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From Neurons to Transcription Factors: the Biological Regulation of Aging

by

Peter Chisnell

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

Neuroscience

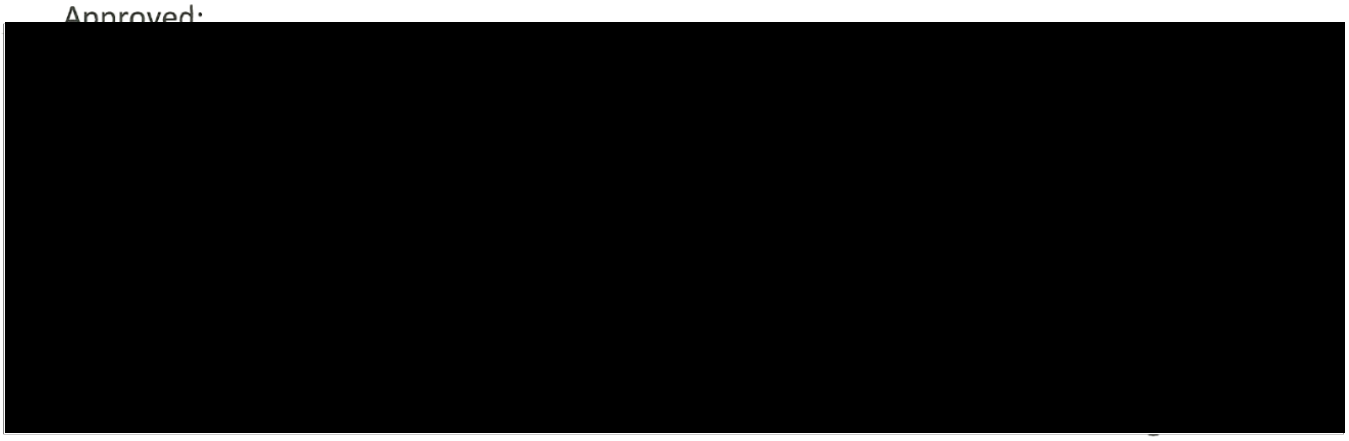
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**Dedication and Acknowledgements**

I would like to acknowledge everyone whose efforts and advice facilitated and improved the quality of this work, including my advisors Cynthia Kenyon and Kaveh Ashrafi, members of my committee Rob Edwards, Noelle L'etoile, and Saul Villeda, and everyone in the Kenyon and Ashrafi labs. All these people not only provided fresh ideas and professional expertise but also friendship and support. I would especially like to thank T. Richard Parenteau, for both his work getting the project off the ground and also for his help in framing and pursuing it, Nina Riehs, for philosophical conversations about aging and for her help optimizing my qPCR protocol, and George Lemieux, whose expertise with genomic sequencing greatly accelerated my discovery of candidate genes.

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**Acknowledgement of Previously Published Materials**

The text of this dissertation includes previously published material from the following three publications:

Chisnell, P (2018). Silencing the ASI gustatory neuron pair extends lifespan. microPublication Biology.

Chisnell, P (2018). Silencing the ASI gustatory neuron pair increases expression of the stress-resistance gene *sod-3* in a *daf-16* and *daf-3* independent manner. microPublication Biology.

Chisnell, P., Parenteau, T.R., Tank, E (2018). The mTOR Target S6 Kinase Arrests Development in Caenorhabditis elegans When the Heat-Shock Transcription Factor Is Impaired. Genetics *210*, 999–1009.

The omitted co-authors listed on the above publications directed and supervised the research that forms the basis for the dissertation. The co-authors listed on the *Genetics* publication conducted the forward genetic screen for suppressor mutations as well as complementation assays. Backcrossing, sequencing, discovery and confirmation of candidate genes, and all further experiments and analysis presented in this thesis was conducted by Peter Chisnell and represents a significant body of work.

**From Neurons to Transcription Factors: the Biological Regulation of Aging**

**By Peter Chisnell**

**Abstract**

Aging is a complex biological process that affects organisms ranging from humans to yeast, and understanding this process holds great potential for reducing the impact of age-related diseases such as heart disease, cancer, and neurodegenerative diseases. In this dissertation I explore both how neurons regulate lifespan and how two genes fundamental to the aging process interact with each other. First, I explore the neuronal regulation of lifespan. While we know that altering neuronal function can alter lifespan, the specific mechanisms by which neurons affect these changes, especially at the level of neuronal communication, remain to be elucidated. I show that reduction in signaling of the *C. elegans* ASI gustatory neuron pair through chemical silencing with tetanus toxin extends lifespan, likely primarily through reducing secretion of a TGFβ ligand (DAF-7), inhibiting the insulin-like signaling pathway, and activating the transcription factor DAF-16. Further results, such as a partial independence from *daf-16*, hint at a neuronal regulation of lifespan more complex than a sensory dial which increases or reduces DAF-7 levels.

Second, I explore how the developmental arrest caused by reduction of activity of the fundamental lifespan gene heat shock factor 1 (*hsf-1)* can be rescued by loss of function of another gene, the ribosomal S6 kinase gene *rsks*-1. *hsf-1,* a regulator of the widely conserved heat-shock response, is not only essential for cellular stress resistance and adult longevity, but also for proper development. However, the genetic mechanisms by which heat-shock transcription factors regulate development are not well understood. I investigated how *C. elegans* strains derived from an unbiased genetic screen ameliorated the developmental-arrest phenotype of a heat-shock factor mutant. Here I show that loss of the conserved translational activator *rsks-1/*S6 Kinase, a downstream effector of mTOR kinase, can rescue the developmental-arrest phenotype of *hsf-1* partial loss-of-function mutants.

Unexpectedly, I show that the rescue is not likely caused by reduced translation, nor by activation of any of a variety of stress-protective genes and pathways. My findings identify an as-yet unexplained regulatory relationship between the heat-shock transcription factor and the mTOR pathway during *C. elegans'* development.

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## Chapter 1: An Introduction to Aging

Aging is a biological process that affects many organisms and has been studied in organisms as diverse as humans and yeast. The precise definition of aging changes slightly depending on the author and publication, but generally speaking, aging is an organism's loss of normal physiological function and increase in mortality rate over time (Hayflick 2007; Flatt 2012). This loss of function can be measured at a cellular level of resolution by a variety of metrics, such as an increase in protein aggregation, dysregulation of cell membrane homeostasis, and alterations in organelle structure. In aggregate, these cellular dysfunctions lead to whole-organism deficits in organ function, immune function, mental acuity, and physical prowess, as well as an increase in risk for a variety of pathologies including heart disease, cancer, and neurodegenerative disease. As more and more people are living to older ages, these conditions constitute an increased burden on healthcare systems as well as an enormous human cost in the suffering they cause. An increased understanding of the biological mechanisms by which aging puts us at risk for these conditions may allow us to develop therapies to help people live longer lives in healthier bodies.

While aging and mortality have captivated humanity as far back as recorded history, as far as I can discern we have been exploring its genetic regulation since the turn of the 20$^{th}$ century. While it had been an anecdotal observation that longevity ran in families, in 1899 Mary Beeton and Karl Pearson published an analysis showing a positive correlation between the mean lifespan of fathers with sons and brothers with each other, suggesting that longevity is at least partially inherited (Beeton and Pearson 1899). Throughout the twentieth century, investigations showed that not only is lifespan inherited, but that a variety of environmental factors can increase it above normal levels in multiple species. These investigations include how temperature affects the lifespan of *Daphnia magna* (MacArthur and Baillie 1929), the effects of diet on flies (Glaser 1923), cockroaches (Haydak 1953), and rats (McCay *et. al* 1939;

Berg and Simms 1961), and the lifespan increase caused by castration, exercise, and mating in rats (Drori and Folman 1975), among many others.

Much of the interest in aging in the 20th century focused on rodents and humans, but after Syndey Brenner's publication of "The genetics of *Caenorhabditis elegans"* in 1974, focus on the soil nematode dramatically increased. *C. elegans* are easily cultivated and genetically tractable; they have a small number of cells (959 in the hermaphrodite) with defined lineages, including a nervous system that produces a diversity of behaviors, and due to their size and translucent body can be easily analyzed via microscopy. Finally, and this is of great importance to the aging field, their median lifespan is approximately 15-20 days, which allows for much faster analysis of lifespan when compared to other organisms such as *Drosophila* (~2-3 months) or mice (~2 years).

