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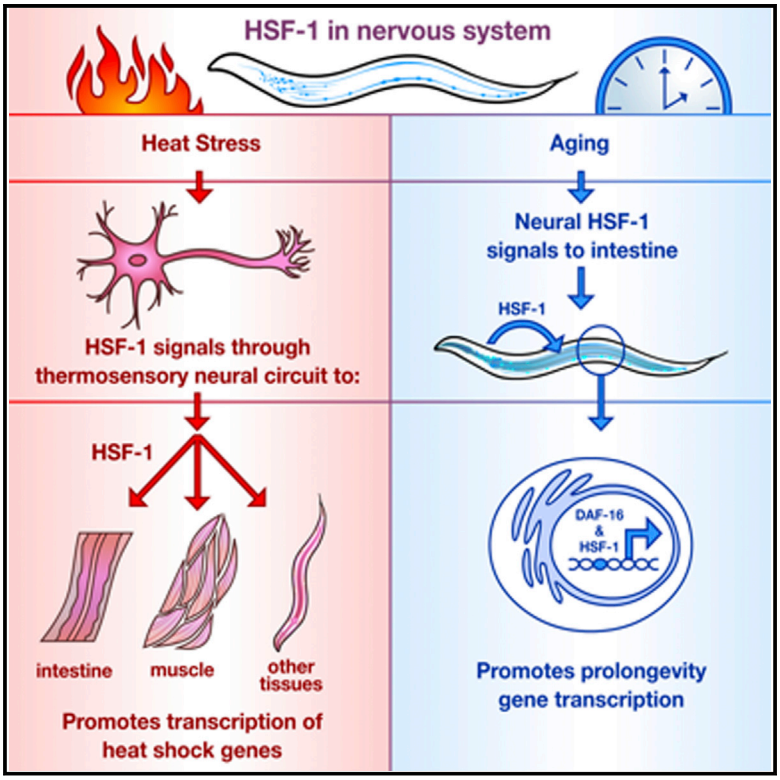
Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity

Graphical Abstract



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In Brief

The heat shock transcription factor, HSF-1, regulates lifespan and stress resistance in *C. elegans*. Douglas et al. find that HSF-1 acts in neurons to emit divergent signals that independently regulate aging and thermotolerance. Thus, a single transcription factor can act within different neurons to modulate distinct protective responses in peripheral tissues.

Highlights

- HSF-1 in the nervous system increases longevity and thermotolerance in *C. elegans*
- Heat protection by neural HSF-1 uses the thermosensory neural circuit, but not DAF-16
- Age determination by neural HSF-1 requires the FOXO, DAF-16, in the intestine
- Distinct signals by neural HSF-1 separate age regulation from thermal protection

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Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity

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SUMMARY

Integrating stress responses across tissues is essential for the survival of multicellular organisms. The metazoan nervous system can sense protein-misfolding stress arising in different subcellular compartments and initiate cytoprotective transcriptional responses in the periphery. Several subcellular compartments possess a homotypic signal whereby the respective compartment relies on a single signaling mechanism to convey information within the affected cell to the same stress-responsive pathway in peripheral tissues. In contrast, we find that the heat shock transcription factor, HSF-1, specifies its mode of transcellular protection via two distinct signaling pathways. Upon thermal stress, neural HSF-1 primes peripheral tissues through the thermosensory neural circuit to mount a heat shock response. Independent of this thermosensory circuit, neural HSF-1 activates the FOXO transcription factor, DAF-16, in the periphery and prolongs lifespan. Thus a single transcription factor can coordinate different stress response pathways to specify its mode of protection against changing environmental conditions.

INTRODUCTION

The long-term health of an organism is linked to its ability to recognize and respond to stresses that arise in its environment. Across evolutionary spectra, organisms have developed complex and highly specialized defense pathways that become transcriptionally activated during times of stress. Often, diverse stress stimuli initiate distinct transcriptional signatures that activate protective and adaptive genes to defend against environmental challenges and restore homeostasis. In metazoa, the upregulation of stress response pathways also requires the coordinated activation of stress response machinery across multiple tissues. Consequently, a hierarchical mode of tissue regulation has evolved in which particular cell types can act as master regulators, initiating protective pathways in peripheral tissues (Wolff et al., 2014).

Organisms are frequently subjected to acute challenges that require a rapid response to potentially lethal conditions. These transient stresses elicit a dramatic cellular reaction with a rewiring of gene expression and a temporary suspension of normal cellular function. Conversely, organisms regularly encounter chronic insults that are not lethal even after long exposures. These prolonged stresses initiate distinct and more-sustained responses that allow for the continuance of most normal cellular functions. The cumulative effect of chronic stress over the lifetime of the organism is known to play a causative role in the onset and severity of many age-related diseases (Failla, 1958; Harman, 1956; Orgel, 1963). However, it is unclear how acute stress responses can alleviate the negative effects of the aging process (Lithgow et al., 1995).

Thermal adaptation in metazoans requires the perception, communication, and initiation of a response across the entire organism. The transcription factor HSF-1 is the key regulator of the cellular and organismal response to heat stress and is conserved in all eukaryotes. It is well-established that HSF-1 mediates a protective transcriptional and translational response to acute heat stress through the induced expression of molecular chaperones (Morimoto, 2008). More recently, it has been shown in the nematode worm that *hsf-1* overexpression in all tissues retards the aging process (Hsu et al., 2003). Thus, mediating stress response pathways by HSF-1 protects against both acute thermal stress and the chronic stresses associated with aging.

In nematodes, thermal adaptation is regulated by a subset of sensory neurons that activate the heat shock response in peripheral tissues (Prahlad et al., 2008). However, the role that HSF-1 plays within the nervous system is not well defined. It is also not clear whether the same sensory neural circuit that controls the heat shock response also controls processes of aging that are tightly associated with heat stress resistance.

