequence-based reagent	*hpk-1F*	This paper	PCR primer	5'-AGTATGCACAGCTCCATCAC
Sequence-based reagent	*hpk-1R*	This paper	PCR primer	5'-CCATTATTGGGACCGGAACA
Sequence-based reagent	*xbp-1F*	This paper	PCR primer	5'-TGCCTTTGAATCAGCAGTGG
Sequence-based reagent	*xbp-1R*	This paper	PCR primer	5'-ACCGTCTGCTCCTTCCTCAATG
Sequence-based reagent	*gei-3F*	This paper	PCR primer	5'-AAGTCCGAGTCGCTGAACAC
Sequence-based reagent	*gei-3R*	This paper	PCR primer	5'- ATGCCTGAATGCTGACGCTC
Chemical compound, drug	isopropylthiogalactoside (IPTG)	GoldBio	I2481c-100	
Chemical compound, drug	TRIzol reagent	Life Technologies	Catalog: 15596026	
Chemical compound, drug	FUdR	Fisher/Alfa Aesar	CAS 50-91-9	
Chemical compound, drug	Aldicarb	Fluka analytical	# 33386	
Chemical compound, drug	serotonin (5-HT)	Sigma	H9623-100mg	
Commercial assay kit	QuikChange II XL Site-Directed Mutagenesis Kit	Agilent	Catalog #200521	
Commercial assay kit	RNeasy Plus Mini Kit	Qiagen	Cat. No.:74034	
Commercial assay kit	cDNA synthesis kit	BioRad	#1708890	
Commercial assay kit	PerfeCTa SYBR green FastMix	Quantabio	# 101414-276	
Commercial assay kit	TruSeq Stranded mRNA	Illumina		
Software, algorithm	Prism	GraphPad	version 7	

Software, algorithm	AxioVision		v4.8.2.0	
Software, algorithm	FastQC	Andrews, 2010		
Software, algorithm	Trimmomatic	Bolger et al., 2014		
Software, algorithm	STAR 2.4.2a	Dobin et al., 2013		
Software, algorithm	featureCounts	Liao et al., 2014	version 1.4.6-p5	
Software, algorithm	RSEM	Li and Dewey, 2011		
Software, algorithm	R statistical software environment	Team, 2013	version 4.0.2	
Software, algorithm	DESeq2	Love et al., 2014		
Software, algorithm	GOSeq	Young et al., 2010		
Software, algorithm	MuDataSeurat	Hao et al., 2021		

759

760 ***C. elegans* strains and details**

761 All strains were maintained at 20°C on standard NGM plates with OP50. For all
762 experiments, animals were grown in 20x concentrated HT115 bacteria seeded on 6cm
763 RNAi plates. Details on the strains, mutant alleles, and transgenic animals used in this
764 study are listed in the Key Resources Table.

765 **Generation of transgenic strains**

766 To assemble tissue-specific constructs for increased *hpk-1* expression, the *hpk-1* cDNA
767 was amplified and cloned under control of the following promoters: hypodermal *dpy-7p*
768 (Gilleard et al., 1997), body wall muscle *myo-3p* (Okkema et al., 1993), pan-neuronal *rab-*
769 *3p* (Nonet et al., 1997), dopaminergic neurons *cat-2p* (Lints and Emmons, 1999),
770 glutamatergic neurons *eat-4p* (Lee et al., 1999), serotonergic neurons *tph-1p* (Sze et al.,
771 2000), and g-aminobutyric acid (GABA)ergic neurons *unc-47p* (Gendrel et al., 2016). In
772 brief, promoter sequences were subcloned from existing plasmids into the *Prab-3p::hpk-*
773 *1* plasmid. To create kinase domain point mutations *K176A* and *D272N*, the *Prab-3p::hpk-*
774 *1* plasmid was used and mutations were performed with a QuikChange II XL Site-Directed
775 Mutagenesis Kit (Agilent).

776 All the assembled plasmids were validated by sequencing prior to microinjection. These
777 constructs were injected at 5 ng/ml together with *myo-2p::mCherry* at 5 ng/ml as co-
778 injection marker and *pBlueScript* at 90ng/ml as DNA carrier.

779 **RNAi feeding**

780 The *hpk-1* RNAi clone was originated from early copies of *E. coli* glycerol stocks of the
781 comprehensive RNAi libraries generated in the Ahringer and Vidal laboratories. The
782 control empty vector (L4440) and *hpk-1* RNAi colonies were grown overnight in Luria
783 broth with 50 µg ml–1 ampicillin and then seeded onto 6cm RNAi agar plates containing
784 5 mM isopropylthiogalactoside (IPTG) to induce dsRNA expression overnight at room
785 temperature. RNAi clones used in this study were verified by DNA sequencing and
786 subsequent BLAST analysis to confirm their identity.

787 **RT-qPCR analysis**

788 For measurement of gene induction, animals were synchronized and grown at 20°C, then
789 isolated at the specified age. For time course of *hpk-1* mRNA upregulation, animals were
790 harvested at specified time points (D2, D4, D6, D8 and D10). For *hpk-1* mRNA regulation
791 at D11 of adulthood, animals were synchronized, grown at 20°C and harvested at D1 and
792 D11 of adulthood. For autophagy genes upregulation, animals were synchronized, grown
793 at 20°C and harvested at D1 of adulthood. After harvesting animals, RNA extraction was
794 followed by using TRIzol reagent (Life Technologies) followed by RNeasy Plus Mini Kit
795 (Qiagen). RNA concentration was measured using a Nanodrop, and RNA preparations
796 were reverse transcribed into cDNA using the BioRad cDNA synthesis kit (#1708890) as
797 per manufacturer's protocol. Quantitative real time PCR was performed using PerfeCTa
798 SYBR green FastMix (Quantabio) with three technical replicates for each condition.

799 Primer sets with at least one primer spanning the exon were used to amplify the gene of
800 interest. *cdc-42* mRNA levels were used for normalization. Fold change in mRNA levels
801 was determined using ΔΔ Ct method (Livak and Schmittgen, 2001). Primer sequences
802 can be found in the Key Resources Table.

803 **Lifespan analysis**

804 Traditional lifespan assays were performed essentially as described in: (Das et al., 2017;
805 Lee et al., 2003). Briefly, animals were synchronized by egg prep bleaching followed by
806 hatching in M9 solution at 20°C overnight. The L1 animals were seeded onto 6cm plates
807 with HT115 bacteria and allowed to develop at 20°C. At L4 stage, FUdR was added to a
808 final concentration of 400 μM. Viability was scored every day or every other day as
809 indicated in each figure. Prism 7 was used to for statistical analyses and the *P* values
810 were calculated using log-rank (Mantel-Cox) method.

811 For lifespan with RNAi-mediated knockdown of TOR (*Figure 8--figure supplement*
812 *2*), animals for the *let-363* (TOR RNAi) condition were first raised on empty vector RNAi
813 during development, and transferred to plates with *let-363* RNAi bacteria at L4 to avoid
814 developmental arrest at L3. In addition to having IPTG present in the media of the RNAi
815 plates, an additional 200uL of 0.2 M IPTG was added directly to the bacterial lawn of all
816 plates and allowed to dry immediately before adding animals. The assays were otherwise
817 performed as just described.

818 For lifespan assays in *Figure 1F*, assay plates and animal populations were
819 prepared as just described, and then lifespan was determined from analysis of time-series
820 image data collected for a given animal at approximately hour intervals throughout adult

821 life on modified Epson V800 flatbed scanners with our instance of the *C. elegans* "lifespan
822 machine" (Oswal et al., 2021; Stroustrup et al., 2013).
823 In all cases: scoring of viability was blinded with respect to genotype and values
824 obtained at previous timepoints, and animals that died due to rupturing, desiccation on
825 the side of the plate or well, or clear body morphology defects were censored.
826 **Thermotolerance assay.**
827 Survival assays at high temperature were conducted as previously described in (Johnson
828 et al., 2014). In brief, synchronized L1 animals were allowed to develop at 20°C and FUdR
829 was added at the L4 stage. At day 1 adulthood, animals were moved to 35°C for a period
830 of 6 and 8 hours. Animals were allowed to recover for 2 hours at 20°C, and viability was
831 scored. In all cases, scoring of viability was blinded with respect to genotype and values
832 obtained at previous timepoints; animals that died due to rupturing, desiccation on the
833 side of the plate or well, or had clear body morphology defects were censored. Statistical
834 testing between pairs of conditions for differences in the number of foci was performed
835 using student's *t* test analysis.
836 **Induction of *hsp-16.2p*::*GFP* after heat shock.**
837 The *hsp-16.2p*::*GFP* animals were heat shocked on day 1 of adulthood for 1 hour at 35°C
838 and imaged after 4 hours of recovery. Images were acquired using a Zeiss Axio Imager
839 M2m microscope with AxioVision v4.8.2.0 software. The GFP a.u. values from the head
840 and intestine of the animals were acquired using the area selection feature from
841 AxioVision software. Two independent trials with a minimum of 20 animals per experiment
842 were performed.
843 **Measurement of autophagosome formation using the *GFP::LGG-1/Atg8* reporter.**

844 *GFP::LGG-1/Atg8* foci formation was visualized as described in (Kumsta et al., 2017).
845 Briefly, L4 stage and day 1 adult animals were raised on HT115 bacteria at 20°C and
846 imaged using a Zeiss Axio Imager M2m microscope with AxioVision v4.8.2.0 software at
847 63X magnification. Two independent trials where at least 20 seam cells from 15-20
848 different animals were scored for *GFP::LGG-1* puncta accumulation. In all cases scoring
849 of puncta was blinded with respect to genotype.