In 1977, Michael Klass showed that *C. elegans'* lifespans, like those of rodents and other organisms, can be regulated by altering the temperature at which they are cultivated and the amount of food they consume (Klass 1977). He later discovered multiple mutant strains of *C. elegans* which lived longer than wild-type animals, although each of his mutant strains showed significant defects in feeding and development due to paralysis of the pharynx or an unknown chemotactic defect causing the animals to avoid food (Klass 1983). Klass argued that the lifespan increase of these mutants was likely secondary to their reduction in food intake, a perturbation already shown to increase lifespan, and questioned whether any genes would be discovered to primarily affect aging. A few years later, Tom Johnson published that a strain from the original Klass screen, animals with a mutation in a gene he called *age-1*, had a 40% increased mean lifespan and showed apparently normal development, movement, and feeding, although the strain did have significantly reduced fertility (Friedman and Johnson 1987). In 1993, Cynthia Kenyon published that a mutation in the gene *daf-2* could double the lifespan of wild-type animals. At high temperatures, *daf-2* mutant animals cause constitutive dauer formation, a long-lived developmental stage normally caused by stressful conditions, but the mutant showed extended lifespan

even at the permissive temperature. This mutation affected lifespan independently of reproduction, and it did not affect animal size, or feeding, providing yet more evidence that mutations which extended lifespan need not cause large-scale defects to normal physiology as Klass had observed. Kenyon *et al.* also found that the lifespan extension depended on the function of another gene, *daf-16*, showing that lifespan could be regulated concertedly by genetic pathways (Kenyon *et al.* 1993).

Later sequencing showed that *daf-2* had sequence similarity to the mammalian insulin receptor and insulin-like growth factor (IGF-1) receptor (Kimura *et al.* 1997) and *daf-16* encoded a FOXO-family transcription factor (Lin *et al*. 1997; Ogg *et al.* 1997), placing both *daf-2* and *daf-16* in the context of the larger insulin and insulin-like growth factor signaling pathway (IIS; reviewed by van Heemst 2010 as well as Murphy and Hu 2013). While the IIS pathway has many components and outputs, a simplified scheme for how it affects lifespan is as follows: in natural conditions, insulin-like peptides (ILPs), primarily expressed in neurons, bind to and either activate (Pierce *et al.* 2001) or inhibit (Murphy *et al.* 2007) DAF-2 activity. Activation of DAF-2 then causes the activation of AGE-1, a phosphoinositide 3-kinase, which in turn causes the activation of PDK-1, a phosphoinositide-dependent kinase, which activates AKT-1, a serine/threonine protein kinase. Finally, AKT-1 phosphorylates and inactivates DAF-16, which activates many protective genes and represses a variety of growth-related genes. In short, mutations of *daf-2* cause a decrease in DAF-2 signaling, thereby activating DAF-16 and increasing lifespan.

A reduction in IIS signaling was the first genetic pathway described to modify lifespan, but it is far from the only pathway. Upon genetic analysis, most of these pathways seem to fit into a similar structure: an upstream signal or perturbation activates a signaling cascade which eventually activates one or more transcription factors necessary for the lifespan phenotype. Differing upstream changes will result in a different strength of phenotype: for instance *daf-2* mutations extend lifespan more than *akt* mutations (Alam *et al.* 2010), but in both cases loss of *daf-16* transcription factor function will cause the

entire effect to be lost no matter the upstream change. This pattern is true of the lifespan extension caused by reduced mTOR signaling, which requires the function of the FoxA transcription factor PHA-4 (Sheaffer *et al.* 2008), loss of germ cells, which requires the activity DAF-12 (Hsin and Kenyon, 1999), reduced respiration, which requires HIF-1 activity (Lee *et al.* 2010), and dietary restriction, which requires differing transcription factors depending on the precise method of restriction (Kenyon 2010). This is not an exhaustive list, as many of these pathways require multiple transcription factors. For instance, loss of germ cells also requires the function of DAF-16 (Hsin and Kenyon 1999), and both a reduction in IIS signaling and mTOR signaling at least partially require the function of the mammalian Nrf homolog SKN-1 (Tullet *et al.* 2008; Robida-Stubbs *et al.* 2012). While there is overlap in the transcription factors which are necessary to extend lifespan, these pathways are considered separate or at least partially separate because they interact additively. For example, a loss of germ cells and a mutation in *daf-2* extend lifespan further than either perturbation alone (Hsin and Kenyon 1999).

The experiments referenced above were all conducted in *C. elegans*, but the major pathways which regulate lifespan are conserved across species. Many of the pathways discussed above have been found to regulate lifespan in multiple organisms, including flies, fish, and rodents. A few interventions, such as dietary restriction, have been tested more widely and even have beneficial effects in primates (Colman *et al.* 2009; Mattison *et al.* 2012). While we cannot directly experiment with human genetics, genetic analysis of long lived humans often reveals DNA variants in genes in the IIS pathway (Kenyon 2010). Otin *et al.* organized these shared aging pathways into what they called the nine "hallmarks of aging," which they classified as genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Focusing on mammals, they considered something a hallmark if "(1) it should manifest during normal aging; (2) its experimental aggravation should accelerate aging; and (3) its experimental amelioration should retard the normal aging process and

hence increase healthy lifespan." This highly conserved nature of lifespan regulation allows for investigation to proceed in multiple organisms according to their particular advantages with the knowledge that the knowledge derived from one will in all likelihood be applicable to another.

While many of the basic underpinnings of the aging process have been worked out, there is much more for us to discover. While we classify pathways based on their requirements for specific transcription factors and additive lifespan effects, there is vast and recurrent crosstalk between pathways that could be understood at a greater resolution. Perturbations that increase lifespan can also be tied intimately to metabolic regulation, fertility, and immune and stress responses. While in some instances these can be separated, it is important to understand the multifaceted functions that genes in these pathways play, especially as we attempt to invent human health interventions. To compound this complexity, all of these systems change their regulation with age and are affected differently in different tissues which themselves signal to one another. All of these complexities are exciting and important directions for future investigations to more fully understand how an organism ages.

During my thesis work at UCSF, I engaged in two projects investigating different aspects of aging. The first, discussed in detail in Chapter 2, was an effort to tease apart the mechanism by which a single pair of neurons responsible for tasting the environment can regulate lifespan. The nervous system reacts dynamically to both internal and external sensory queues and directs whole-body responses to them, including responses which can increase lifespan. In Chapter 3, I use the power of genetics to discover how a mutation incompatible with life can be resisted and explore the previously unknown cellular channel of communication that mediates this resistance.

## Chapter 2: How Neurons Regulate Lifespan

**Introduction:**

The longevity of an organism can be regulated by its sensory nervous system. This finding was first shown in 1999 when Javier Apfeld published that mutant *C. elegans* with widespread dysfunction in their sensory system lived much longer than controls. Lifespan increases were seen in a multitude of mutants, eleven of which caused disruption to cilia, which are required for the vast majority of sensory neurons to function, and four of which caused various deficits in sensation by neurons within the amphid, the primary sensory organ of *C. elegans*. Apfeld further showed that reducing sensory input to the animal via the laser-ablation of the amphid sheath cell could also increase the lifespan of an otherwise genetically normal animal. While the various methods of sensory disruptions had a large effect on lifespan, they did not have a measurable effect on development, growth, feeding, or reproduction, suggesting that it is not an obvious large-scale change in physiology but a lack of sensory input *per se* that increases lifespan. The lifespan increase largely required the function of *daf*-16/FOXO, and did not further extend the lifespan of *daf-2* mutants, suggesting that sensory input modifies lifespan through altering signaling in the IIS pathway.

While the neuronal regulation of lifespan was first discovered in *C. elegans*, it is by no means unique to that organism (Alcedo *et. al* 2013). Mutant *Drosophila* with olfactory defects show an increased lifespan (Libert *et al.* 2007), and mice with a genetically-overheated hypothalamus showed increased lifespan without an effect on caloric intake (Conti *et al.* 2006). Nervous systems are responsible for sensing and reacting to both changes in external conditions as well as internal state, and understanding how these processes impact health and longevity may have wide-spread implications for understanding and intervening in the variety of deleterious human health conditions that occur with age. A unique advantage to studying the neuronal regulation of lifespan in *C. elegans* is that instead of having neuronal nuclei made up of thousands of neurons to perform a function, each nematode has 302

neurons, bilateral pairs of which are largely responsible entire functions on their own. Fully understanding this simpler system may give us an understanding of the basic inputs and outputs that are being regulated by much larger groups of neurons in other organisms.

In *C. elegans*, even single pairs of neurons can have detectable effects on lifespan. At the ambient oxygen concentration of 20%, loss of the BAG neuron pair, which detects low levels of oxygen, can increase lifespan, whereas loss of the URX neuron pair, which detects high levels of oxygen, can decrease lifespan (Liu and Cai 2013). Further investigation proved these effects were dependent upon oxygen concentration, showing normal URX function is to increase lifespan in response to high oxygen whereas BAG decreases lifespan in response to low oxygen. These neuronal effects on lifespan oppose the normally toxic effects of high oxygen concentration and beneficial effects of low oxygen concentration on lifespan, suggesting that animals compensate for environmental change to maintain a specific length of life.