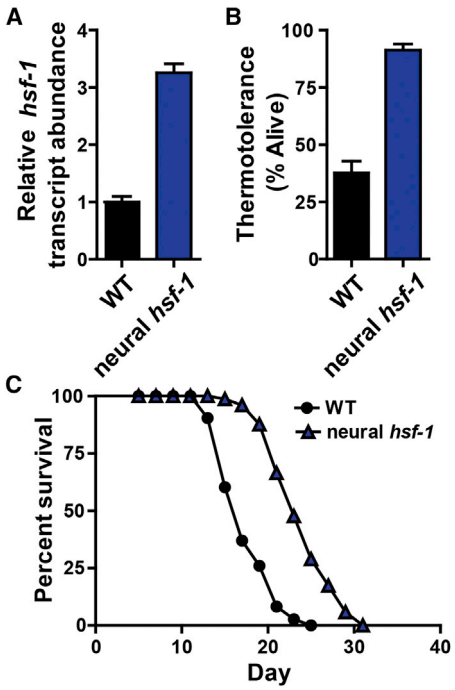

Figure 1. Neural Overexpression of *hsf-1* Protects *C. elegans* against Heat Stress and Aging

(A) Transcript abundance of *hsf-1* determined by quantitative RT-PCR analysis of wild-type (N2) and *rab-3p*::*hsf-1* transgenic animals (AGD1289). Error bars represent the SEM.

(B) Thermotolerance of wild-type and *rab-3p*::*hsf-1* transgenic worms shifted from permissive (20°) to heat shock (34°) temperatures for 14 hr. Error bars represent the SEM.

(C) Lifespan survival curves of wild-type and *rab-3p*::*hsf-1* transgenic strains at permissive temperatures (20°). Lifespan statistics are found in Table 1.

RESULTS

Neural Overexpression of *hsf-1* Promotes Heat Stress Resistance and Longevity

To explore these questions, we examined whether increasing *hsf-1* levels exclusively in the worm nervous system was sufficient to mediate protection against acute thermal stress and the aging process. Transgenic worms were generated that ectopically overexpressed *hsf-1* throughout the nervous system (Figures 1A and S1A–S1C). This level of *hsf-1* overexpression in neurons was sufficient to extend worm lifespan and protect against heat shock treatments (Figures 1B, 1C, S1D, and S1E; Table 1).

To gain insight into the neural-signaling pathways responsible for thermotolerance and longevity assurance, heat-shock-responsive transcriptional targets were examined under conditions of either acute heat stress or aging. We first utilized a transgenic reporter worm that expresses GFP under the promoter of the HSF-1 target gene, *hsp-16.2*, a member of the alpha β crystalline family of small chaperones (Link et al., 1999). Upon application of heat stress, animals robustly induced the expression of GFP driven by the *hsp-16.2* promoter compared to non-heat-treated worms (Figure 2A). Elevating neural *hsf-1* expression enhances the heat shock response throughout all worm tissues. These results strongly suggest that HSF-1 activity in neurons communicates to peripheral tissues and enables a more-robust transcriptional response to heat stress. Through large-particle flow cytometry, fluorescent quantification of individual worms reveals that heat shock induces expression of *hsp-16.2p*::*GFP* over 2-fold higher in worms that overexpressed *hsf-1* in the nervous system (Figures 2B and S2A). Similar increases of endogenous *hsp-16.2*, *hsp70a*, and *hsp-70b* transcript levels were observed upon heat shock in worms overexpressing neural *hsf-1* (Figures 2C, S2B, and S2C). Similar expression patterns of HSP-16 protein levels were also observed (Figure 2D). Genome-wide transcriptomics further confirmed that *hsf-1* overexpression in the nervous system bolsters transcription of numerous heat-responsive genes compared to wild-type animals (Figure S2D). The ability of worms overexpressing neural *hsf-1* to mount a more-robust heat shock response in peripheral tissues is consistent with the hypothesis that thermal protection is conferred by the heat shock response.

Loss of the Thermosensory Neural Circuit Disrupts Thermotolerance, but Not Longevity

We examined the role that heat-inducible chaperones might play in lifespan determination when *hsf-1* is overexpressed in the nervous system. We predicted that induction of heat-responsive genes would be correlated with neural *hsf-1* overexpression. Perplexingly, only minor differences in heat-responsive elements were observed at permissive temperatures between control animals and those overexpressing neural *hsf-1* (Figures 2B–2D and S2B–S2D). Thus, elevating *hsf-1* levels in the nervous system confers longevity without induction of the canonical heat-shock-responsive chaperones. Furthermore, RNAi knockdown of *hsp-16* expression, previously linked to age determination (Walker and Lithgow, 2003), did not alter lifespan extension by neural *hsf-1* (Figure S3A; Table 1). These data suggest that *hsf-1* overexpression elicits a divergent pathway in response to the aging process. Similar responses have been reported for HSF-1 in the context of reduced insulin/IGF-1 signaling (Hsu et al., 2003).

Because lifespan extension and thermotolerance appear divergent, we speculated that mutations might exist that abolish thermal protection but retain lifespan extension. To test this hypothesis, the thermosensory neural circuit was disrupted and the ability of neural *hsf-1* overexpression to enhance heat tolerance and extend lifespan was assessed. Genetic ablation of the AIY interneuron through a *ttx-3* mutation severs the thermosensory neural circuit and dampens the activation of heat shock responders in peripheral tissues (Prahlad et al., 2008). As expected, in the *ttx-3* mutant background, neural *hsf-1* overexpression no longer enhanced heat tolerance (Figure 2E). Surprisingly, neural *hsf-1* overexpression was still capable of prolonging lifespan in the *ttx-3* mutant (Figure 2F; Table 1). Thus, an intact thermosensory circuit is required for neural *hsf-1*-overexpressing worms to protect against heat stress but is dispensable for age regulation. More importantly, these data challenge the idea that HSF-1 regulates the aging process through the same heat shock response mechanisms and suggest that HSF-1 initiates an alternative transcriptional pathway to combat the stress of aging.