850 **Measurement of neurodegeneration in motor neurons.**

851 Animals carrying the *unc-25p::GFP* reporter strain to visualize VD and DD motor neurons
852 were synchronized and allowed to developed at 20°C and FUdR was added at L4 stage.
853 At L4, D2 and D9 of adulthood, animals were imaged in a Zeiss Axio Imager M2m
854 microscope and the number of animals showing axonal breaks in the VD and DD motor
855 neurons were scored. Axonal breaks were defined as an area of discontinued
856 fluorescence in either the ventral or dorsal nerve cords.

857 **Polyglutamine aggregation in neurons and locomotion analyses.**

858 The visualization and quantification of the progressive decline in proteostasis in the
859 nervous system (specifically the nerve ring) was performed as described in (Brignull et
860 al., 2006; Lazaro-Pena et al., 2021). In brief, synchronized *rgef-1p*::*polyQ40*::*YFP* L4
861 animals were treated with FUdR. Then, z-stack images from the nerve ring were acquired
862 using a Zeiss Axio Imager M2m microscope with AxioVision v4.8.2.0 software at 63X
863 magnification. The fluorescent foci form compressed *z*-stack images from 20 animals per
864 technical replicate were scored blind every other day from four to eight days of adulthood.

865 To assess locomotion capacity, at day two of adulthood, 40 animals were
866 transferred to a drop of 10ul of M9 solution and the number of body bends performed in

867 a period of 30 seconds from each animal was scored (as described in (Lazaro-Pena et
868 al., 2021)). Statistical testing between pairs of conditions for differences in the number of
869 foci was performed using student's *t* test analysis.

870 **Polyglutamine aggregation in muscle and paralysis analyses.**

871 The visualization and quantification of the progressive decline in proteostasis in muscle
872 tissue was performed as described in (Lazaro-Pena et al., 2021; Morley et al., 2002).
873 Briefly, synchronized *unc-54p*::*Q35*::*YFP* L4 animals were treated with FUdR. Then,
874 fluorescent foci from 20 animals per technical replicate were scored blind daily from days
875 one to three of adulthood. To assess paralysis, at days 5, 7, 9, and 11 of adulthood,
876 prodded animals that responded with head movement (and were therefore still alive) but
877 no backward or repulsive motion were scored as paralyzed (as described in (Lazaro-Pena
878 et al., 2021)). Statistical testing between pairs of conditions for differences in the number
879 of foci was performed using student's *t* test analysis. Two to five independent transgenics
880 lines were tested.

881 **Aldicarb assay.**

882 The aldicarb assay was performed as described in (Mahoney et al., 2006; Oh and Kim,
883 2017). Plates containing Aldicarb 1mM were prepared the day before the assay and
884 stored at 4°C. Animals were synchronized by picking at L4 stage and placing them in a
885 plate with HT115 bacteria. After 24 hours, one aldicarb plate per strain were brought out
886 of 4°C and a small drop of OP50 was placed in the center of the plate a let dry for 30
887 minutes. Approximately 25 animals were placed on each plate, and the number of
888 paralyzed animals were scored every hour up to 5 hours. The assay was performed at
889 room temperature.

890 **Exogenous 5-HT immobilization assay.**

891 Animals were synchronized by picking at L4 stage and placing them in a plate with HT115
892 bacteria. After 24 hours, serotonin (5-HT) was dissolved in M9 buffer to a 20mM
893 concentration (Hart, 2006). Twenty worms were placed on a glass plate well with 400ul
894 of 20mM serotonin for 10 minutes. The same number of control animals were placed in
895 400ul of M9 buffer. The locomotion of worms (mobilize or immobilized) was annotated at
896 the following time points: 2, 4, 6, 8, and 10 minutes. The assay was performed at room
897 temperature.

898 **Sample preparation and sequencing for RNA-Seq.**

899 10 cm RNAi plates were seeded with 1 mL of 10x concentrated HT115 EV bacteria from
900 an overnight culture, and allowed to dry for 1-2 days. N2 or *hpk-1(pk1393)* Approximately
901 1,000 synchronized L1 animals were added to each plate (~3000 animals per condition
902 across multiple plates). At the L4 stage, 600 µL of 4 mg/mL FUdR was added. Animals
903 were kept at 20 °C for the duration of the experiment.

904 Day 2 adult animals were collected in M9 buffer, washed 2x in M9, and a final wash
905 with DEPC-treated RNase-free water. Approximately two times the volume of the animal
906 pellet of TRIzol® reagent was added to each animal preparation, followed by brief mixing
907 and freezing overnight at -80 °C. Tubes were then allowed to partially thaw, and were
908 vortexed for five minutes to assist with disrupting the cuticle. Samples were transferred
909 to new tubes, and 200 µL of chloroform per 1 mL of TRIzol was added, followed by 20s
910 vortexing; the tubes settled at RT for 10 minutes. Supernatants were transferred to new
911 tubes, and an equal amount of 100% ethanol was added and mixed before proceeding to
912 column purification with the Qiagen RNAeasy Mini kit according the manufacturer's

913 instructions. Samples were eluted with 30 – 50 µL of DEPC-treated water, and checked
914 for initial concentration and quality with a Nanodrop ND-1000 spectrophotometer.
915 Biological replicate samples were prepared from independently synchronized populations
916 of animals.

917 Isolated RNA was provided to the University of Rochester Genomics Research
918 Center for library preparation and sequencing. Prior to library preparation, RNA integrity
919 of all samples was confirmed on an Agilent 2100 Bioanalyzer. Libraries for sequencing
920 were prepared with the Illumina TruSeq Stranded mRNA kit, according to the
921 manufacturer's instructions. Quality of the resulting libraries was checked again with a
922 Bioanalyzer prior to sequencing to ensure integrity. Sequencing was performed on an
923 Illumina HiSeq2500 v4, yielding an average of approximately 31 million single-ended 100
924 bp reads per sample. Quality of the output was summarized with FastQC (Andrews, 2010)
925 and reads were trimmed and filtered with Trimmomatic to remove adapter sequence and
926 any low-quality content occasionally observed toward the ends of reads (Bolger et al.,
927 2014). After filtering out low-quality reads, an average of 30 million reads per sample
928 remained, and were used for the rest of the analysis.

929 **Analysis of *hpk-1* null and wild-type control RNA-Seq dataset.**

930 RNA-Seq reads were aligned to the *C. elegans* genome (assembly WBcel235) with STAR
931 2.4.2a (Dobin et al., 2013) using gene annotation from Ensembl (version 82)
932 (Cunningham et al., 2022). An average of 90.2% of reads per sample were uniquely and
933 unambiguously mapped to the genome. These alignments were used as input to
934 featureCounts (version 1.4.6-p5) for gene-level counts (Liao et al., 2014). Ambiguous or
935 multi-mapping reads, comprising an average of approximately 9% of the reads per

936 sample, were not included in the gene-level count results. Transcript-level quantification
937 was also performed with RSEM (Li and Dewey, 2011) to obtain TPM (Transcripts Per
938 Million) expression estimates.

939 Further analysis was performed primarily in the R statistical software environment
940 (version 4.0.2) (Team, 2013), utilizing custom scripts and incorporating packages from
941 Bioconductor. Both count and TPM expression matrices were filtered to remove genes
942 with low or no expression, keeping genes with at least 10 read counts in at least one
943 sample. Genes were further filtered to keep only those annotated as protein coding,
944 pseudogene, ncRNA, lincRNA, antisense, or snRNA, which could have been included in
945 the sequenced libraries after poly-A selection; 96.3% of genes included in the final set
946 are protein-coding. Gene identifiers have been updated as necessary from newer
947 versions of WormBase (WS284) for integration with recent resources and datasets.

948 Differential expression analysis was performed with DESeq2, and the optional fold-
949 change shrinkage procedure was applied to moderate fold-changes for low-expression
950 genes (Love et al., 2014). Genes significantly differentially expressed between *hpk-*
951 *1(pk1393)* and N2 animals were taken as those with FDR-adjusted p-value less than 0.05
952 and absolute value of $\log_2$ foldchange of at least 1 (on a linear scale, down- or up-
953 regulated by at least two-fold). Functional and over-representation analysis for
954 differentially expressed genes was performed with GOSeq (Young et al., 2010)using gene
955 sets and pathways from the Gene Ontology (Harris et al., 2004), KEGG (Kanehisa and
956 Goto, 2000), and Reactome (Jassal et al., 2020).

957 **Integration with public tissue-specific and single-cell RNA-Seq datasets.**

958 <u>*C. elegans* Aging Atlas and CenGen single-cell datasets</u>

959 We obtained differential expression results for old (days 8, 11, 15) vs young (days 1, 3,
960 5) animals across 200 cell clusters from the Aging Atlas single-cell RNA-Seq project
961 (Roux et al., 2022). Significantly differentially expressed genes within each cluster were
962 taken as those with adjusted p-value < 0.05, and $\log_2$ fold-change magnitude of at least
963 0.5 (approximately 1.4 fold up- or down-regulated on a linear scale). Additional neuron
964 identity information was obtained by mapping Aging Atlas neuron clusters to clusters
965 identified in late-larval stage animals in the CeNGEN single-cell neuronal transcriptome
966 project (Taylor et al., 2021), assisted by correlations between clusters of the two datasets
967 already provided with the Aging Atlas. Chemical signaling subtypes of neurons were
968 determined by expression of marker genes commonly used in the field for fluorescent
969 labeling of neurons: *tph-1* for serotonergic, *unc-47* for GABAergic, *eat-4* for glutamatergic,
970 *cat-2* for dopaminergic, *cat-2* for cholinergic. Where a high correlation had been
971 established between a cluster in the Aging Atlas and CeNGEN, a signaling type was
972 assigned if the cluster expressed the associated marker gene at TPM greater than 75
973 based on the provided pre-normalized expression matrix available from the CeNGEN
974 website. Ten Aging Atlas neuron clusters did not have an obvious corresponding cluster
975 in the CeNGEN dataset; for these clusters neuron signaling class was based on marker
976 genes having average cluster counts > 0.5 across all cells in a cluster and, where
977 possible, a consensus of the six Aging Atlas timepoints.