While the lifespan-effects of BAG and URX antagonize each other, they seem to be mechanistically separable, mediated through different guanylate cyclases and with no known common downstream target. In contrast, some neurons affect lifespan in concert with other neurons. For instance, the ablation of the olfactory neuron pair AWA causes a small increase in mean lifespan (11%), and the ablation of another olfactory neuron pair AWC has no significant effect on lifespan, but if both are ablated there is a larger lifespan increase than either alone (26%). Similarly, the ablation of the gustatory neuron pair ASI increases mean lifespan by 19%, and the ablation of the gustatory neuron pair ASJ has no significant effect on lifespan, but if both are ablated together then animals no longer show an increased lifespan. As was the case with the widespread sensory reduction experiments from Apfeld *et al.*, *daf-16*/FOXO was partially required for lifespan increase caused by olfactory-neuron ablation and fully required in the case of gustatory-neuron ablation (Alcedo and Kenyon 2004), suggesting that

sensory input on multiple neurons is somehow integrated and transmitted into a whole-organism response through cell-nonautonomous activation of *daf-16*.

Ablation of ASI is likely to be increasing lifespan through a loss of *daf-7*/TGFβ signaling. ASI is the only neuron which expresses *daf-7*/TGFβ under normal experimental conditions (Meisel *et al.* 2014), and mutation of *daf-7*/TGFβ causes an increase in lifespan (average mean increase of 23.8% over 6 replicates, max 39.3%) (Shaw *et al.* 2007) through signaling to downstream transcription factors *daf-3*/SMAD and *daf-16*/FOXO. Consistent with this, Shaw *et al.* also showed that *daf-7*/TGFβ mutation increases DAF-16 localization to the nucleus and the expression of the DAF-16-target gene *sod-3*, two measurements of DAF-16 activity.

While neuronal ablation can have lifespan effects specific to the presence or absence of other neurons, the signals that are mediating these effects and whether those effects are truly a matter of neuronal communication has yet to be explored. In this chapter I will discuss the first half of my thesis work where I investigated the connection between the neurons ASI and ASJ and lifespan. I show that a transgenic strain expressing tetanus toxin specifically in ASI, a perturbation which should disrupt (silence) chemical signaling from ASI, exhibits an increase in lifespan much like animals subjected to ablation of the neuron pair. I further show that this silencing effect increases expression of the DAF-16-target gene *sod-3*. Interestingly, this increase did not require *daf-16* or *daf-3*. Further investigation of the genetic epistasis of the lifespan phenotype was complicated by lack of replicability of the phenotype, especially in a strain in which the transgenic construct had been integrated into the genome. This difficulty was not resolved by the use of automated lifespan machines, even though they have a 48x increase in resolution over by-hand lifespan assays. Despite these difficulties, I will argue that both silencing of ASI and ablation of ASI are similar in that they fully require the function of *daf-3* to increase lifespan, and the majority of their lifespan increase, but perhaps not all, is also mediated through *daf-16.* My studies re-emphasize that perturbation of single gustatory neuron pairs can cell-nonautonomously

modify whole-animal physiology to increase lifespan. In addition, differences measured between ablation and silencing of neurons as well as differences with the *daf-7* mutation provide clues that the neuronal regulation of lifespan is likely more complicated than the regulation of a single secreted factor. Finally, my studies are also a cautionary tale showing that deeper dissection of small increases in lifespan can be hampered by the inherent stochasticity in the length of *C. elegans* lifespan.

**Methods and Materials:**

*C. elegans strains*

Wild-Type (N2)

CF4126: *muEx641*[pPC30(p*gpa-4*::GFP::Tetx) + p*unc-122*::RFP]

CF4138: *muIs84* [pAD76(p*sod-3*::GFP)]; *muEx641* [pPC30(p*gpa-4*::GFP::Tetx) + p*unc-122*::RFP]

CF4152: *daf-16(mu86)*I; *muEx641*[*Punc-122*::RFP + pPC30(*Pgpa-4*::GFP::Tetx)]

CF4153: *kri-1(ok1251)*I; *muEx541*[*Punc-122*::RFP + pPC30(*Pgpa-4*::GFP::Tetx)]

CF4154: *muEx642*[pPC31 (*Ptrx-1*::GFP::Tetx) + *Podr-1*::RFP]

CF4170: *daf-16(mu86)*I; *muIs84*[pAD76(*sod-3*::gfp)]; *muEx641*[*Punc-122*::RFP + pPC30(*Pgpa-4*::GFP::Tetx)]

CF4171: *kri-1(ok1251)*I; *muIs84*[pAD76(*sod-3*::GFP)]; *muEx541*[*Punc-122*::RFP + pPC30(*Pgpa-4*::GFP::Tetx)]

CF4172: *daf-3(mgDF90)*X; *muIs84*[pAD76*(sod-3*::GFP)]; *muEx541*[*Punc-122*::RFP + pPC30(*Pgpa-4*::GFP::Tetx)]

CF4281: *oyIs84* [*Pgpa-4*::TU#813 + *Pgcy-27*::TU#814 + *Pgcy-27*::GFP + *Punc-122*::DsRed]

CF4282: *muIs224*[*Pgpa-4*::GFP::Tetx + *Punc-122*::RFP]

CF4283: *muIs225* [*Ptrx-1*::GFP::Tetx + *Podr-1*::RFP]

CF4284: *muIs224*[*Pgpa-4*::GFP::Tetx + *Punc-122*::RFP]; [rec caspases under *Pgpa-4*, *Pgcy-27* + *Pgcy-27*::GFP]

CF4285: *muIs225* [*Ptrx-1*::GFP::Tetx + *Podr-1*::RFP]; [rec caspases under *Pgpa-4*, *Pgcy-27* + *Pgcy-27*::GFP]

CF4286: N2E; *muIs224*[*Pgpa-4*::GFP::Tetx + *Punc-122*::RFP]; *muIs225*[*Ptrx-1*::GFP::Tetx + *Podr-1*::RFP]

*Dauer assay*

Adult animals were allowed to lay eggs at 20°C for 2-3 hours, after which adults were removed and eggs were incubated at 25°C for 48 hours. Animals were then washed off plates and incubated for 1 to 2 hours in 1% SDS, which is lethal to non-dauer animals. After incubation the animals were washed, re-plated, and kept at 15°C overnight before being scored as dauers or non-dauers.

sod-3 *expression assay*

Animals collected from the same plate were divided into experimental or control conditions based on expression or lack thereof of RFP. Animals were then paralyzed with 10mM levamisole for 10 minutes, corralled together into rows, and then imaged at 50x under bright field and to measure GFP fluorescence. Images contained ~5 animals per image. Light level and exposure time (108ms) were selected so as to provide the brightest pictures without any saturation and were kept constant both within and across experiments. To analyze the images in as unbiased a manner as possible, the entire body of each animal was outlined under a brightfield image in imageJ and then the average intensity of that outline was measured in the corresponding fluorescent image. A large section of the background fluorescence was measured and this number was subtracted from all measurements. Significance was measured by comparing the control and experimental group utilizing a two-tailed t-test. While the tetanus toxin construct itself was bound to GFP, the intensity of that construct was exceedingly dim and we required the use of an RFP co-injection marker to separate Tetx-expressing animals from controls at magnifications of 50x and lower. In addition, the increase in expression was not restricted to the ASI

neuron pair alone but also appeared throughout the head as well as the nerve cord, parts of the vulva, and the tail, all locations the p*sod-3*::GFP construct is expressed under control conditions (Libina et al. 2003). Due to both these pieces of evidence, we conclude that the increased GFP measured is due to the effects of tetanus toxin in ASI.

*RNAi:*

HT-115 RNAi bacteria were obtained from existing Ahringer and Vidal libraries, or in the case of *daf-16* RNAi a clone created in the Dillin lab was used. The control for all RNAi experiments was L4440 vector. For each experiment, single colonies were grown overnight at 37°C in 3 mL LB with 100µg/mL carbenicillin and 10µg/mL tetracycline. The next day, the resulting stationary-phase cultures were diluted tenfold with LB containing 100µg/mL carbenicillin and grown for two hours at 37°C. 1M IPTG was then added to the culture to reach a final concentration of 2mM, after which 100µL of the resulting culture were spotted onto NGM plates. The bacteria were grown on these plates overnight at 30°C. Animals were placed on plates the following day in all experiments except those involving a drug condition, in which case drugs were added and left to diffuse into the plate for an additional day before the addition of experimental and control animals.

*Real-time qPCR:*

RNA extraction, cDNA preparation, and real-time qPCR were performed as described previously (Van Gilst *et al.* 2005) from 1 biological replicate of each condition animals were grown to young adulthood before being washed and flash-frozen for RNA extraction. Data were standardized to a tubulin control primers.