Table 1. Statistical Analysis of *C. elegans* Lifespan Data

Figure	Strain, Treatment	Mean Lifespan ± SEM (Median Lifespan in Days)	75th % (Day)	Observed/Total	% Lifespan Increase	p Value Log Rank (Mendel-Cox)
1C	N2	17.3 + 0.4 (17)	22	88/100		
1C	AGD1289 (neural *hsf-1*)	23.1 + 0.4 (24)	27	68/101	33.5	<0.0001
2F	AGD1008 non-transgenic	18.0 + 0.4 (18)	20	69/86		
2F	AGD1008 (neural *hsf-1*)	23.2 + 0.5 (24)	26	58/88	28.8	<0.0001
2F	*ttx-3*(*ks5*)	17.5 + 0.4 (18)	20	88/96	2.8	0.7231
2F	AGD1449 (neural *hsf-1 ttx-3*(*ks5*))	22.0 + 0.6 (22)	24	59/85	22.2	<0.0001
3D	N2	17.5 + 0.4 (17)	21	73/100		
3D	AGD1289 (neural *hsf-1*)	23.9 + 0.4 (23)	27	70/103	36.6	<0.0001
3D	*daf-16*(*mu86*)	15.8 + 0.4 (15)	19	61/100	−9.7	0.0022
3D	AGD1217 (neural *hsf-1*, *daf-16*(*mu86*))	16.6 + 0.3 (17)	19	72/100	−5.1	0.0578
4A	AGD1272 (neural *daf-16*)	18.7 + 0.4 (19)	22	68/108		
4A	AGD1273 (neural *daf-16*, neural *hsf-1*)	18.2 + 0.6 (17)	22	57/105	−2.6	0.6775
4B	AGD1276 (intestinal *daf-16*)	16.7 + 0.5 (17)	19	53/104		
4B	AGD1277 (intestinal *daf-16*, neural *hsf-1*)	22.7 + 0.5 (22)	26	51/110	35.9	<0.0001
4C	AGD1278 (muscle *daf-16*)	16.8 + 0.5 (17)	19	71/107		
4C	AGD1279 (muscle *daf-16*, neural *hsf-1*)	18.5 + 0.6 (19)	22	52/94	10.1	0.0429
5A	N2	18.5 + 0.2 (18)	20	90/114		
5A	AGD1289 (neural *hsf-1*)	27.6 + 0.4 (28)	31	113/123	51.9	<0.0001
5A	*hsf-1*(*sy441*)	16.8 + 0.2 (18)	18	100/114		
5A	AGD1471 (*hsf-1*(*sy441*), neural *hsf-1*)	18.4 + 0.2 (18)	20	91/120	9.5	<0.0001
S1D	N2	17.1 + 0.4 (16)	20	76/102		
S1D	AGD1053 (neural *hsf-1*)	21.2 + 0.6 (20)	25	72/100	24	<0.0001
S1D	AGD1054 (neural *hsf-1*)	21.4 + 0.4 (22)	25	76/100	25.2	<0.0001
S1E	N2	19.1 + 0.5 (20)	22	79/102		
S1E	AGD1441 (neural *hsf-1*)	22.2 + 0.7 (22)	26	62/78	16.2	0.0002
S3A	N2, vector RNAi	18.5 + 0.3 (19)	21	83/109		
S3A	AGD1289 (neural *hsf-1*), vector RNAi	23.0 + 0.3 (22)	24	94/113	24.3	<0.0001
S3A	N2, *hsp16.1* RNAi	19.6 + 0.3 (19)	22	92/105		
S3A	AGD1289 (neural *hsf-1*), *hsp-16.1* RNAi	23.5 + 0.3 (24)	26	95/106	19.9	<0.0001
S3B	N2, vector RNAi	19.3 + 0.4 (19)	22	68/103		
S3B	AGD1289 (neural *hsf-1*), vector RNAi	23.7 + 0.4 (25)	25	74/111	22.8	<0.0001
S3B	N2, *pha-4* RNAi	17.6 + 0.5 (19)	19	43/104		
S3B	AGD1289 (neural *hsf-1*), *pha-4* RNAi	22.9 + 0.4 (22)	25	77/103	30.1	<0.0001
S3C–S3E	N2, vector RNAi	17.0 + 0.4 (16)	20	90/108		
S3C–S3E	AGD1289 (neural *hsf-1*), vector RNAi	22.4 + 0.3 (22)	24	57/105	31.8	<0.0001
S3C	N2, *xbp-1* RNAi	18.6 + 0.4 (18)	22	85/98		
S3C	AGD1289 (neural *hsf-1*), *xbp-1* RNAi	23.2 + 0.4 (24)	26	63/98	24.7	<0.0001
S3D	N2, *skn-1* RNAi	15.3 + 0.2 (16)	16	89/98		
S3D	AGD1289 (neural *hsf-1*), *skn-1* RNAi	22.2 + 0.3 (22)	26	85/108	45.1	<0.0001
S3E	N2, *ubl-5* RNAi	17.1 + 0.4 (18)	20	91/100		
S3E	AGD1289 (neural *hsf-1*), *ubl-5* RNAi	21.0 + 0.4 (20)	24	67/91	22.8	<0.0001

HSF-1 Signals Heterotypically to DAF-16 in the Periphery for Increased Longevity

Intrigued by the possible separation of thermotolerance and aging, we sought to identify alternative transcriptional pathways that regulate aging independently of the heat stress response. To gain insight into alternate pathways, we analyzed genes that were significantly upregulated in multiple long-lived, *hsf-1*-overexpressing strains (Baird et al., 2014). Promoter analysis was performed on these gene data sets to identify possible transcription-factor-binding elements associated with an alternative cellular process or pathway. As expected, many of the promoters contained HSEs (heat shock elements). We also