978 Normalized single-cell aging time-course gene expression was obtained by
979 processing raw counts in the H5AD file from the Aging Atlas resource website
980 (http://c.elegans.aging.atlas.research.calicolabs.com/data). Briefly, the H5AD data from
981 SCANPy was read in and converted into a Seurat object with MuDataSeurat

982 (https://github.com/PMBio/MuDataSeurat) (Hao et al., 2021). SCTransform, as
983 implemented in Seurat, was used to normalize raw counts for library size differences,
984 yielding log-scale normalized expression values for each cell that were used for further
985 analysis.

986 Genes uniquely expressed in neurons, or significantly enriched compared to other
987 tissues were obtained from tissue-specific bulk RNA-Seq reported by (Kaletsky et al.,
988 2018) due to the additional dynamic range provided over current single-cell sequencing
989 technology.

990 *C. elegans* kinome, transcription factors, neuronal genome, and longevity-associated
991 genes

992 A collection of *C. elegans* kinases was assembled based on Zaru *et al.* 2017 (Zaru et al.,
993 2017) and cross-referenced against current gene annotation in WormBase version
994 WS284 (Harris et al., 2020) to keep only protein-coding genes and remove pseudogenes
995 or "dead" genes. This produced a list of 438 kinases in the *C. elegans* kinome (*Figure 2-*
996 *-figure supplement 4, Supplementary File 5*). *C. elegans* transcription factors were
997 identified from a previously published compendium (Nye et al., 1989). Genes with known
998 or predicted functions important to neurons, and their associated roles, were assembled
999 from tables curated in (Hobert, 2013). Genes with lifespan phenotypes primarily from
1000 perturbation studies in *C. elegans* utilizing RNAi or mutant strains were assembled from
1001 annotation in WormBase (WS282) as well as the GenAge database (de Magalhaes and
1002 Toussaint, 2004).

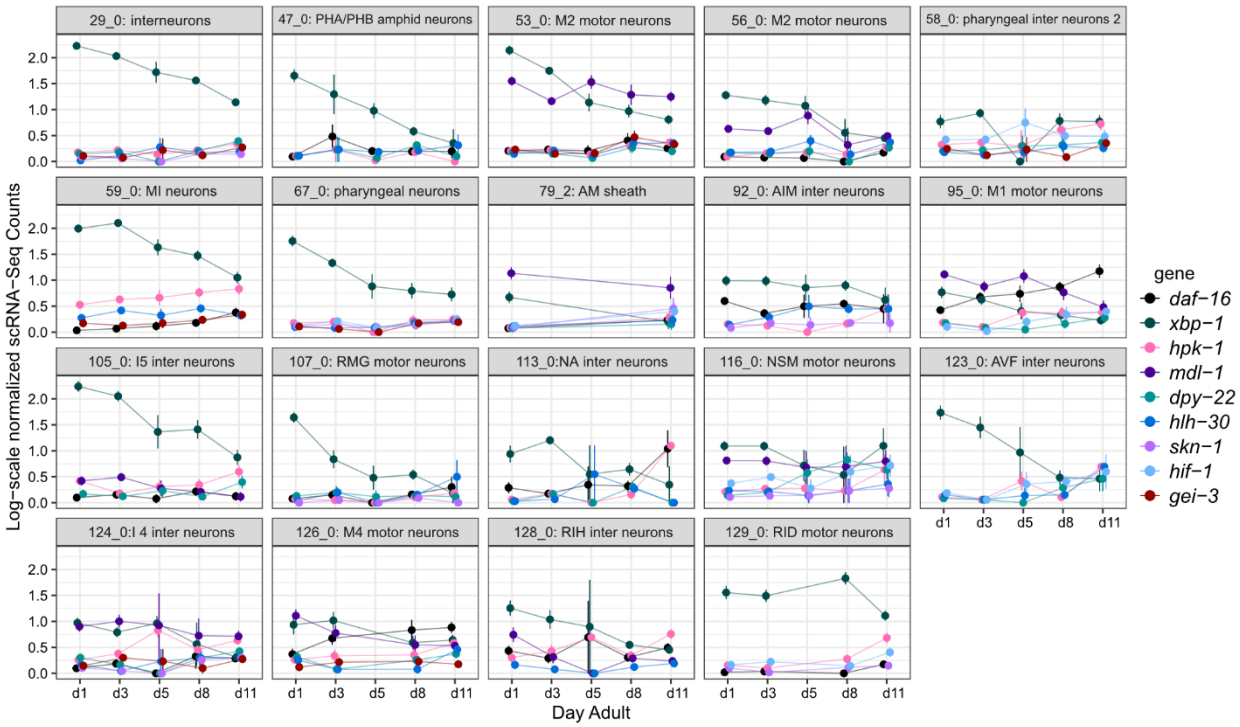
1003 **Statistical analysis for *in vivo* assays.**

1004 Unless otherwise specified, GraphPad PRISM version 7 software was used for statistical
1005 analyses. Data were considered statistically significant when *P* value was lower than 0.05.
1006 In figures, asterisks denote statistical significance as (*, $P < 0.05$, **, $P < 0.001$, ****, $P <$
1007 0.0001) as compared to appropriate controls. N number listed in figure legend indicates
1008 the number of one representative experiment among all biological trials.

1009 **Appendices.**

1010 **Appendix 1.** While single-cell RNA-Seq is a powerful approach already, well-documented
1011 challenges remain with respect to consistency in cell captures and differentiating
1012 biological noise from technical artifacts such as “drop-outs” from low numbers of average
1013 reads per cell (Hwang et al., 2018, 2021; Ke et al., 2022; Nayak and Hasija, 2021)(refs).
1014 We attempted to identify gene-gene correlations within cell clusters across individual ages
1015 based on the *C. elegans* Aging Atlas dataset and found that inconsistency in the number
1016 of cells per cluster across timepoints limits our ability to identify many robust and
1017 significant associations- such variability is likely technical in nature, as it is not globally
1018 consistent across cell clusters for a given timepoint. In most cases, when aggregated into
1019 “young” and “old” sample groups, as was done for differential expression analysis in the
1020 original Aging Atlas analysis (Roux et al., 2022) the groups represent comparable
1021 numbers of total cells for most clusters. The trends we have observed at the level of
1022 individual time-points indicate that the age-associated up-regulation of *hpk-1* does not
1023 always occur at the same time point across cell clusters and is not necessarily tightly
1024 linked to the upregulation of longevity-associated transcription factors in the same cluster
1025 (*Appendix 1 Figure 1*). Thus, the age-associated upregulation of pro-longevity factors in
1026 many cell clusters may not be due to a highly coordinated regulatory process, but rather

a distributed response to accumulating local stress or damage. More work will be necessary to resolve if such late-life expression changes indeed contribute to maintenance of wild-type lifespan and healthspan, and the mechanism of the changes thereof (e.g., increased transcription, down-regulation of miRNA targeting, epigenetic changes) and the factors that mediate these changes.



Appendix 1--Figure 1. *hpk-1* and longevity-associated transcription factors are up-regulated in overlapping cell-clusters, but timing of the age-associated changes varies between cell clusters.
Per-age expression summary for cell clusters with four or more differentially expressed longevity-associated transcription factors, of the 48 cell clusters in which *hpk-1* is upregulated in old vs young animals. Each sub-plot shows log-scale normalized scRNA-Seq expression (mean and SEM) for the indicated cluster. Only genes which were significantly differentially expressed in the cluster are shown (old vs young animals, up- or down-regulated). Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022).

Appendix 2. Loss of *hpk-1* upregulates expression of a number of neuronal genes including those involved in: neuropeptide signaling, neurotransmission, release of

1046 synaptic vesicles, calcium homeostasis, and function of sensory neurons. In addition to
1047 upregulation of *ins* neuropeptides, 12 FMRFamide-related (flp) peptides, 19
1048 neuropeptide-like proteins (*nlp*), nine neuropeptide maturation genes (e.g., *egl-3*
1049 (proprotein convertase subtilisin/kexin type 2) and *egl-21*(carboxypeptidase E)), and nine
1050 putative GPCR neuropeptide receptors were also significantly upregulated in *hpk-1* null
1051 mutants. Neurotransmitters are signaling molecules that trigger a response by binding to
1052 ionotropic or metabotropic neurotransmitter receptors in recipient neurons or tissues; we
1053 find that mainly the latter are upregulated in *hpk-1* null mutant animals, including:
1054 acetylcholine (*gar-1, gar-2* and *gar-3*), GABA (*gbb-2*) and glutamate (*mgl-3* and
1055 uncharacterized *C30A5.10*). In total nine metabotropic and two ionotropic glutamate
1056 receptors, 14 vesicular glutamate transport family genes, nine acetylcholine receptor
1057 ligand-gated ion channels, and nine GABA receptor ligand-gated ion channels were
1058 upregulated. Additionally, *ace-2*, *ace-3* and *cha-1*, involved in acetylcholine degradation
1059 and synthesis (respectively), were upregulated. *comt-4*, a catechol-O-methyltransferase
1060 expected to be involved in the degradation of dopamine, was significantly down-
1061 regulated.