*Statistical Analyses:*

On all dot plots and bar graphs, bars represent the mean, and error bars represent the SEM. For lifespan assays, log-rank tests were used to test for significance. For *sod-3* expression experiments, a two-tailed Student's t-test was used to test for significance.

*Lifespan Analysis:*

Life span analysis was performed at 20°C as described previously (Apfeld and Kenyon 1998). Animals were grown on OP50 *E. coli* or HT-115 RNAi bacteria and transferred to fresh plates on the first day of adulthood ("day 1") and every 2 days thereafter. Every two days, starting on day 1, animals were scored. Animals that moved were scored as alive, animals that did not move, even after being prodded with a platinum wire, were scored as dead, and animals that could not be found or displayed phenotypes such rupturing or bagging were censored.

**Results:**

*Silencing the gustatory neuron pair ASI increases lifespan*

To test whether disrupting the signaling of ASI, as opposed to ablating it entirely, could increase lifespan, I expressed the tetanus toxin light chain (Tetx) under the ASI-specific promoter *pgpa-4* (Figure 2.1 A; Jansen *et al.* 2004). Tetanus toxin silences, or disrupts, neurotransmission by blocking the release of both small clear-core vesicles and large dense-core vesicles, but should not affect communication through gap junctions, which do not require vesicles to transmit electrical current (Schiavo *et al.* 1992; McMahon *et al.* 1992). The stochastic inheritance of the transgenic construct caused adults expressing the construct to produce progeny with and without Tetx in ASI. This allowed for comparison of animals raised to adulthood on the same plates and produced by the same group of parents. Tetx in ASI significantly extended lifespan in otherwise wild-type animals across five replicates, with median

lifespan increasing by an average of 14.9% (Figure 2.1 B, C). All lifespans discussed in this section are recorded in Table 2.1.

In order to reduce variance in the lifespan measure, more efficiently cultivate and select animals for experiments, and lay the groundwork for more complicated experiments involving both ASI and ASJ, I integrated the extrachromosomal array expressing Tetx in ASI into the animals' linear DNA via UV radiation. Unfortunately, after backcrossing, this strain tended to have a smaller lifespan increase than the extrachromosomal strain from which it was derived. Across five replicate experiments, the average increase in median lifespan was only 9.8%, and only three out of the five experiments yielded statistically significant results between wild-type and Tetx strains by the log-rank test (Figure 2.1 D).

*The automated lifespan machine*

As a part of my thesis work I constructed and operated automated lifespan machines (Stroustrup *et al.* 2013). These machines scan sixteen plates of animals once each hour over the course of a lifespan experiment. These machines not only allow a large number of experiments to be run simultaneously, but they also produce higher-resolution measurements (Figure 2.1 B vs. C) and better statistical power. Constructing the machines required me to physically modify the chassis and recalibrate the focal length of commercially available scanners as well as laser-cutting rubber sheets to hold plates of worms in position on glass plates precisely. They also required managing communication between multiple computers to run the scanners, store the data, and analyze the results. The instructions for modifying the scanners and the software used to run them and analyze the results were all created by Stroustrup *et al.*; still, implementation of them in the Kenyon lab was a substantial project. The machines were effective and useful, however some software issues (such as scanners spontaneously stopping) and rubber sealing issues (resulting in desiccated plates) remained chronic barriers that needed to be managed.

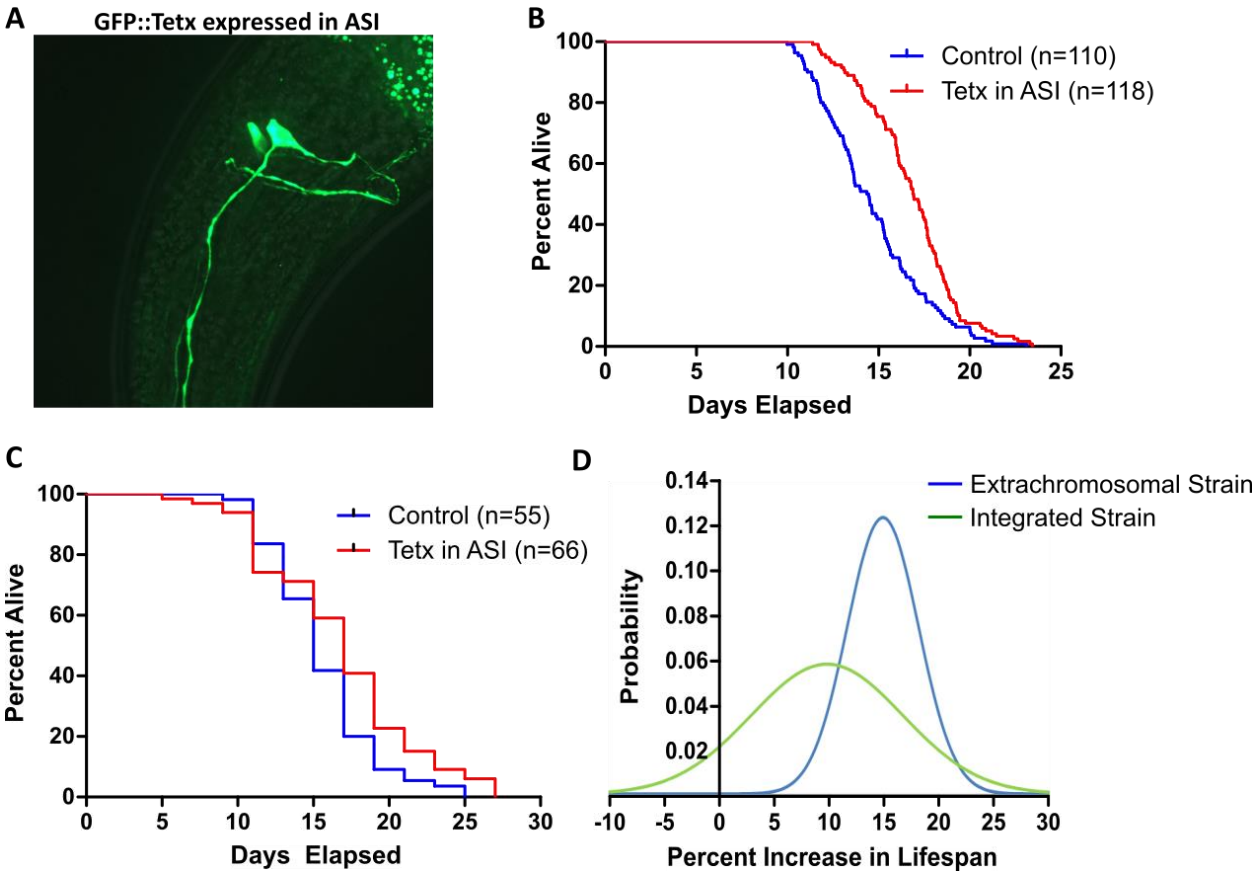



**Figure 2.1:** Silencing the ASI neuron extends lifespan. (A) Image of the *pgpa-4::*Tetx::GFP construct expressed only in the ASI neuron pair, 600x magnification. (B) Tetx in ASI increases lifespan, measured by the automated lifespan machine, $p<0.0001$ by log-rank test. (C) Tetx in ASI increases lifespan, measured by hand, $p=0.0125$ by log-rank test. (D) Plots of the normal distributions of percent increase in median lifespan of Silenced ASI vs. wild-type. The two plots represent two strains, one that expresses the Tetx construct through an extrachromosomal array, and the other in which that array has been integrated into the genome (mean and standard deviation based on five replicates for each strain).

*Silencing ASI increases expression of the* daf-16-*target gene* sod-3

The extended lifespan caused by ablation of ASI requires the function of *daf-16* (Alcedo and Kenyon 2004). I tested to see if silencing ASI increased the expression of any *daf-16*-target genes, both to determine if *daf-16* was activated by silencing ASI with Tetx as well as to develop an assay, faster than lifespan assays, to explore the genetic epistasis of effects caused by silencing ASI. A preliminary RT-qPCR experiment revealed that silencing ASI increased expression of *sod-3* and *mtl-1* but not that of another DAF-16-regulated gene, *dod-8*. These changes in expression were lower in all cases than the increase produced by ablation of ASI (Figure 2.2). Because *sod-3* showed the highest increase in response to the silencing of ASI, I then tested if this effect could be seen with a transcriptional fluorescent reporter by silencing ASI in a strain that expressed GFP under the *sod-3* promoter. Tetx in ASI caused a significant increase in *sod-3* expression under these conditions (Figure 2.3 A,B), but surprisingly this did not require DAF-16 or DAF-3 activity (Figure 2.3 C, D). The *kri-1(ok1251)* null mutation, which prevents the lifespan extension of germline-defective animals but not *daf-2* mutants, also had no effect on *sod-3* expression (Figure 2.3 E). The quantifications shown are for whole-body fluorescence, but similar trends were seen when only the head or only the anterior intestine were quantified (data not shown). All *sod-3* experiments were conducted with an extrachromosomal Tetx construct.