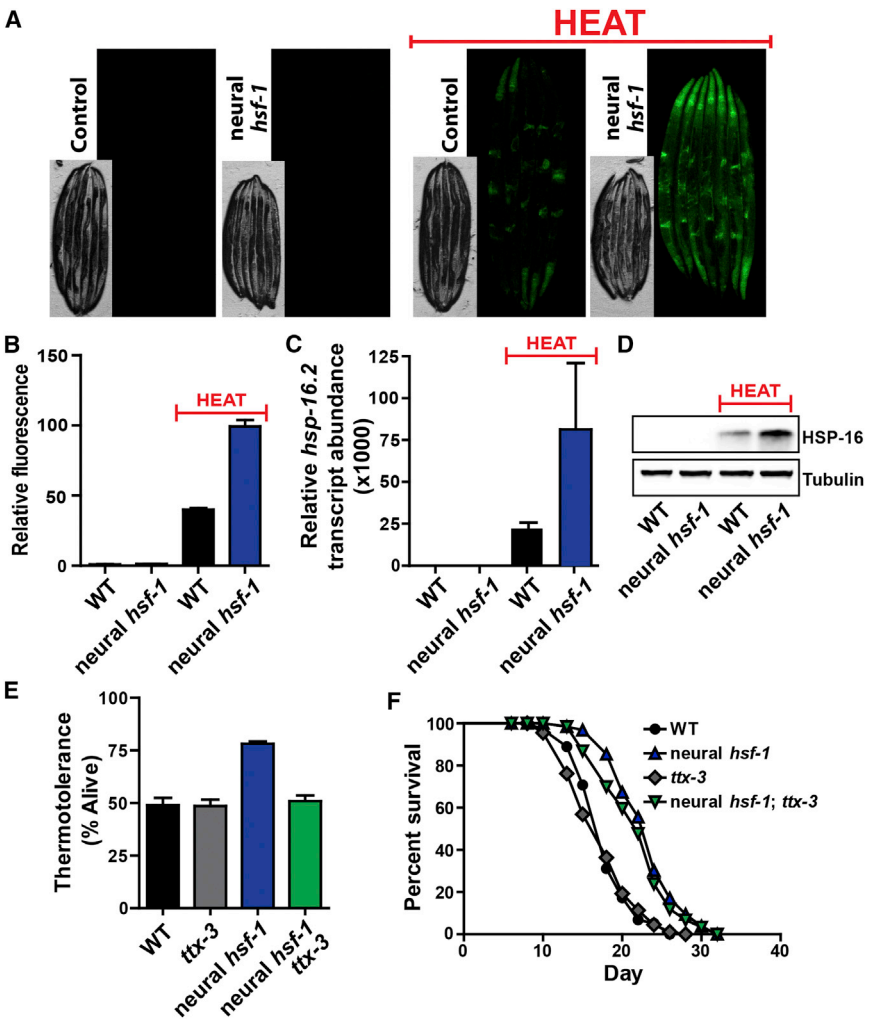

Figure 2. Neural *hsf-1* Overexpression Enhances Heat-Inducible Chaperone Expression in All Tissues and Requires an Intact Thermosensory Circuit for Heat Protection, but Not Lifespan Extension

(A) Fluorescent microscopy of *C. elegans* expressing GFP from the *hsp-16.2* promoter in control (CL2070) and *rab-3p*::*hsf-1* transgenic animals (AGD1448) at permissive (20°C) and heat shock (34°C) temperatures.

(B) Large-particle flow cytometry was used to quantify GFP fluorescence from strains used in (A). Error bars represent the SEM.

(C) Transcript levels of endogenous *hsp-16.2* determined by quantitative RT-PCR from day 1 adult wild-type and *rab-3p*::*hsf-1* transgenic animals (AGD1289). Error bars represent the SEM.

(D) Western blot analysis of endogenous HSP-16 from strains used in (C).

(E) Thermotolerance of WT, *ttx-3*(*ks5*), *rab-3p*::*hsf-1* (AGD1289), and *rab-3p*::*hsf-1*;*ttx-3*(*ks5*) (AGD1449) animals was assessed at 34°C. Error bars represent the SEM.

(F) Lifespan survival was performed at 20°C on strains used in (E). Lifespan statistics are found in Table 1.

observed a significant enrichment in DAF-16-associated elements (DAEs) (Table S1). The forkhead (FOXO) transcription factor DAF-16 is an essential component in the insulin/IGF-1-signaling cascade. This systemic process enables organisms to maintain glucose and energy homeostasis at optimal levels. HSF-1 has been linked to the insulin-signaling pathway and DAF-16 (Chiang et al., 2012; Hsu et al., 2003; Morley and Morimoto, 2004), yet it remains unclear how these essential processes function together.

Disrupting insulin signaling through various mutations in different transduction components enhances longevity (Kenyon, 2011). Moreover, this lifespan extension requires the activation of DAF-16 to drive the pro-longevity transcriptional response. The canonical DAF-16 target gene superoxide dismutase-3 (*sod-3*) exhibits expression levels highly correlative with lifespan extension (Henderson et al., 2006; Sánchez-Blanco and Kim, 2011). Because neural *hsf-1* overexpression does not activate a heat shock response at permissive temperatures, we hypothesized that *hsf-1* might be capable of activating DAF-16 target genes. Fluorescence was examined in transgenic worms that expressed GFP under the control of the *sod-3* promoter. In this manner, temporal and spatial aspects of *sod-3* transcriptional activity were analyzed in different worm tissues.

Elevated expression of *hsf-1* in the nervous system increased GFP fluorescence in all worm tissues harboring the transcriptional *sod-3* reporter (Figure 3A). We observed that overexpression of neural *hsf-1* yields twice as much GFP fluorescence (Figures 3B and S4A). GFP expression was not only elevated in the nervous system by neural *hsf-1* overexpression but also in peripheral tissues. The *daf-16*(*mu86*)-null allele prevented neural *hsf-1* overexpression from increasing GFP fluorescence of the *sod-3* reporter strain (Figures 3A, 3B, and S4A). Induction of endogenous *sod-3* transcripts by neural *hsf-1* overexpression was also *daf-16* dependent (Figure 3C). Thus, neural *hsf-1* overexpression drives FOXO-dependent activation of *sod-3* at permissive temperatures in all worm tissues.

Taken together, these results suggest that *hsf-1* combats acute heat stress through the activation of a transcellular heat shock network and *hsf-1* in the nervous system initiates an independent signal to activate DAF-16 in peripheral tissues to extend lifespan. To disprove this hypothesis, we determined the dependence of *daf-16* upon lifespan extension by neural *hsf-1*. Consistent with separation of thermotolerance and aging, *hsf-1* overexpression in the nervous system was incapable of extending worm lifespan in *daf-16* mutants (Figure 3D; Table 1). In contrast, stress-responsive transcription factors such as *pha-4*, *xbp-1*, *skn-1*, and *ubl-5* were dispensable for neural *hsf-1*-mediated lifespan extension (Figures S3B–S3E; Table 1). The ability of increased neural *hsf-1* to modulate *sod-3*

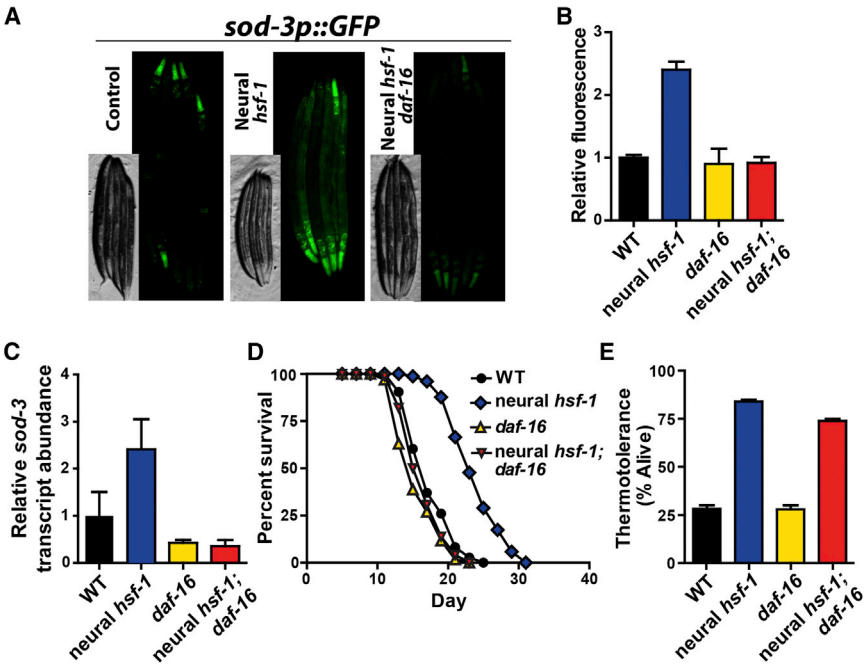

Figure 3. *daf-16* Is Required for Neural *hsf-1* to Induce *sod-3* Expression in Peripheral Tissues and Extend Lifespan but Is Dispensable for Increased Thermotolerance