1062 Synaptic transmission, the formation and release of synaptic vesicles, carry
1063 neurotransmitters and rely on proteins necessary for docking, priming and fusing to the
1064 axon terminal plasma membrane, which releases cargo into the synaptic cleft.
1065 Interestingly, loss of *hpk-1* causes the upregulation of synaptic proteins such as *unc-13*,
1066 *unc-10* and *rab-3*, which are essential for vesicle priming, recruitment and sequestration
1067 of vesicles, respectively. The process of vesicle release is highly dependent on a Ca^{2+}
1068 signal detected by synaptotagmins proteins, which promotes vesicle fusion to the plasma

1069 membrane (Deak, 2014). In *hpk-1* null mutant animals, we observed upregulation of
1070 synaptotagmin *snt-1*, *snt-3* and *snt-4* Ca^{2+} sensor proteins for vesicle release.

1071 Neuronal function is regulated by changes in calcium levels: a decrease in calcium
1072 uptake reduces neuronal function by a reducing neurotransmitter release (Scriabine and
1073 Kazda, 1989). In contrast, increasing calcium influx triggers activation of proteases and
1074 phospholipases, resulting in cell damage and cell death (Cheung et al., 1986; Siesjo,
1075 1989). Voltage-gated Ca^{2+} channels (VGCCs) are composed of 24 transmembrane
1076 alpha-1 subunits and associate with auxiliary beta-1 and alpha-2-delta subunits. *hpk-1*
1077 null mutant animals have increased expression of two of five alpha-1 subunits (*egl-19* and
1078 *unc-2*), and both alpha-2-delta subunits (*unc-36* and *tag-180*). Previous research
1079 describes the role of *egl-19* voltage-gated Ca^{2+} channel in the induction of the heat stress
1080 response (Silva et al., 2013): moderate upregulation of acetylcholine signaling improves
1081 muscle proteostasis by the activation of HSF-1, triggered by the *egl-19*-dependent Ca^{2+}
1082 influx.

1083 Neuronally expressed cilia are essential for cell motility, sensory perception and
1084 signaling. The assembly and maintenance of sensory cilia depends on IFT: bidirectional
1085 movement of cargo to and from the base and tip of the axoneme. The *C. elegans* IFT
1086 machinery is composed of: anterograde IFT motors, heterotrimeric kinesin-II and
1087 homodimeric OSM-3, retrograde dynein motors; and the IFT-A, -B and BBSome particle
1088 complexes (Hao et al., 2009; Scholey et al., 2004). Loss of *hpk-1* induces the expression
1089 of a high number of IFT machinery components including: *klp-11* (kinesin II motor), *che-*
1090 *3* (dynein motor), three and seven members of the IFT-A and -B complexes (respectively),
1091 and five members of BBSome complex. In mammals, there is a link between primary cilia

signaling and age-related brain disease, including neurodegenerative disorders (Ma et al., 2022).

Data availability.

All data supporting the findings of this study are available within the article, its Supplementary Information files, or the peer-review file. RNA-Seq data for *hpk-1(pk1393)* and N2 is available from GEO, accession GSE220744. Data analysis scripts have been deposited at: https://github.com/samuelsonlab-urmc/hpk1_manuscript_2023.

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1118 does not necessarily represent the official views of the National Institutes of Health.
1119

1120 **Video 1. HPK-1 functions within the nervous system to regulates healthspan.**
1121 Video of wild-type, *hpk-1(pk1393)* null animals, and *hpk-1* null animals with neuronal
1122 expression of *hpk-1* (*rab-3p::HPK-1*). Videos were generated from consecutive images
1123 taken approximately every 15 minutes over the course of the animals lifespan using the
1124 Lifespan Machine method (Stroustrup et al., 2013).

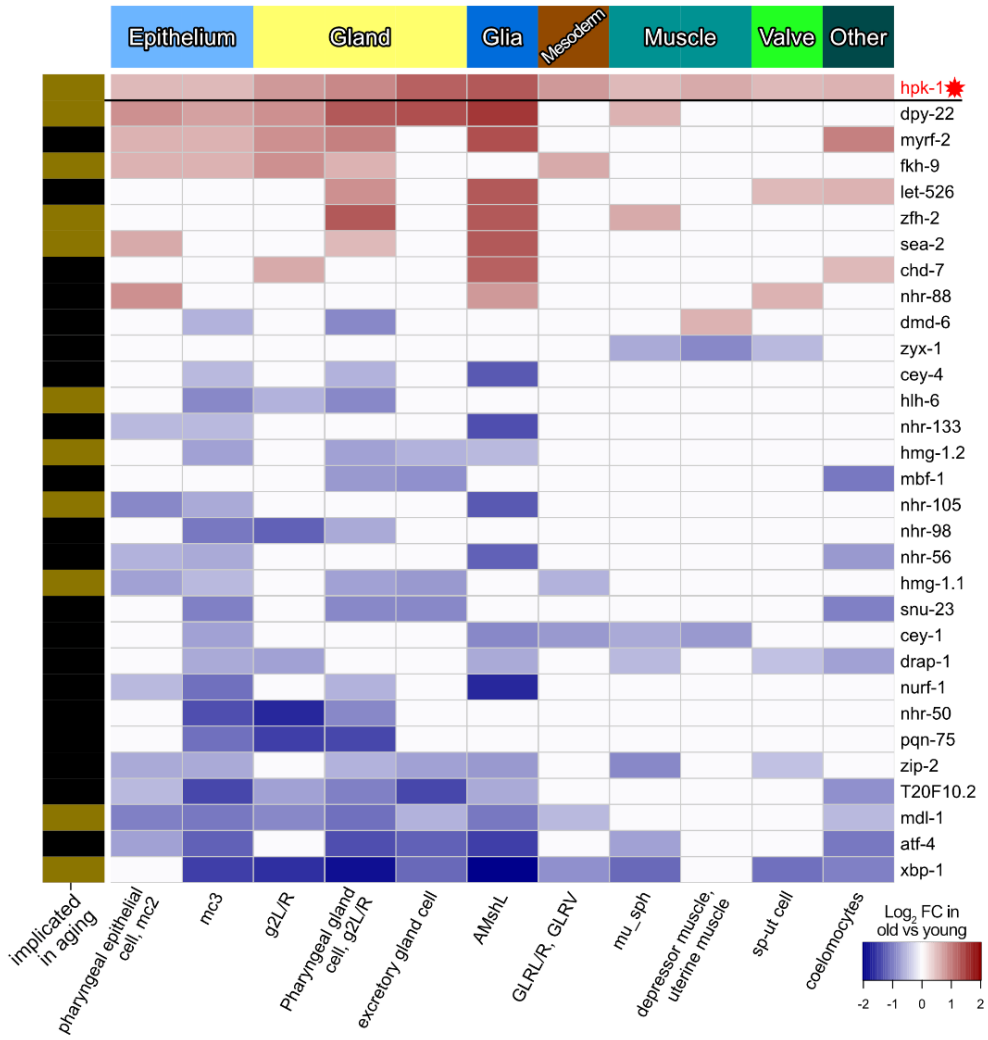

Figure 2--Figure Supplement 1. During normal aging *hpk-1* is differentially expressed along with transcription factors in cell clusters corresponding to tissues outside of the nervous system.

Heat map of non-neuronal cell clusters in which *hpk-1* expression increases during normal aging along with TFs differentially expressed in 3 or more neuron cell-clusters. Fold-changes of a given TF from old versus young animals are shown, upregulation with aging in red and downregulation with aging in blue. Neuronal types, neurotransmitter subtypes are indicated in the top, names of the cell cluster or individual neuronal pair are given at the bottom, and whether a TF has previously been implicated in aging is indicated by the black and gold on the left side (genes with gold bars have previously published aging-associated phenotypes). Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022) and differential expression with aging was based on adjusted p-values <0.05 and a $\log_2$ FC magnitude of at least 0.5 (between days 8,11,15 and days 1,3,5). Note *hpk-1* is included for reference and is a transcriptional co-factor, but is not a *bona fide* transcription factor. See *Supplementary Files 5 and 6* for dataset.