*Silencing of ASI, rather than ablating ASI, partially reduces the requirement of* daf-16/FOXO

Because silencing ASI did not require the function of *daf-16* or *daf-3* to increase the expression of *sod*-3, I wondered if these genes were also dispensable for the lifespan phenotype. I tested the lifespan-effects of *daf-16* RNAi on both silenced and ablated-ASI conditions as well as the lifespan-effect of the null mutation *daf-16(mu86)* on the silenced ASI strain (Figure 2.4). In all cases, I found that *daf-16* mutants exhibited the expected reduction of lifespan when compared with the wild type condition (an average 22.7% reduction in median lifespan). In two experiments, I tested the lifespan-effects of

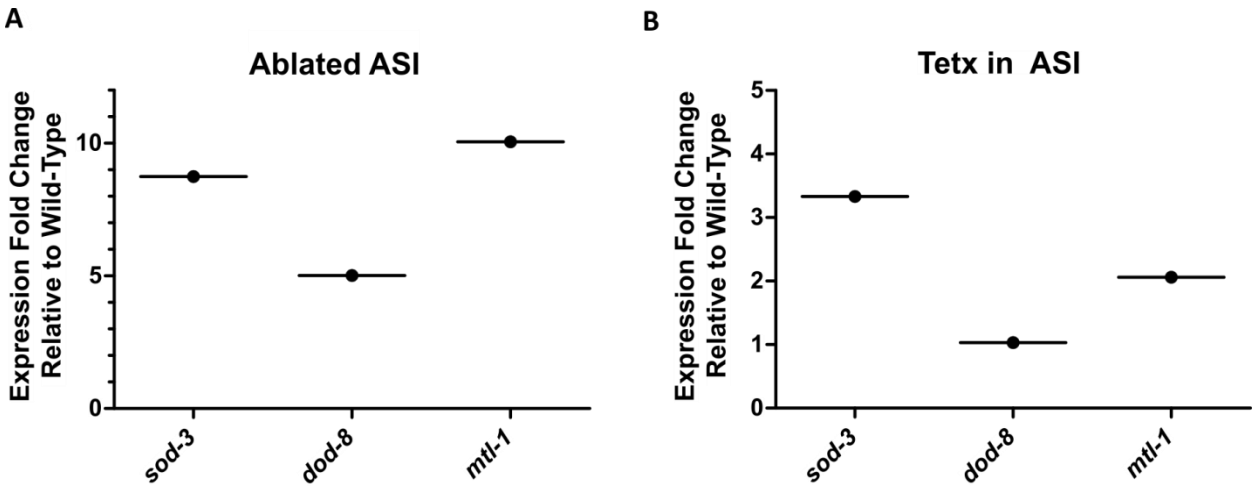



**Figure 2.2:** Ablation, more than silencing, of ASI increases expression of *daf-16*-target genes. (A) Ablation of ASI increases expression of *sod-3*, *dod-8*, and *mtl-1* in a single RT-qPCR experiment. (B) Tetx in ASI increases expression of *sod-3* and *mtl-1* but non *dod-8* in a single RT-qPCR experiment.

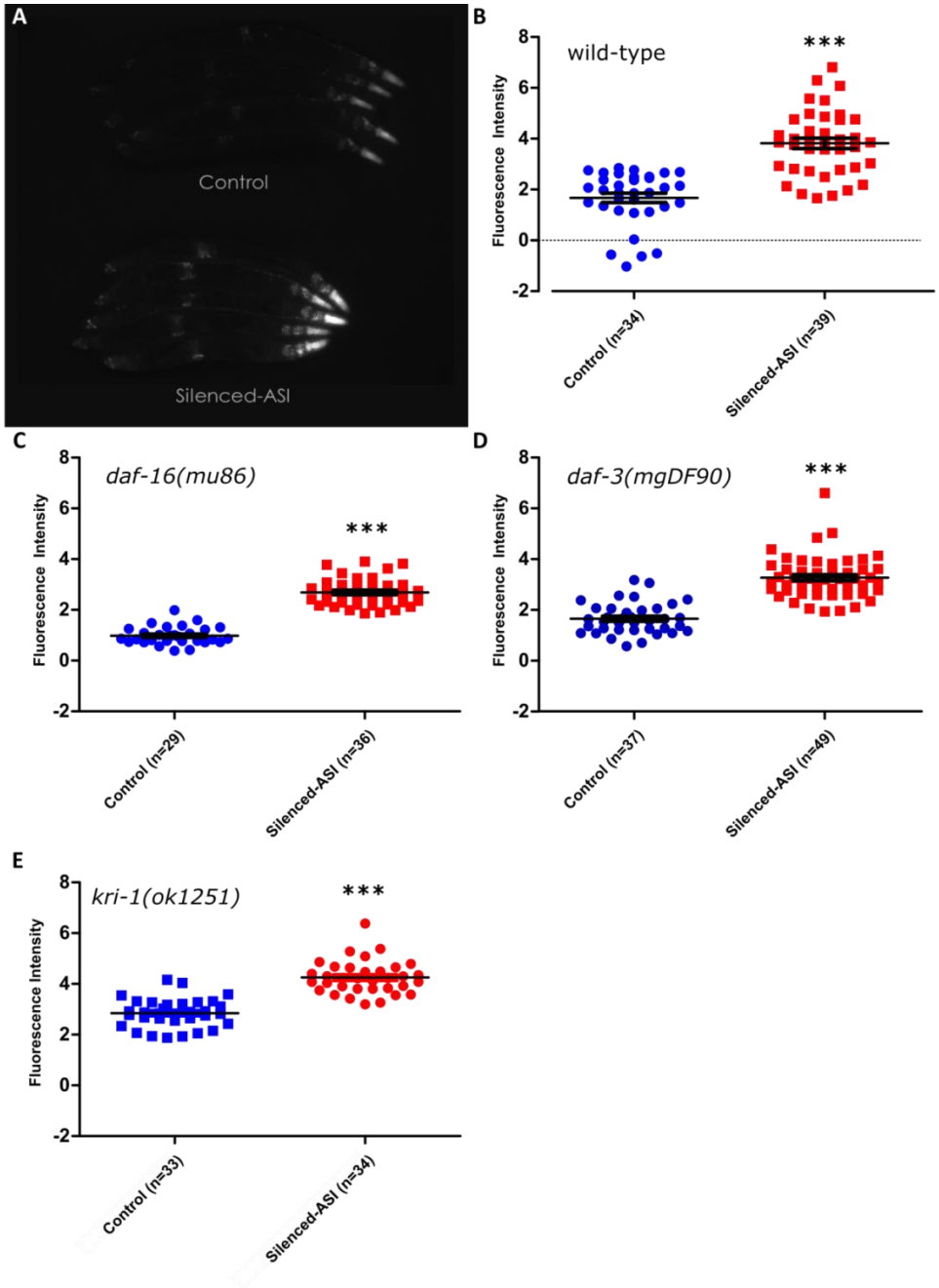



**Figure 2.3:** Silencing ASI increases *sod-3* expression independently of *daf-16*, *daf-3*, or *kri-1*. (A) Image of the increase in *sod-3* expression caused by silencing of ASI, 50x magnification. (B) Silencing ASI significantly increases whole-body *psod-3*::GFP fluorescence in a wild-type background, ***p<0.001 by Student's t-test. (C) Silencing ASI significantly increases whole-body *psod-3*::GFP fluorescence in a *daf-16(mu86)* null background, ***p<0.001 by Student's t-test. (D) Silencing ASI significantly increases whole-body *psod-3*::GFP fluorescence in a *daf-3(mgDF90)* null background, ***p<0.001 by Student's t-test. (E) Silencing ASI significantly increases whole-body *psod-3*::GFP fluorescence in a *kri-1(ok1251)* null background, ***p<0.001 by Student's t-test.