(A) Fluorescent microscopy of *C. elegans* expressing GFP from the *sod-3* promoter in control (CF1553), *rab-3p*::*hsf-1* (AGD1198), and *rab-3p*::*hsf-1*; *daf-16*(*mu86*) (AGD1457) animals at 20°C.

(B) Quantification of GFP fluorescence from strains used in (A) as determined by large-particle flow cytometry. Error bars represent the SEM.

(C) Transcript levels of endogenous *sod-3* determined by quantitative RT-PCR from day 1 adult WT, *daf-16*(*mu86*), *rab-3p*::*hsf-1* (AGD1289), and *rab-3p*::*hsf-1*; *daf-16*(*mu86*) (AGD1217) animals. Error bars represent the SEM.

(D) Lifespan survival was assessed at 20°C for strains used in (C). Lifespan statistics are found in Table 1.

(E) Thermotolerance was determined at 34°C for strains used in (C) and (D). Error bars represent the SEM.

levels in a *daf-16*-dependent manner correlates with its ability to extend animal lifespan. Although *daf-16* possesses thermal protective properties (Volovik et al., 2014), *daf-16* was not required for neural *hsf-1*-overexpressing worms to protect against heat stress (Figure 3E). Moreover, expression of the *sod-3* stress-responsive gene was not heat inducible and likely represents a distinct stress response pathway utilized by neural *hsf-1* during the aging process (Figures S4B–S4D).

The ability of *hsf-1* to regulate the heat shock response and aging are separable. Removal of the thermosensory circuit through a *ttx-3* mutation blocks heat resistance but has no effect on increased longevity. Conversely, eliminating *daf-16* function does not affect thermotolerance but abolishes lifespan extension by neural *hsf-1* overexpression. Therefore, distinct signaling events are communicated across an organism from *hsf-1* in the nervous system in response to acute heat stress compared to the chronic stress of aging.

Intestinal *daf-16* Is Sufficient to Extend Lifespan by Neural *hsf-1* Overexpression

Neural *hsf-1* regulates *daf-16* in peripheral tissues; however, it is not clear in what tissues *daf-16* is required for lifespan extension. By rescuing *daf-16* expression in individual worm tissues in an otherwise null *daf-16*(*mu86*) mutant, we tested whether restoring *daf-16* activity in a particular tissue was sufficient to drive the lifespan extension by neural *hsf-1* overexpression. Expression of *daf-16* was rescued in the nervous system, intestine, and body-wall muscle (Libina et al., 2003). Rescuing expression of *daf-16* in the nervous system was not sufficient for neural *hsf-1* overexpression to extend lifespan or enhance GFP fluorescence of the *sod-3* transcriptional reporter (Figures 4A and S4E; Table 1). This demonstrates that *daf-16* in the nervous system is not required for neural *hsf-1* to communicate to peripheral tissues. In contrast, *daf-16* expression exclusively in the intestine enabled neural *hsf-1* overexpression to both extend lifespan and modestly enhance GFP fluorescence in the *sod-3* transcriptional reporter (Figures 4B and S4E; Table 1). Additionally, neural *hsf-1* acted specifically through the intestine, as *daf-16* expression in body-wall muscles did not extend lifespan or induce the *sod-3* reporter (Figures 4C and S4E; Table 1). Therefore, neural *hsf-1* functions cell non-autonomously in lifespan regulation via a signaling mechanism that requires the activity of *daf-16* in intestinal cells. DAF-16 has previously been shown to play an important role in the worm intestine to influence lifespan (Libina et al., 2003). The communication between neural *hsf-1* and *daf-16* in the periphery does not require *daf-16* in the nervous system, indicating that *hsf-1* regulates *daf-16* in a transcellular manner (Figure 5B).

Although *daf-16* activity is required in the intestine for neural *hsf-1*-overexpressing worms to extend lifespan, it is unclear whether *hsf-1* also functions in the peripheral, non-neural tissues to mediate longevity assurance. Thus, *hsf-1* activity in peripheral tissues was reduced through a hypomorphic *hsf-1*(*sy441*) allele in which the carboxy-terminal transactivation domain has been removed through a premature stop codon (Hajdu-Cronin et al., 2004). Overexpressing full-length *hsf-1* in the nervous system was not able to extend the lifespan of the hypomorphic *hsf-1*(*sy441*) animals (Figure 5A; Table 1). These results indicate that *hsf-1* activity in the nervous system is not sufficient to increase lifespan on its own, but rather communication of neural *hsf-1* to peripheral cells expressing *hsf-1* is essential for longevity assurance.

DISCUSSION

Stress encompasses a wide spectrum of insults for which cells have evolved highly specialized responses to both combat the