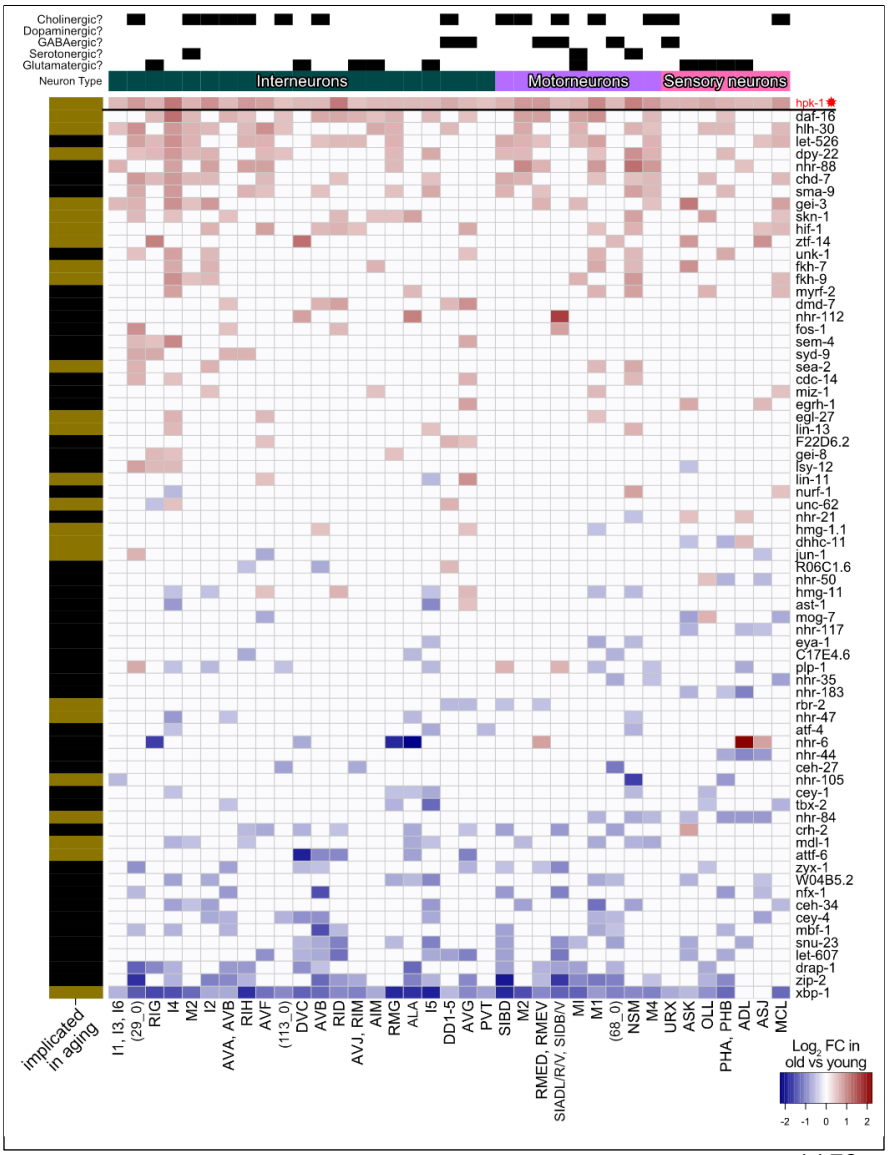

Figure 2--Figure Supplement 2. *Hpk-1* and all transcription factors differentially expressed in overlapping cell clusters within the *C. elegans* nervous system during aging. Heat map of neuronal cell clusters in which *hpk-1* expression increases during normal aging along with TFs differentially expressed in 3 or more neuron cell-clusters. Fold-changes of a given TF from old versus young animals are shown, upregulation with aging in red and downregulation with aging in blue. Neuronal types, neurotransmitter subtypes are indicated in the top, names of the cell cluster or individual neuronal pair are given at the bottom, and whether a TF has previously been implicated in aging is indicated by the black and gold on the left side (genes with gold bars have previously published aging-associated phenotypes). Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022) and differential expression with aging was based on adjusted p-values <0.05 and a $\log_2$ FC magnitude of at least 0.5 (between days 8,11,15 and days 1,3,5). Note *hpk-1* is included for reference and is a transcriptional co-factor, but is not a *bona fide* transcription factor. See *Supplementary Files 5 and 6* for dataset.

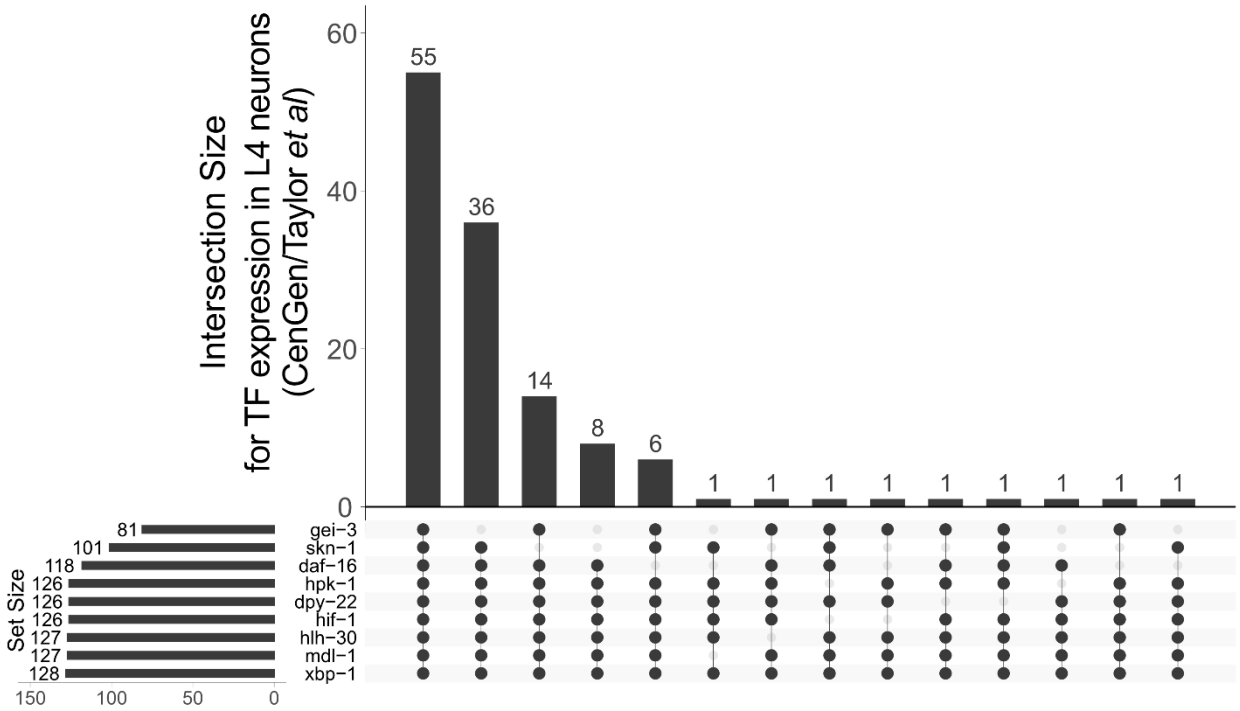

Figure 2--Figure Supplement 3. *hpk-1* is broadly co-expressed with longevity-associated transcription factors in late larval stage animals.
Vertical bars show the number of neurons expressing TFs or *hpk-1* in the intersection indicated by the filled dots. The number of neurons each individual gene is found expressed is given by the horizontal bars, “set size”. Primary data for this analysis was generated in the CenGEN project, expression was determined based on their “medium” stringency threshold of average TPM per neuron type ≥ 0.1 (Taylor et al., 2021).