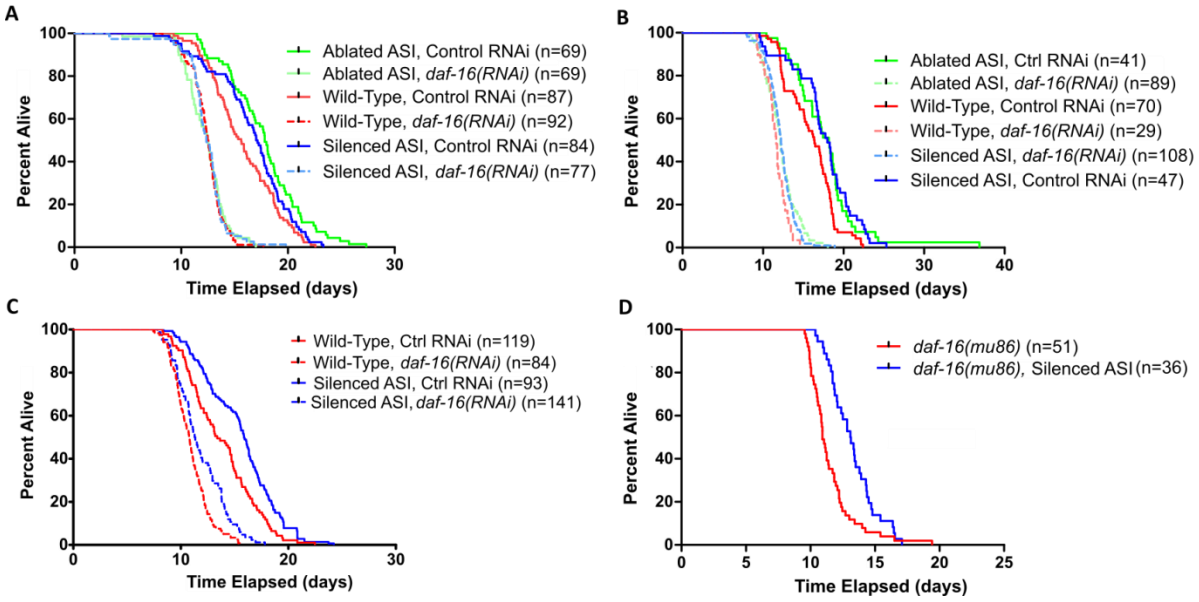



**Figure 2.4** The effects of silencing and ablating ASI in *daf-16* reduction of function conditions. (A) Neither ablation nor silencing of ASI (integrated strain) increase lifespan in animals grown on *daf-16* RNAi by log rank test. (B) Both ablation and silencing of ASI (integrated strain) significantly increase the lifespan of animals grown on *daf-16* RNAi, $p<0.03$ by log-rank test before multiple comparisons. (C) Silencing ASI in animals grown on *daf-16* RNAi significantly increases lifespan, $p<0.001$ by log-rank test before multiple comparisons. (D) Silencing ASI in the *daf-16(mu86)* null background significantly extends lifespan, $p<0.001$ by log-rank test.

ablating ASI in animals grown on *daf-16*-RNAi bacteria. These experiments yielded mixed results, with one showing no significant difference and the other a significantly different lifespan curve with a 4% increase in median lifespan (Figure 2.4 A, B). Across seven replicates, I found that silencing ASI in a *daf-16* null background or in animals grown on *daf-16* RNAi produced a significant difference in lifespan in four, or just over half, of the cases with an average increase in median lifespan of 6.5% (Figure 2.4 A-D). The lack of reproducibility is likely explained by the drastic reduction in lifespan increase when compared with conditions in which DAF-16 function is normal. Because there is still often a detectable difference, I argue that signaling through *daf-16* mediates most of the lifespan increase of silencing ASI but not the entirety of it. I did not conduct enough experiments in which ASI was ablated to know if the very small but significant increase in lifespan in one of two experiments was due to random chance, or if ablation of ASI also has a partially *daf-16*-independent increase in lifespan.

*Both silencing and ablation of ASI require* daf-3/*SMAD to increase lifespan*

While silencing and ablation of ASI may not entirely require the function of *daf-16*/FOXO to extend lifespan lifespan, their lifespan-extension phenotypes both fully required the function of *daf-3*/SMAD, a transcription factor required for *daf-7/*TGFβ mutation to increase lifespan. In no experiments did I see a significant increase in reduced-ASI function over wild type lifespan in *daf-3*-reduction-of-function backgrounds (Figure 2.5 A,B).

*Activity of KRI-1 is not required for silencing of ASI to increase lifespan*

ASI ablation has been shown to reduce the number of germ cells in *C. elegans* (Dalfó *et al.* 2012), and a loss of germ cells has been shown to increase lifespan through the activity of the KRI-1 adaptor protein (Berman and Kenyon 2006). I tested if this was a possible mechanism by which silencing ASI increases lifespan by silencing ASI in the *kri-1(ok1251)* null background (Figure 2.5 C). In the only

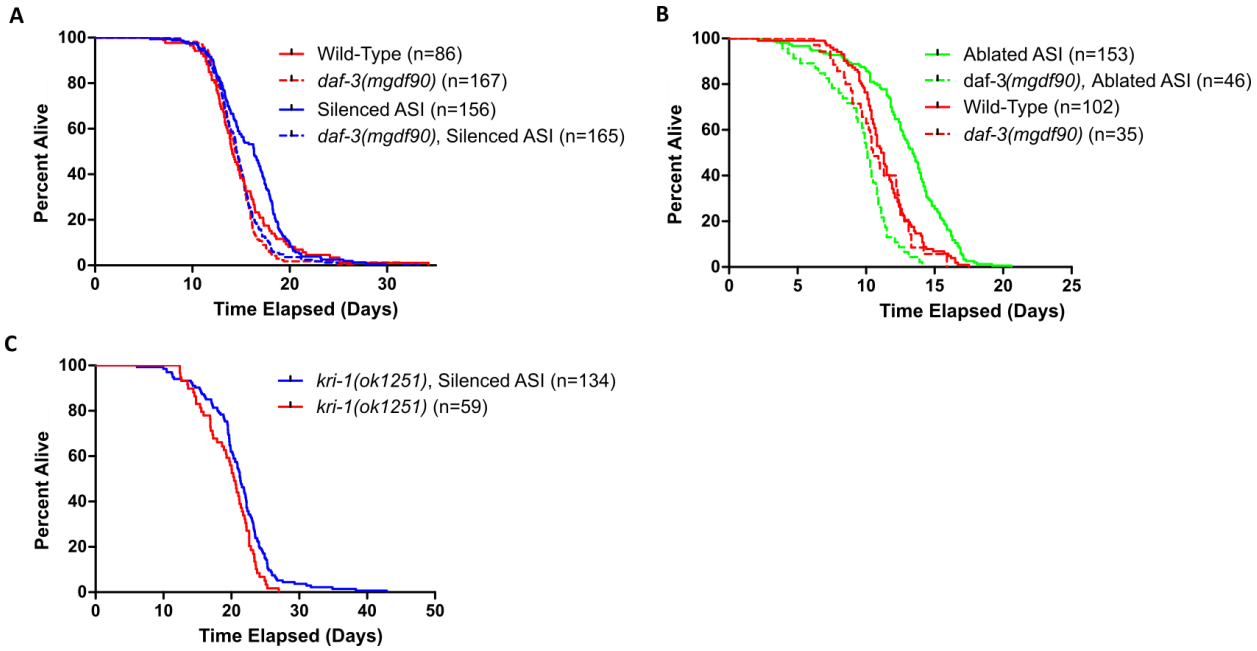



**Figure 2.5:** *daf-3,* but not *kri-1*, is required for silencing ASI to extend lifespan. (A) Silencing of ASI failed to increase lifespan in the *daf-3(mgdf90)* null background. (B) Ablation of ASI failed to increase lifespan in the *daf-3(mgdf90)* null background. (C) Silencing of ASI significantly increased lifespan in the *kri-1(ok1251)* null background, p=0.01 by log-rank test.

lifespan I conducted with this strain, I saw that Tetx significantly increased lifespan in the *kri-1(ok1251)* background. Strangely, the control *kri-1(ok1251*) single mutants seemed to have a much longer lifespan than wild-type animals, a phenotype either not reported in the literature or due to background differences in the strains.

*The effects of silencing the ASJ neuron pair on lifespan*

Similar to the strain with Tetx in ASI, I created a strain in which the gustatory neuron pair ASJ was silenced by expressing Tetx under the ASJ-specific promoter *ptrx-1*. Previous experiments by Alecdo and Kenyon (2004) showed that ablation of ASJ had no effect on wild-type lifespan but completely eliminated the lifespan extension caused by ablation of ASI. I saw very different results with silencing of ASJ. Silencing of ASJ produced a significant decrease in wild-type lifespan, no effect on the lifespan of animals with ablated ASI, and only a small effect on the lifespan of animals with silenced-ASI (Figure 2.6A).