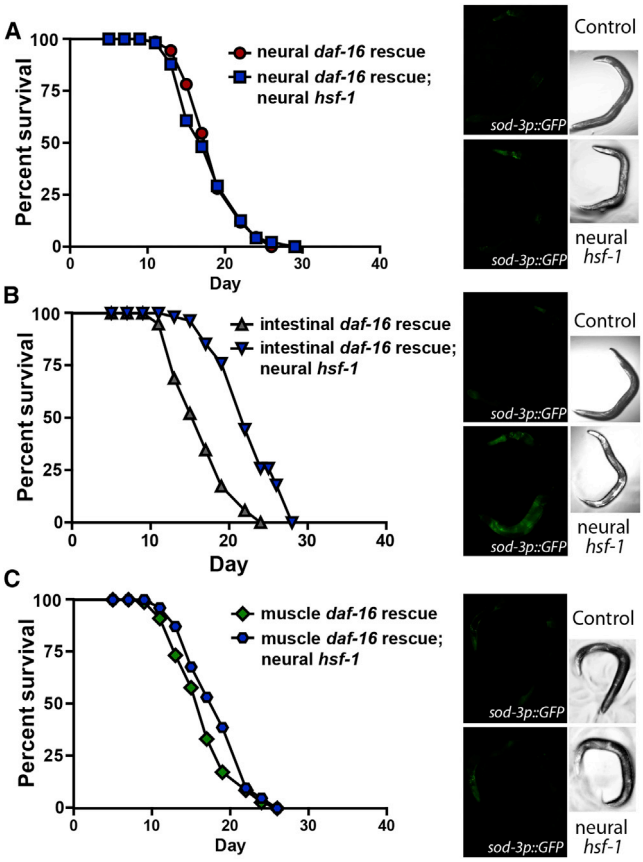

Figure 4. Neural Overexpression of *hsf-1* Requires *daf-16* in the Intestine to Activate *sod-3* and Extend Lifespan

(A–C) Lifespan survival curves and representative *sod-3p*::*GFP* fluorescent micrographs of *rab-3p*::*hsf-1* transgenic animals with different tissue-specific *daf-16* rescues in an otherwise *daf-16*(*mu86*) mutant background. Expression of *daf-16* is ectopically restored in individual tissues of *daf-16*(*mu86*)-null animals including the (A) nervous system (AGD1273), (B) intestine (AGD1277), and (C) body-wall muscle (AGD1279). Lifespan statistics are found in Table 1.

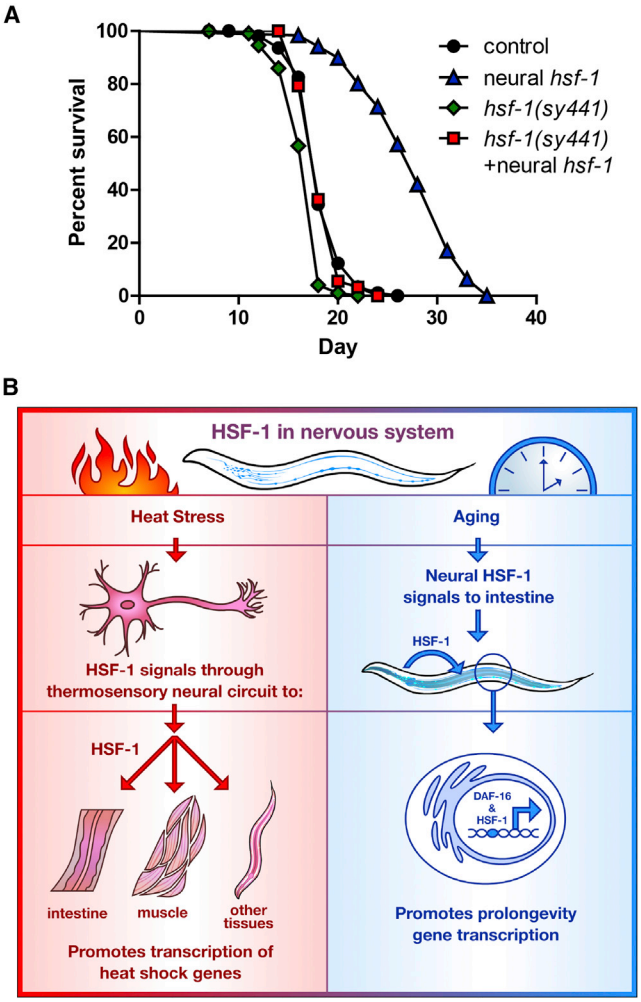

Figure 5. *hsf-1* Is Required in Peripheral Tissues for Neural *hsf-1*-Overexpressing Worms to Extend Lifespan

(A) Lifespan survival curves of WT and *rab-3p*::*hsf-1*-overexpressing nematodes (AGD1289) in the presence or absence of the hypomorphic *hsf-1*(*sy441*) mutation (AGD1471). Lifespan statistics are found in Table 1.

(B) Model depicting how heterotypic signals by neural *hsf-1* separate thermal protection from age regulation. Under thermal stress, HSF-1 in the nervous system signals to peripheral tissues through the thermosensory neural circuit and enhances the heat shock response to protect worms (left model). Conversely, neural *hsf-1* functions independently of the thermosensory circuit to generate a transcellular signal that activates DAF-16 and HSF-1 in the intestine and drives pro-longevity gene expression (right model).

immediate stress and initiate recovery mechanisms. With the evolution of multicellularity, organisms developed a hierarchical mode of stress response regulation across tissues. This normally includes a master tissue, which can both sense the particular stress and transmit the appropriate response to the pertinent peripheral tissues. Cell non-autonomous signaling of stress responses includes numerous mechanisms, such as the insulin-signaling pathway (Libina et al., 2003), germline ablation (Hsin and Kenyon, 1999), mitochondrial unfolded protein response (UPR) (Durieux et al., 2011; Owusu-Ansah et al., 2013), ER UPR (Deng et al., 2013; Taylor and Dillin, 2013), and the heat shock response (Prahlad et al., 2008). To date, each signaling mechanism arises in a particular tissue and functions in a homotypic manner whereby a single signaling cascade conveys stress responsive cues to all pertinent tissues. Consistent with these responses, neural *hsf-1* signaling directly regulates *hsf-1* targets in peripheral tissues to mount the heat shock response upon acute stress. In contrast, neural *hsf-1* initiates a distinct response under aging to coordinate *daf-16* activity in peripheral tissues in addition to *hsf-1*. Thus, *hsf-1* communicates in a heterotypic manner to regulate different stress response pathways. Given the vast array of stresses that *hsf-1* has been reported to protect against (Hsu et al., 2003; Morimoto, 2008), it is reasonable that *hsf-1* can optimally tailor stress response machinery to combat either acute or chronic insults.

Links between *hsf-1* and components of the insulin/IGF-1-signaling pathway exist in multiple experimental paradigms including aging, proteotoxicity, and thermotolerance (Hsu et al., 2003; McColl et al., 2010; Morley and Morimoto, 2004). From these reports, *hsf-1* function has been modeled to reside

downstream of, or in parallel with, the insulin-signaling pathway. Herein, we provide evidence suggesting that *hsf-1* functions upstream of *daf-16* signaling. Furthermore, mutations abolishing distinct types of neural vesicular release, *unc-13* and *unc-31*, extend worm lifespan in a *daf-16*-dependent manner (Ailion et al., 1999; Gems and Riddle, 2000). We observe that animals harboring the *unc-13* or *unc-31* mutation induce *sod-3* expression (Figure S4F), suggesting that reduced neural secretion of insulin-like peptides activates *daf-16* in distal tissues to promote longevity. Because of this functional redundancy, the signaling mechanism by which neural *hsf-1* communicates to the periphery remains unclear but could include any of the 39 insulin-like peptides found in *C. elegans*.