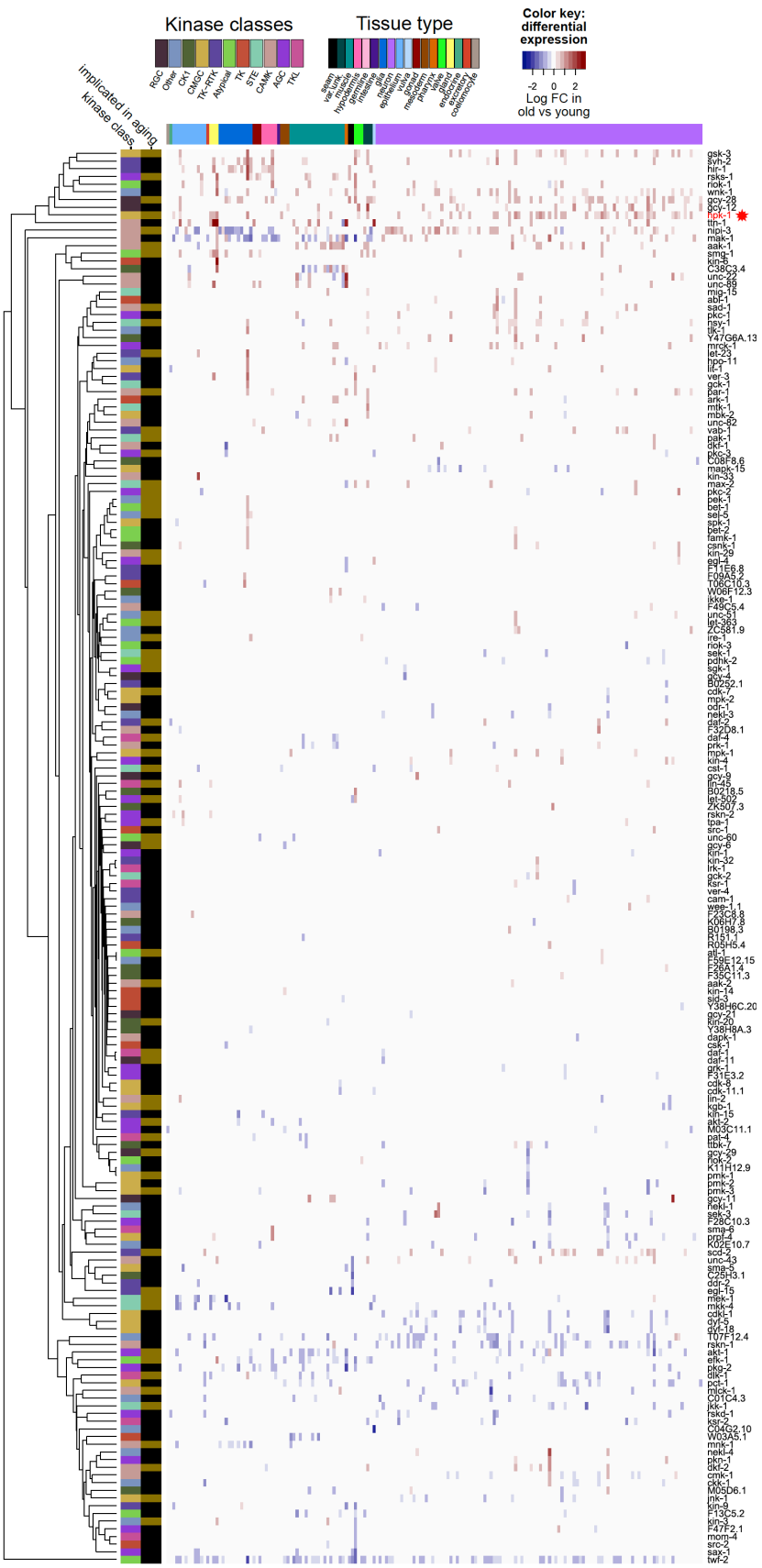

Figure 2--Figure Supplement 4. Only a limited subset of kinases change expression during normal aging.
Heat map of 184 kinases that change expression during normal aging in any cell cluster in the aging atlas dataset, of the 438 total kinases identified in *C. elegans*. Increased (red) and decreased (blue) expression with aging of a given kinase is shown. Major tissue types, kinase classes, as well as whether a kinase has previously been implicated in aging is indicated in the top and side bars, respectively. Primary data for this analysis was generated in the *C. elegans* Aging Atlas (Roux et al., 2022) and differential expression with aging was based on adjusted p-values <0.05 and a $\log_2$ FC magnitude of at least 0.5 (between days 8,11,15 and days 1,3,5). See *Supplementary Files 5 and 6* for dataset.

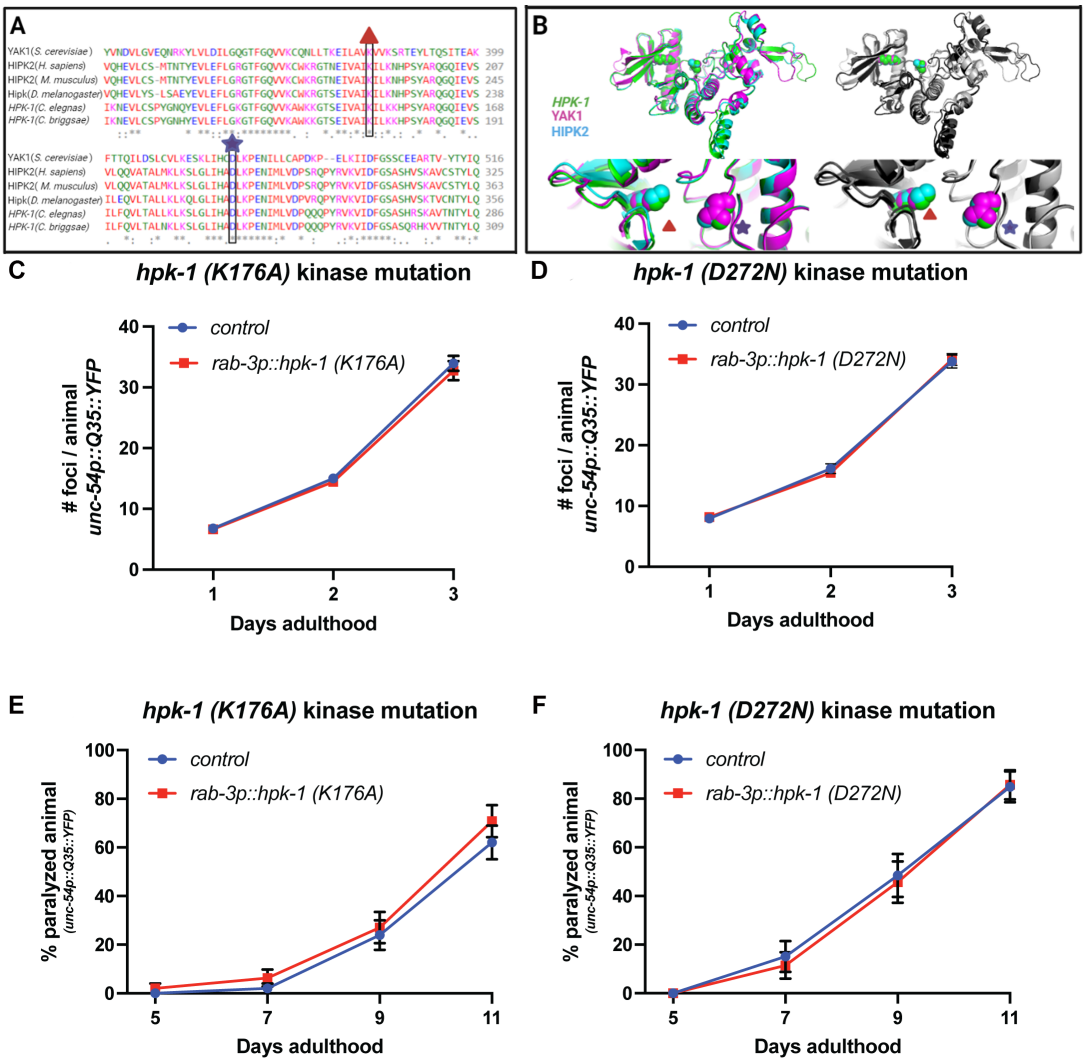

Figure 5--Figure Supplement 1. HPK-1 kinase activity is necessary to improve proteostasis.

(A) Multiple sequence alignment of the HPK-1 kinase domain across evolutionarily distant species. Alignment was conducted using Clustal Omega (Sievers et al., 2011), with sequence IDs: HIPK2 (NP_073577.3), HPK-1 (F20B6.8a), HIPK2 *(M. musculus*, NP_001281073.1), HIPK (*D. melanogaster*, CG17090), HPK-1 (*C. briggsae*, CBP26904), and Yak1 (SGDID:S000003677). **(B)** Overlap between the predicted tertiary structures of the kinase domain of *C. elegans* HPK-1 (green), *S. cerevisiae* Yak1 (pink), and *H. sapiens* HIPK2 (blue). The K176A and D272N mutations are indicated by a red triangle and purple star, respectively, falling within the catalytic domain (He et al., 2010). Predicted structures for these genes were downloaded from AlphaFold (Jumper et al., 2021; Varadi et al., 2022): HPK-1(Q8MQ70 HIPK_CAEEL), Yak1 (A0A7C8V8P7_ORBOL), (H7BXX9_HUMAN). Highly conserved regions observed using multiple sequence alignment where then aligned in 3D using PyMol alignment tool (DeLano, 2002). **(C-F)** Quantification of foci in muscle (C, D) and paralysis rate (E, F) of transgenic animals with neuronal overexpression of *hpk-1(K176A)* (C, E) and *hpk-1(D272N)* (D, F), respectively. (n≥20 for C, n≥20 for D, n≥48 for E and n≥30 for

1247 F). *T* test analysis was performed between transgenic and non-transgenic animals at every
1248 time point. See *Supplementary Files 12 and 13* for details.

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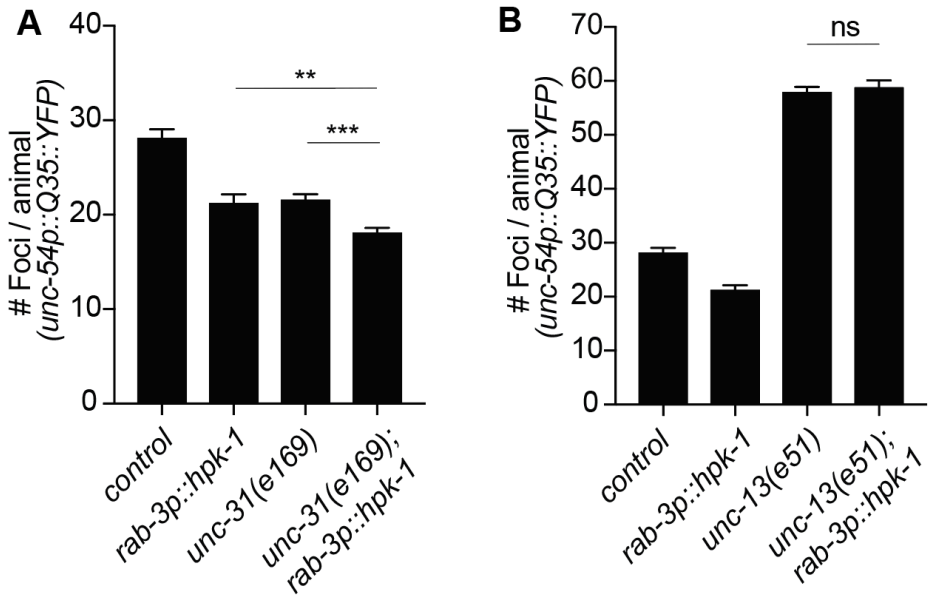

1250
1251
1252 **Figure 5--Figure Supplement 2. HPK-1 cell-nonautonomous regulation of**
1253 **proteostasis depends on neurotransmitter signaling.**
1254 (**A)** Quantification of *polyQ* foci in muscle of wild type and neuropeptide secretion
1255 mutants, *unc-31(e169)* (n≥21), **(B)** Quantification of foci in muscle of wild type and
1256 neurotransmitters secretion mutants, *unc-13(e51)* (n≥21). *T* test analysis with ***P* < 0.01
1257 and ****P* < 0.001. See *Supplementary File 12* for details.