*The strain with the integrated Tetx in ASI construct shows resistance to* hsf-1 *RNAi*

A reduction in the transcription factor *hsf-1* significantly reduces lifespan on its own, and is required for many interventions to increase lifespan, including a reduction of IIS (Morley and Morimoto 2004) and reduction of mTOR signaling (Seo *et al.* 2013). Therefore I was surprised to find, in each of two experiments, that silencing of ASI (but not ablation) significantly increased the lifespan of animals grown on *hsf-1* RNAi (Figure 2.6 B). I conducted the experiment again on the extrachromosomal strain with Tetx in ASI and found in that case the lifespan extension entirely required the function of *hsf-1* (Figure 2.6C), pointing towards an explanation where disruption of function at the genomic site of integration or perhaps a mutation linked to the site of integration grants the animals resistance to the harmful effects of *hsf-1* RNAi.

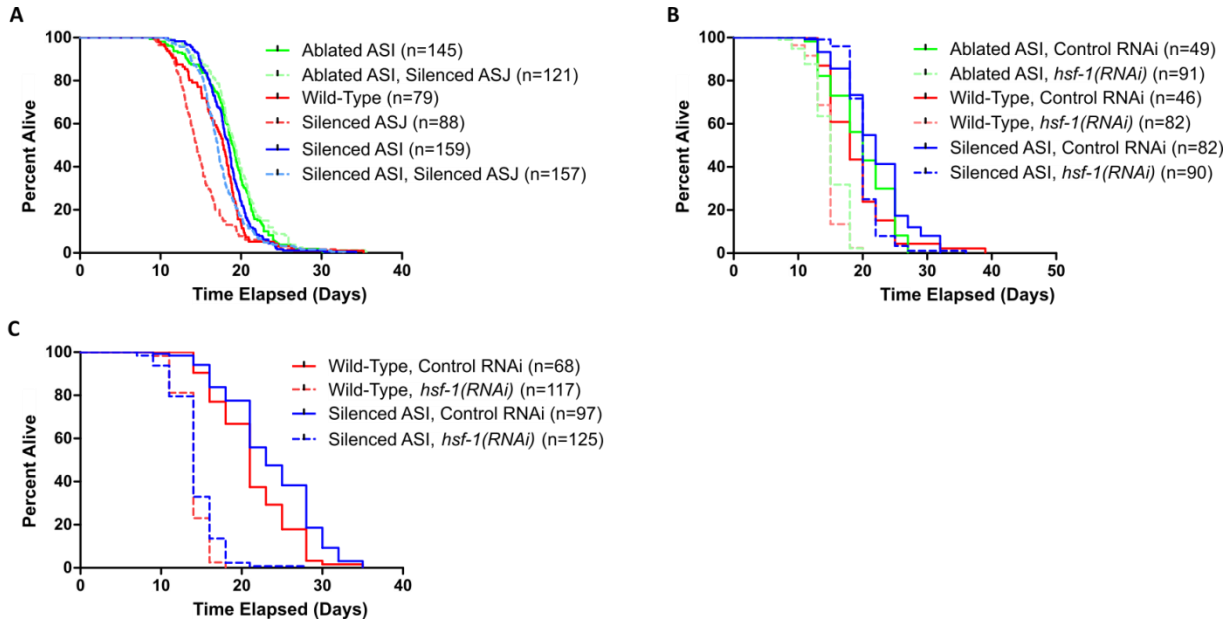



**Figure 2.6:** Effects of silencing ASJ and *hsf-1* RNAi on the lifespan extension caused by silencing of ASI. (A) Silencing ASJ reduces the lifespan of wild-type, reduces the lifespan of animals with Silenced ASI to a lesser extent, and does not at all reduce the lifespan of animals with ASI ablated ($p<0.001, p=0.003, p=0.22$, respectively by log-rank test before multiple comparisons). (B) Silencing ASI (integrated strain) increases the lifespan of animals grown on *hsf-1* RNAi, $p<0.0001$ by log-rank test. (C) Silencing ASI (extrachromosomal strain) does not increase the lifespan of animals grown on *hsf-1* RNAi.

*Does tetanus toxin actually block vesicle-based signaling?*

While Tetx has previously been shown to be functional in *C. elegans* (Macosko *et al.* 2009), I wanted to see if I could measure its effects on a phenotype other than lifespan. I attempted this in two ways: first by comparing the effect of *daf-7* mutation, ASI ablation, and silencing of ASI on dauer formation at 25 $^{o}$C, and second by trying to measure the secretion of DAF-26 from ASI in a *pgpa*-4::*daf-26*::RFP strain. In the dauer assay, *daf-7* mutants showed a high level of dauers, as previously reported (Figure 2.7; Ren *et al.* 1996). I was surprised to find that ablation of ASI showed fewer dauers than *daf-7* mutants, and animals with silenced-ASI showed no dauer formation whatsoever (Figure 2.7).

I attempted to measure the level of secretion from ASI by measuring the intensity of RFP signal from coelomocytes in animals with RFP-tagged DAF-26 expressed in ASI. Unfortunately, even after multiple attempts and consultation with multiple parties that had conducted the assay, I could not see a reliable change in signal between positive and negative controls.

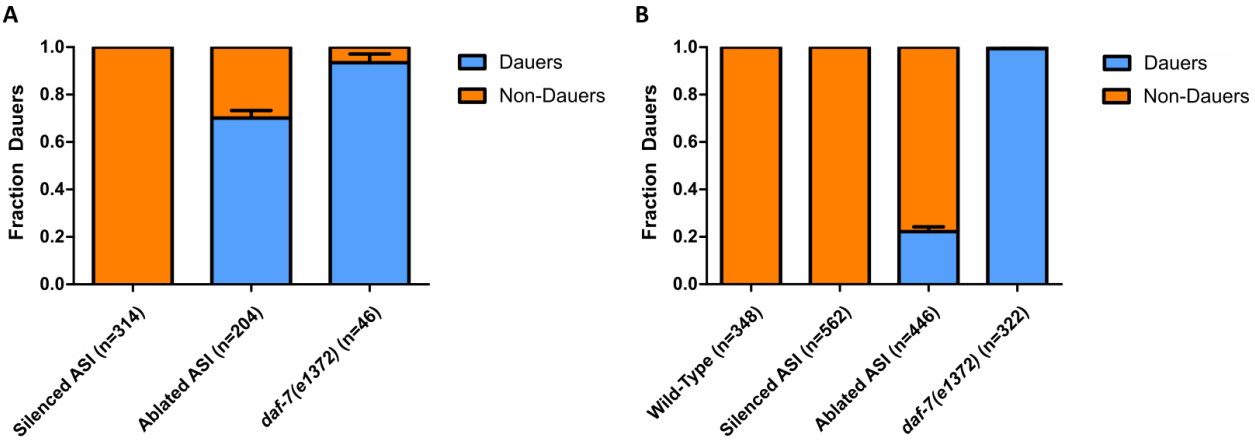



**Figure 2.7:** Effect of silencing and ablating ASI on dauer formation. (A,B) Silencing ASI produces no dauers, ablating ASI produces some dauers, and *daf-7* mutation produces constitutive dauer formation at 25 °C.

**Discussion:**

Numerous publications have shown that, across species, the nervous system plays an important role in the regulation of lifespan. *C. elegans* provide an excellent opportunity to explore how single neurons can shape the course of health and longevity, and while some neurons have been discovered which can affect lifespan and some important pathways by which they mediate this effect have been established, the field is still in its infancy. What signals are being detected, how they are being processed and measured, and which signaling compounds finally mediate a shift in lifespan are open questions that remain to be explored.

In this chapter I have shown that disruption of signaling from the gustatory neuron pair ASI through either ablation of the neuron or silencing of the neuron with tetanus toxin can extend the lifespan of *C. elegans.* I have further shown that this extension completely requires the function of *daf-3*/SMAD and mostly requires the function of *daf-16*/FOXO, two transcription factors required for mutation of *daf-7/*TGFβ, a secreted factor only expressed in ASI under normal conditions, to extend lifespan. While ASI has been shown to affect the germ cell pool size in *C. elegans*, the increased lifespan of silenced-ASI animals in a *kri-1(ok1251)* null background show that this is unlikely the mechanism by which ASI-silencing increases lifespan.

Despite the fact that silencing ASI, ablating ASI, and mutation of *daf-7*/TGFβ all share the requirement of *daf-3*/SMAD to increase lifespan, it is interesting to note that these conditions are not identical. In terms of lifespan, *daf-16* is entirely required for *daf-7* mutation to increase lifespan, but only seems partially required for the increase caused by disruption of ASI signaling. Whether this *daf-16*-independent lifespan increase is mediated by another secreted molecule from ASI or some effect mediated by loss of communication to another neuron remains unknown. The *daf-16*-independent component of the lifespan increase also reinforces the idea that ASI is playing a more complex role than simply altering DAF-7 secretion in response to sensory input. Whether this *daf-16*-independent increase

in lifespan is mediated by a chemical signal(s) from ASI or a downstream effect of neuronal communication to other neurons remains to be seen.