Within the nervous system, *hsf-1* could function at multiples steps to modulate insulin signaling: either by regulating the production, processing, trafficking, or secretion of insulin peptides during conditions of chronic stress. Future studies are needed to elucidate the molecular mechanisms by which *hsf-1* overexpression in neurons could possibly regulate insulin biogenesis. Expanding these concepts to mammalian systems, it will be interesting to understand how bolstering *hsf-1* activity in the brain influences more localized insulin secretion within the nervous system versus insulin biogenesis in pancreatic β cells. Either course of action could profoundly affect energy homeostasis and provide a novel, long-term mode of diabetes intervention.

The process of aging is due, in part, to protein misfolding events and a general deterioration in the quality of the proteome. In support of this hypothesis, metastable proteins that can fold and function in youthful cells begin to misfold upon aging, losing functionality (Ben-Zvi et al., 2009). A similar phenomenon appears in numerous age-onset neurodegenerative disorders, in which the aging brain can no longer maintain disease-linked proteins in properly folded, functional states and misfolding leads to multimerization of the disease proteins and neural death (Douglas and Dillin, 2010; Morimoto, 2008). In these studies, the levels of *hsf-1* and its chaperone target genes directly correlated with the age onset of different neurodegenerative models. Thus, by extension, it was hypothesized that *hsf-1* regulates the aging process by modulating chaperone network components to directly influence the folding state of the proteome (Morimoto, 2008). Our data suggest an alternate method by which *hsf-1* can regulate the aging process. In this model, *hsf-1* activates the FOXO transcription factor, which initiates a pro-longevity stress response that is distinct from the heat shock response. Although we cannot entirely exclude the possible involvement of the chaperone network in lifespan extension by neural *hsf-1* overexpression, we did not observe an enhancement in heat-responsive elements. Further investigation is needed to uncover stress-responsive genes within the DAF-16 activation cascade that are responsible for longevity assurance.

EXPERIMENTAL PROCEDURES

C. elegans Strains and Maintenance

All strains were maintained at 15°C on the *E. coli* strain, OP50. The following strains were used in this work: wild-type (N2), *ttx-3*(*ks5*), *daf-16*(*mu86*), *hsf-1*(*sy441*), *daf-2*(*e1370*), AM101 (rmIS110[*gref-1*p::Q40::YFP]; AGD440 (N2; uthEx457[*rab-3*p::tdTomato); *rol-6*(*su1006*)]); AGD1008 (uthEx663[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1441 (uthEx741[*rgef-1*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1289 (uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1053 (uthIS365[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1054 (uthIS366 [*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1449 (*ttx-3*(*ks5*); uthEx663[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1471 (*hsf-1*(*sy441*); uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), CL2070 (dvln70[pCL25 (*hsp-16.2*p::GFP); pRF4(*rol-6*)]), AGD1448 (dvln70[pCL25 (*hsp-16.2*p::GFP); pRF4(*rol-6*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), CF1553 (muls84[pAD76(*sod-3p*::GFP)]), AGD709 (*daf-16*(*mu86*); muls84[pAD76(*sod-3p*::GFP)]), AGD1198 (muls84[pAD76(*sod-3p*::GFP)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1457 (*daf-16*(*mu86*); muls84[pAD76(*sod-3p*::GFP)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]; AGD1217 (*daf-16*(*mu86*); uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]; AGD1272 (*daf-16*(*mu86*); muEx169[*unc-119p*::*GFP*::*daf-16*, *rol-6*(*su1006*)]); AGD1273 (*daf-16*(*mu86*); muEx169[*unc-119p*::*GFP*::*daf-16*, *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]); AGD1276 (*daf-16*(*mu86*); muEx211[pNL213(*ges-1*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]); AGD1277 (*daf-16*(*mu86*); muEx211[pNL213(*ges-1*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1278 (*daf-16*(*mu86*); muEx212[pNL212(*myo-3*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]), AGD1279 (*daf-16*(*mu86*); muEx212[pNL212(*myo-3*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]), AGD1309 (*daf-16*(*mu86*); muEx169[*unc-119p*::*GFP*::*daf-16*, *rol-6*(*su1006*)]; muls84[pAD76(*sod-3p*::GFP)]), AGD1313 (*daf-16*(*mu86*); muEx169[*unc-119p*::*GFP*::*daf-16*, *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]; muls84[pAD76(*sod-3p*::GFP)]), AGD1311 (*daf-16*(*mu86*); muEx211[pNL213(*ges-1*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; muls84[pAD76(*sod-3p*::GFP)]), AGD1315 (*daf-16*(*mu86*); muEx211[pNL213(*ges-1*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]; muls84[pAD76(*sod-3p*::GFP)]), AGD1312 (*daf-16*(*mu86*); muEx212[pNL212(*myo-3*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; muls84 [pAD76(*sod-3p*::GFP)]), AGD1316 (*daf-16*(*mu86*); muEx212[pNL212(*myo-3*p::*GFP*::*daf-16*), *rol-6*(*su1006*)]; uthIS368[*rab-3*p::*hsf-1*; *myo-2*p::tdTomato]; muls84[pAD76(*sod-3p*::GFP)]), AGD1475 (unc-13(e450); muls84[pAD76(sod-3p::GFP)]), and AGD1476 (unc-31(e928); muls84[pAD76(sod-3p::GFP)]).

Wild-type (N2), *ttx-3*(*ks5*), and *daf-16*(*mu86*) CF1553 and CL2070 strains were obtained from the *Caenorhabditis* Genetics Center. For generation of transgenic overexpression strains, *hsf-1* cDNA was inserted downstream of the neural promoters *rab-3* or *gref-1* and upstream of the *unc-54* 3′ UTR. Neural *hsf-1* DNA plasmid constructs were injected at 2 ng/μl along with a co-injection marker (*myo-2p*::tdTomato) at 10 ng/μl to make transgenic overexpression worms.