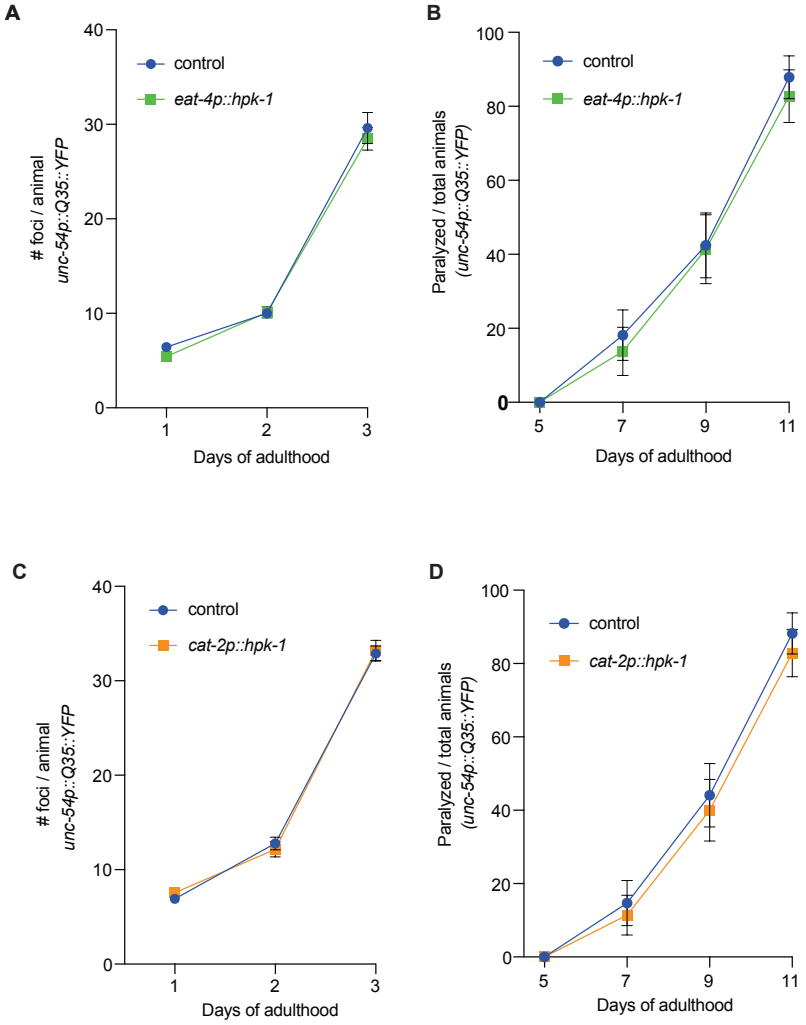

Figure 7--Figure Supplement 1. *Hpk-1* expression in glutamatergic and dopaminergic neurons is not sufficient to prevent the decline in proteostasis. **(A** and **B)** Quantification of number of foci (A) and paralysis rate (B) of animals expressing *hpk-1* in the glutamatergic neurons (*eat-4p::hpk-1*; green line) and control non-transgenic siblings (blue line) (n≥18 for A and n≥29 for B). **(C** and **D)** Quantification of number of foci (C) and paralysis rate (D) of animals expressing *hpk-1* in the dopaminergic neurons (*cat-2p::hpk-1;* orange line) and control non-transgenic siblings (blue line) (n≥16 for C and n≥34 for D). See *Supplementary Files 12 and 13* for details and additional trials.

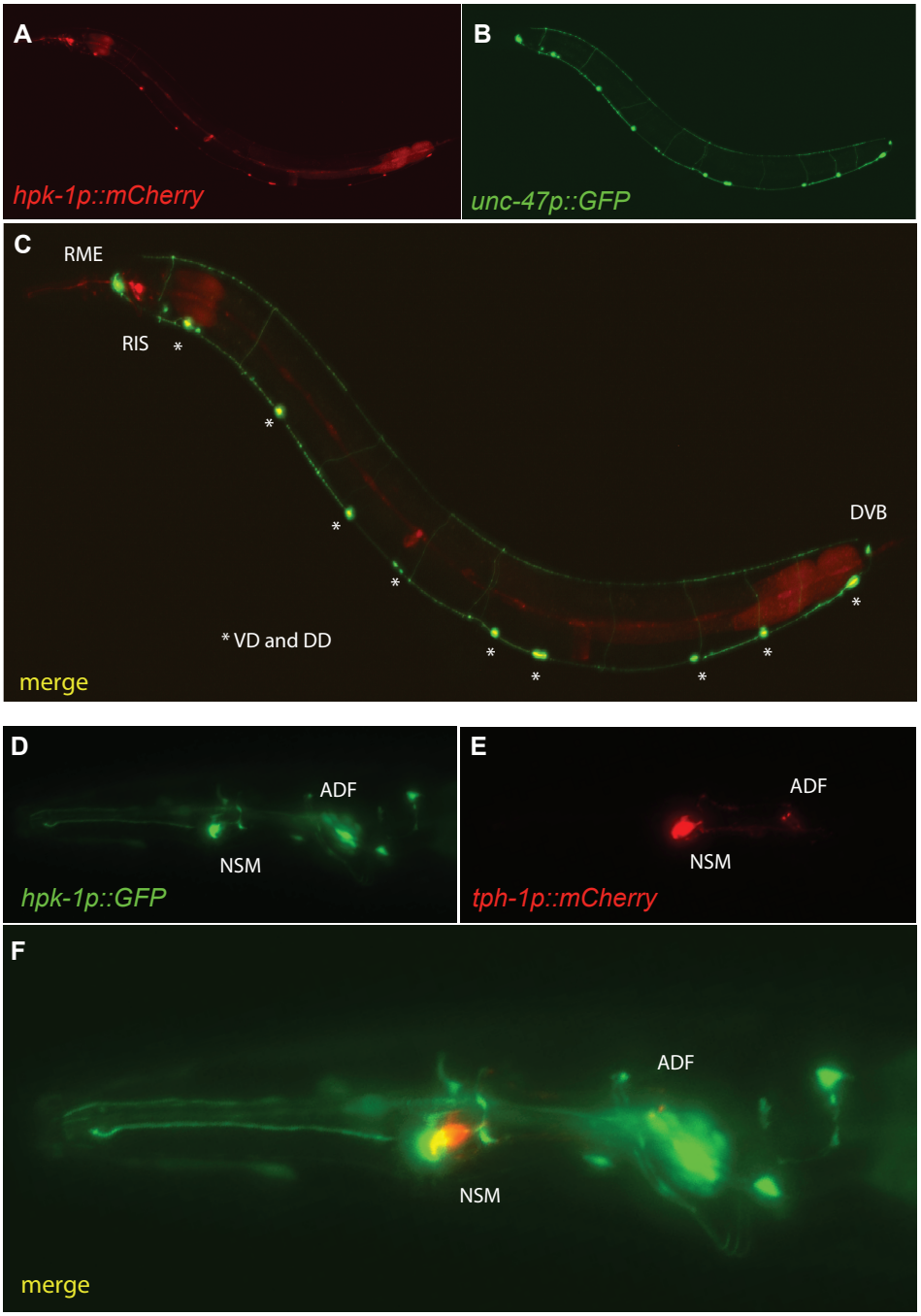

Figure 7--Figure Supplement 2. *Hpk-1* is expressed in GABAergic and serotonergic neurons. Representative fluorescent micrographs of day 1 adult animals co-expressing *hpk-1p::mCherry* and *unc-47p::GFP* **(A-C)**; and *hpk-1p::GFP* and *tph-1p::mCherry* **(D-F)**.

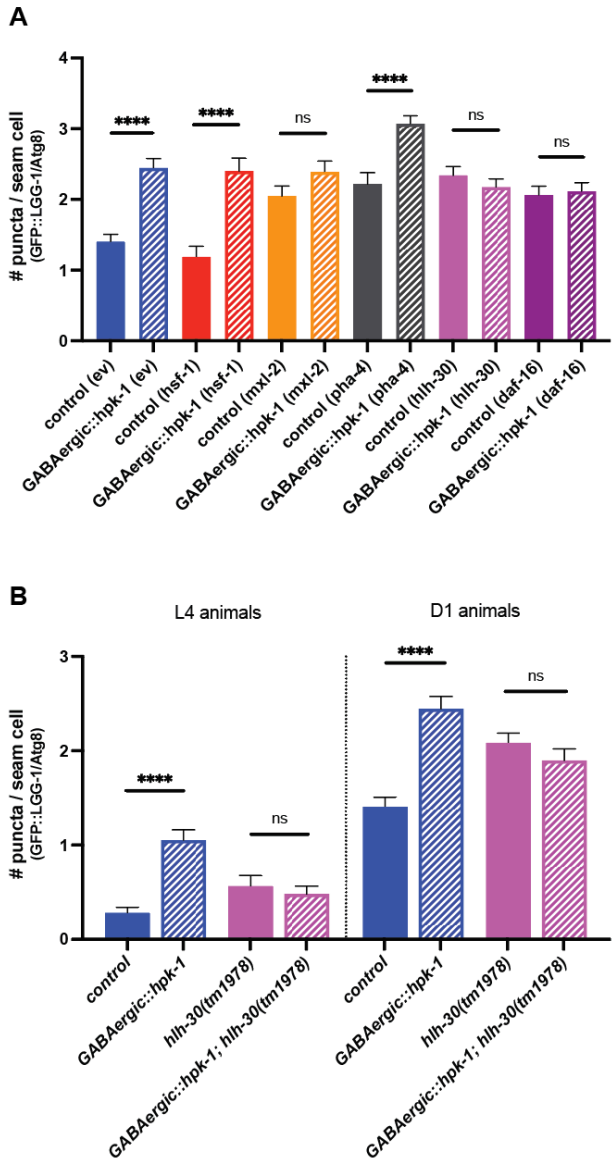

Figure 8--Figure Supplement 1. HLH-30 (TFEB), MXL-2 (Mlx), and DAF-16 (FoxO) are required for HPK-1 mediated induction of autophagy.
(A) Quantification of puncta in seam cells of *unc-47p::hpk-1* and non-transgenic control animals expressing *lgg-1p::GFP::LGG-1/Atg8* after either RNAi treatment with empty vector (ev), *hsf-1*, *mxl-2, pha-4, hlh-30,* or *daf-16* (n≥28). *T* test analysis with ****$P < 0.0001$. **(B)** Quantification of puncta in seam cells of *unc-47p::hpk-1* and non-transgenic control animals expressing *lgg-1p::GFP::LGG-1/Atg8* in otherwise wild-type or *hlh-30(tm1978)* mutant animals at L4 (left) or day 1 of adulthood (right) (n≥57). *T* test analysis with ****$P < 0.0001$. See *Supplementary File 15* for details and additional trials.

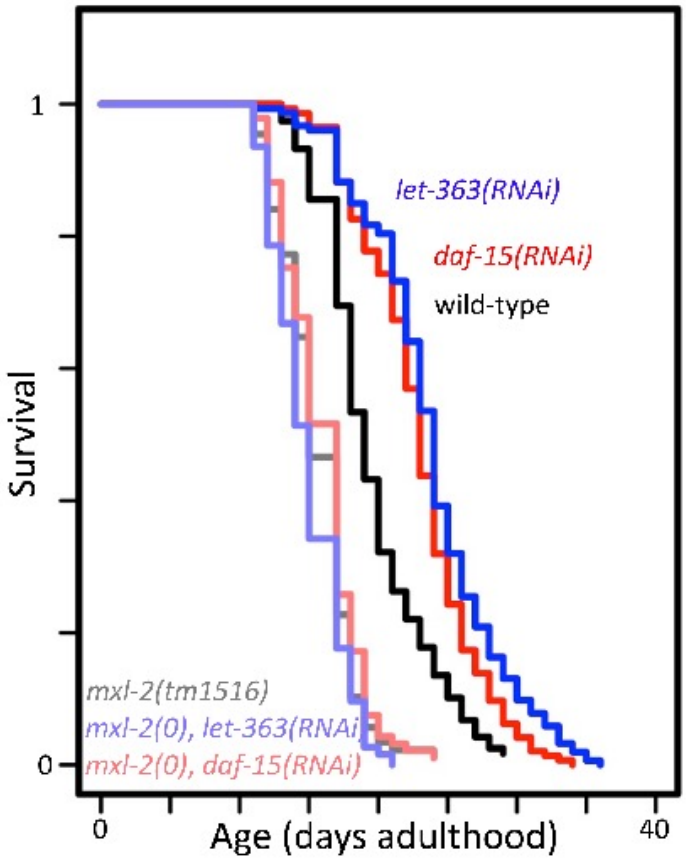

Figure 8--Figure Supplement 2. ***mxl-2*** **is required for decreased TORC1 signaling to increase longevity.**

Lifespan of wild-type (N2, darker shades) or *mxl-2(tm1516)* null mutant animals (lighter shades) treated with either empty vector (black/gray), *let-363(RNAi)* (blues), or *daf-15(RNAi)* (reds). Lifespan graph is representative of one trial with two replicates. Curves were plotted using WormLife (https://github.com/samuelsonlab-urmc/wormlife) (Cornwell et al., 2018). See *Supplementary File 1* for details and additional trial.

Supplementary File 1. ***Lazaro-Pena SF01 Lifespan assays.xlsx.*** Trials, data, strains, genotypes, number of animals, and statistics for lifespan data presented in Figures 1a-d, f, 8b,f, and 8-S2.

Supplementary File 2. ***Lazaro-Pena SF02 Body bends assays.xlsx.*** Experimental data, strains, genotypes, number of animals and statistical analysis for data presented in Figures 1e, and 5c,e.

Supplementary File 3. ***Lazaro-Pena SF03 Thermotolerance assays.xlsx.*** Trials, data, analysis, strains, genotypes, number of animals, and statistics for thermotolerance data presented in Figures 1g, and 8a,e.

Supplementary File 4. ***Lazaro-Pena SF04 RT-qPCR assays.xlsx.*** Trials, data, analysis, strains, genotypes, RNAi conditions, and statistics for RT-qPCR expression analysis presented in Figures 2a,e, and 6l.

Supplementary File 5. ***Lazaro-Pena SF05 TFs DE with aging in hpk-1 DE clusters.xlsx.*** Transcription factors differentially expressed in old vs young animals in the same cell clusters where *hpk-1* has significant expression changes with age in the Aging Atlas dataset. This includes a summary of which clusters exhibit upregulation and which exhibit downregulation for a TF, and if the gene has been previously shown to have aging or lifespan associated phenotypes in RNAi or loss-of-function mutant experiments. Primary data was from the *C. elegans* Aging Atlas, and association to aging phenotypes was determined based on curated annotations from WormBase and GenAge. File is associated with Figures 2, 2-S1, 2-S2, and 2-S4.

Supplementary File 6. ***Lazaro-Pena SF06 C elegans kinome aging DE.xlsx.*** Kinases differentially expressed in old vs young animals out of any of the 200 clusters with DE analysis results from the Aging Atlas dataset. Out of 438 kinases identified in *C. elegans*, 186 have significant age-associated expression changes in at least one Aging Atlas cell cluster. For these kinases, we summarized the number of age-associated up- and down-regulated changes, if they have been previously identified as having aging or lifespan phenotypes in loss-of-function experiments, and the fold-changes in *hpk-1* null vs WT animals for significant changes in our bulk RNA-Seq experiment. Primary data was from the *C. elegans* Aging Atlas, and association to aging phenotypes was determined based on curated annotations from WormBase and GenAge. File is associated with Figures 2, 2-S1, 2-S2, and 2-S4.

Supplementary File 7. ***Lazaro-Pena SF07 Neurodegeneration assay.xlsx.*** Experimental data, strains, genotypes, number of animals/cells, age and statistical analysis for data presented in Figures 3b-d.

Supplementary File 8. ***Lazaro-Pena SF08 Aldicarb assay.xlsx.*** Experimental data, strains, genotypes, number of animals, hours on aldicarb and statistical analysis for data presented in Figure 3e.

Supplementary File 9. ***Lazaro-Pena SF09 Serotonin assay.xlsx.*** Experimental data, strains, genotypes, number of animals, minutes on 5-HT and statistical analysis for data presented in Figure 3f.

Supplementary File 10. ***Lazaro-Pena SF10 hpk-1(0) and N2 D2 RNA-seq.xlsx.*** Differential expression analysis results for RNA-Seq of *hpk-1(pk1393)* null mutant animals compared to wildtype, presented in this manuscript. ***Sheet 1:*** differential expression for all 16,828 genes considered to be expressed in any sample in the experiment. ***Sheet 2:*** the subset of genes with significant expression changes between groups (adjusted p-value < 0.05, log2 fold-change magnitude ≥ 1). Additional columns in both sheets include a summary of the raw and normalized expression for each group, and the human homologs from OrthoList, where applicable. File is associated with Figure 4.

Supplementary File 11. ***Lazaro-Pena SF11 hpk-1 DE genes with neuron-associated function annotations.xlsx.*** Of the genes significantly differentially expressed in *hpk-1(pk1393)* null mutant animals vs wildtype, we found 280 overlap a set of genes with important functions in neurons (Hobert, O. (2018). *The neuronal genome of Caenorhabditis elegans*). For these genes, table includes a summary of their function, the differential expression fold-change and raw expression level in the *hpk-1(pk1393)* vs wildtype RNA-Seq comparison, and the clusters from the Aging Atlas dataset with significant age-associated expression changes (where applicable). File is associated with Figure 4.

Supplementary File 12. ***Lazaro-Pena SF12 Polyglutamine assays.xlsx.*** Trials, data, analysis, strains, genotypes, number of animals, age and statistical analysis of polyglutamine foci formation data presented in Figures 5b,d,g,i,l, 7a,c, 5-S1c,d, -S6a,b, and 7 -S1a,c.

Supplementary File 13. ***Lazaro-Pena SF13 Paralysis assays.xlsx.*** Trials, data, analysis, strains, genotypes, number of animals, age and statistical analysis of data for paralysis onset in animals expressing *polyQ::YFP* presented in Figures 5h,j, 7b,d, 5-S1e,f, and 7-S1b,d.

Supplementary File 14. ***Lazaro-Pena SF14 Induction of molecular chaperone.xlsx.*** Trials, fluorescent quantification, analysis, strains, genotypes, number of animals, heat shock conditions, and statistical analysis of data presented in Figure 6c,e.

Supplementary File 15. ***Lazaro-Pena SF15 Induction of autophagosomes.xlsx.*** Trials, autophagosome quantification, analysis, strains, genotypes, number of cells, age and statistical analysis of data presented in Figures 6g,i,k, 8d,h,l,j, and 8-S1.

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