Western Blot Analysis

Age-synchronized worms were cultivated on nematode growth (NG) plates containing the *E. coli* strain, OP50, at 20°C until day 1 adulthood. Worms were washed off the plate with M9 buffer pre-heated to 34°C, collected, and incubated in a 34°C water bath for 15 min. Worms were centrifuged at 1,000 × *g* for 30 s and moved back to NG plates seeded with OP50 bacteria at 20°C. Worms were allowed 1.5 hr of recovery at 20°C before worms were collected and frozen in liquid nitrogen for further processing.

Worm extracts were generated by glass bead disruption in non-denaturing lysis buffer (150 mM NaCl, 50 mM HEPES [pH 7.4], 1 mM EDTA, 1% Triton X-100, and protease inhibitor cocktail without EDTA [Roche]). Crude lysates were subject to centrifugation at 10,000 × *g* at 4°C for 5 min. The supernatant was supplemented with 2× SDS sample buffer containing 50 mM Tris-Cl (pH 6.8), 2 mM EDTA, 4% glycerol, 2% SDS, Coomassie Blue, and protease inhibitor cocktail without EDTA (Roche). Samples were boiled for 10 min and resolved by SDS-PAGE. Proteins levels were monitored by standard immuno-blotting procedures with α-Hsp-16.2 (kind gift from Lithgow Lab) and α-tubulin (Sigma T6074) antibodies.

Transcript Analysis

Total RNA was isolated from synchronized populations at day 1 of adulthood using Qiazol (QIAGEN) and then further purified with the RNeasy mini kit (QIAGEN). cDNA was synthesized using the QuantiTect kit (QIAGEN). SybrGreen was used for quantitative PCR as described in the SsoAdvanced SYBR Green Supermix protocol (Bio-Rad). Experiments were repeated with

three biological repeats and analyzed using the comparative Ct method. Internal controls utilized a geometric mean of *cdc-42*, *pmp-3*, and *Y45F10D.4*. The Roche Universal ProbeFinder online tool was used to design primers. Primer sequences are as follows:

cdc-42 forward 5′- AGGAACGTCTTCCTTGTCTCC −3′
cdc-42 reverse 5′- GGACATAGAAAGAAAAACACAGTCAC −3′
pmp-3 forward 5′- CGGTGTTAAAACTCACTGGAGA −3′
pmp-3 reverse 5′- TCGTGAAGTTCCATAACACGA −3′
Y45F10D.4 forward 5′- AAGCGTCGGAACAGGAATC −3′
Y45F10D.4 reverse 5′- TTTTTCCGTTATCGTCGACTC −3′
hsf-1 forward 5′- TTTGCATTTTCTCGTCTCTGTC −3′
hsf-1 reverse 5′- TCTATTTCCAGCACACCTCGT −3′
hsp-16.2 forward 5′- TCCATCTGAGTCTTCTGAGATTGTTA −3′
hsp-16.2 reverse 5′- TGGTTTAAACTGTGAGACGTTGA −3′
hsp-70a (C12C8.1) forward 5′- CGGTATTTATCAAAATGGAAAGGTT −3′
hsp-70a (C12C8.1) reverse 5′- TACGAGCGGCTTGATCTTTT −3′
hsp-70b (F44E5.4) forward 5′- TGCACCAATCTGGACAATCT −3′
hsp-70b (F44E5.4) reverse 5′- TCCAGCAGTTCCAGGATTTC −3′
pat-10 forward 5′- TCGAGGAGTTCTGGGAGTTG −3′
pat-10 reverse 5′- TTGTAGATCAGCGATTTTAAAGGA −3′
sod-3 forward 5′- CACTGCTTCAAAGCTTGTTCA −3′
sod-3 reverse 5′- ATGGGAGATCTGGGAGAGTG −3′.

RNA for global sequencing analysis was prepared using Illumina TruSeq RNA Sample Prep Kit (Illumina). Paired-end sequencing was performed on an Illumina HiSeq 2000, and data were analyzed with CLC Genomics Workbench 7.0.4 software.

Promoter Analysis

We used RSAT (Thomas-Chollier et al., 2011) to ask for overrepresented sequences of length 6, 7, or 8 upstream of our ORF start sites, within 1.5 kb or until the preceding ORF, whichever was closer. e-value is a multiple testing corrected estimate of the probability of this degree of overrepresentation.

Lifespan Analysis

Lifespan experiments were conducted at 20°C as previously described (Wilkinson et al., 2012), and a minimum of three independent experiments were performed under every condition. Worms were fed different *E. coli*, OP50, or HT115 for experiments involving RNAi knockdown of gene expression. Tissue-specific *daf-16* rescue lifespans were performed on OP50. The pre-fertile period of adulthood was considered day 0. Worms were transferred to fresh plates every second day until day 12. To prevent excessive worm censorship, 5-fluoro-2′-deoxyuridine, FUDR, was supplemented into growth media of lifespan experiments involving the hypomorphic *hsf-1*(*sy441*). Lifespan analysis on Prism 6 and JMP software was used for statistical analysis to determine significance calculated using the log rank (Mantel-Cox) method.

Thermotolerance Assay

Synchronized day 1 adult worms were placed at 34°C for 12–14 hr on plates spotted with OP50 *E. coli* or HT115 for RNAi. Worms were then scored for viability. At least 80 worms were used per genotype, and experiments were repeated at least three times. Prism 6 software was used for statistical analysis.

RNAi Feeding

Worms were fed from hatch HT115 *E. coli* containing an empty vector control or expressing double-stranded RNA. RNAi strains were taken from the Vidal library if present or the Ahringer library if absent from the Vidal library. All RNAi clones were sequence verified prior to use and knockdown verified previously (Carrano et al., 2009; Durieux et al., 2011; Panowski et al., 2007; Taylor and Dillin, 2013).

Microscopy and Fluorescence Analysis

For fluorescence microscopy, worms were anesthetized with 10 mM levamisole and images were captured using a Leica DM6000 B microscope and Hamamatsu ORCA-ER camera. We also used a COPAS Biosort (Union Biometrica) to measure individual day 1 worm length, width, and GFP fluorescence. At least 500 worms were measured per genotype and pooled in three biological replicates. We normalized fluorescence by worm size to compare between genotypes.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.07.026.

AUTHOR CONTRIBUTIONS

P.M.D., N.A.B., M.S.S., and S.U. performed the experiments and analyzed the data. M.A.M. and B.K.K. assisted P.M.D. on bioinformatics analysis. P.M.D., N.A.B., S.C.W., and A.D. wrote the manuscript. P.M.D. and N.A.B. contributed equally.

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