*daf-7* mutants and silenced-ASI animals show a difference not only in lifespan but also in dauer phenotype. At 25$^{o}$C the vast majority of *daf-7* mutant animals formed dauers, whereas only some animals with ASI ablated did so and no animals with silenced-ASI were found to do so. A likely explanation for this is that while ASI is the primary neuron responsible for DAF-7 secretion under normal conditions, it is not the only neuron capable of doing so (Meissel *et al.* 2014), and therefore some compensatory mechanism to increase DAF-7 secretion in response to specific kinds of reduced-SASI signaling may exist to regulate the prevalence of dauers. *daf-7*-mutant animals, having a defect in the gene itself, would not have access to any such compensatory mechanism. Despite ablation and silencing of ASI having a similar lifespan phenotype, the difference in dauer phenotype shows that the perturbations are physiologically different, perhaps due to some residual, but reduced, chemical signaling that exists in ASI expressing Tetx, physiologically important signaling mediated through gap junctions, or some unknown response to the presence of the neuron cell body itself. Results from the attempted secretion assays would have helped to narrow down the possible explanations, but unfortunately they did not render meaningful results.

The *sod-3* expression data is perhaps the most surprising. A canonical *daf-16*-target gene, in the silenced-ASI condition it shows a robust increase in expression that is independent of *daf-16*. Additionally, the expression pattern of *sod-3* itself is interesting. Previous studies have shown that *daf-2* mutant animals have an increase throughout the entire body (Libina *et al.* 2003), whereas *daf-7* animals show an increase in expression but only in tissues that are seen to express GFP under normal conditions, namely the head, nerve cord, vulva, and tail (Shaw *et al.* 2007). Despite this similarity, the increase in *sod-3* expression does not require *daf-3*, a transcription factor necessary for the *daf-7* mutant

phenotypes of dauer and lifespan. It is unclear through which mechanism, therefore, silencing ASI is increasing *sod-3* expression or what that might mean physiologically for the animal.

Silencing and ablation show even more dissimilarity in the case of ASJ. Ablation of ASJ shows a reduction in the lifespan of ASI-ablated animals, but no effect on wild-type animals, whereas silencing ASJ seems to show the opposite effect. This observation is subject to numerous caveats, including the fact that I did not measure the lifespan effects of ablating ASJ in my own hands as well as the fact that I only conducted the lifespan of silenced-ASJ animals once. However, if this observation proves replicable it re-emphasizes the complicated nature of neurons, which are not simple on or off switches but rather can increase or decrease the secretion of a variety of signaling molecules in response a diversity of inputs. Understanding this complex nature fully will require more precise tools than tetanus toxin or whole-cell ablation.

While integration can lead to a stable transgenic strain more easily grown in large quantities, my experience shows that the integration process comes with caveats. It is important to recover multiple integrated strains and test them to find the one with the strongest phenotype so that replicability is not lost, especially in the case of a small lifespan increase. It seems somewhat unlikely to me that the random site of integration would provide resistance to the highly harmful lifespan effect of knocking down *hsf-1* with RNAi, but this too could have been avoided if multiple integrants had been recovered. Additionally, the mechanism by which this strain is resistant to *hsf-1* RNAi remains an interesting and unexplored line of inquiry.

In conclusion, the silencing of ASI signaling leads to a significant lifespan increase similar to that of ablating ASI. This increase seems to be mostly mediated through TGFβ signaling, but it is also partially *daf-16* dependent. The silenced-ASI strain increases *sod-3* in a *daf-16*-independent manner and shows no dauer phenotype. These findings, along with preliminary but similarly surprising findings with the silenced-ASJ strain, reveal that the neuronal regulation of lifespan is likely a complex phenotype

differentially affected by wide-scale silencing of chemical signaling with tetanus toxin versus ablation of the entire neuron.

## Chapter 3: Communication between the two Fundamental Lifespan Regulators mTOR and Heat Shock

**Introduction:**

The health and longevity of an organism depends on robust proteostatic machinery to keep proteins functioning properly. One major source of cellular quality control is the heat-shock response (HSR). The heat-shock response increases expression of a variety of chaperones in response to many stresses, including heat and heavy metals. These so-called heat-shock proteins are coordinately regulated by one (in yeast and invertebrates) or multiple (in vertebrates) transcription factors called heat-shock factors. In addition, heat-shock factors act under normal conditions to promote longevity and developmental growth to adulthood.

In *S. cerevisiae, C. elegans*, and *Drosophila*, a loss of heat-shock factor leads to developmental defects at non-stressful temperatures. In yeast, defects in growth caused by lack of HSF activity can be rescued by restoring basal expression levels of two heat-shock proteins, Hsp70 and Hsp90, but additional Hsf1 target genes are required for resistance to heat stress (Solís *et al.* 2016). In *Drosophila*, loss of Hsf causes arrest at the first or second larval-instar stage of development, as well as defects in oogenesis (Jedlicka *et al.* 1997). Unlike in yeast, Jedlicka *et al.* found that this essential developmental function in *Drosophila* was not mediated through canonical heat-shock genes. In *C. elegans,* HSF-1 is needed for progression past the L2-L3 larval stage, and it has been shown to regulate a developmental program that is distinct from the heat-shock response by binding to a promoter sequence different from the canonical sequence (Li *et al.* 2016).

Heat-shock factors are important but not essential for mouse development. Mouse cells lacking HSF1 or both HSF1 and HSF2 can be cultured and show normal constitutive transcription of heat-shock proteins, although cells without HSF1 are sensitive to heat stress (McMillan *et al.* 1998; Solís *et al.* 2016; Zhang *et al.* 2002). *In vivo, Hsf1*(-/-) mice display additional phenotypes, including growth retardation,

female infertility, prenatal lethality (Xiao *et al.* 1999, Christians *et al.* 2000), and neurological defects (Santos and Saraiva 2004, Takaki *et al.* 2006), with the penetrance of prenatal lethality partially regulated by the genetic background. *Hsf2*(-/-) mice also display abnormalities, in both gametogenesis and brain structure (Chang *et al.* 2006).

While these studies establish that a heat-stress-independent activity of heat-shock factor is inextricably tied to development, the genetic pathways and mechanisms by which heat-shock factors promote growth and development remain largely unexplored. To address this question in an unbiased way, we conducted a genetic screen in *C. elegans* for suppressor mutations that allow *hsf-1(sy441)* mutant animals to grow to adulthood. The *sy441*-mutant HSF-1 protein lacks its transactivation domain, severely blunting its ability to regulate canonical heat-shock response genes (Hajdu-Cronin *et al.* 2004). These animals grow well at lower temperatures (15-20°C in our hands), but when grown at temperatures of 25°C or higher, they display a developmental phenotype similar to that of *hsf-1* loss-of-function mutants, arresting at the L2-L3 stage (Li et al. 2016).

In this study, we show that mutations in multiple complementation groups allow developing *sy441* mutants to reach adulthood. These mutations dramatically postpone, but do not eliminate, growth arrest within the strain's lineage, as the rescued adults produce progeny that fail to progress through development. We show that one of these mutations is a putative null allele of *rsks-1*/S6Kinase, a translational activator regulated by the mechanistic Target of Rapamycin mTOR. We find that knockdown of various mTOR-pathway components can also rescue the phenotype. Unexpectedly, we find no evidence that phenotypic rescue is mediated by inhibition of translation. Nor does rescue appear to be due to the activation of other stress responses such as the ER unfolded-protein response or the mitochondrial unfolded-protein response; nor is it due to the activation of various stress-resistance pathways known to extend adult lifespan.

We find that the rescue mediated by loss of S6 Kinase is dependent upon residual activity of the mutant HSF-1(sy441) protein, and that transgenically-increased expression of the *hsf-1(sy441)* allele is sufficient on its own to rescue development. However, we see no evidence that the S6 kinase mutation increases expression of *hsf-1* nor any of a variety of target genes we examined. We conclude that loss of S6 kinase could potentially elevate other targets of *hsf-1* or, alternatively, provide other factors that allow low levels of HSF-1 activity to sustain growth to adulthood.

**Materials and Methods**:

C. elegans *strains:*

Wild-type (N2)

VB654: *rsks-1(sv31);* svEx136[*unc-36(+) rsks-1(+) sur-5::gfp*]

AGD794: *hsf-1(sy441)*; uthIs225[*sur-5p::hsf-1(sy441); myo2p::tdTomato*]

CF3951: *hsf-1(sy441)*

CF4522: *hsf-1(sy441)* with an unidentified suppressor mutation

CF4523: *hsf-1(sy441)* with an unidentified suppressor mutation

CF4524: *hsf-1(sy441)* with an unidentified suppressor mutation

CF4525: *hsf-1(sy441); rsks-1(mu482)*

CF4526: *hsf-1(sy441)* with an unidentified suppressor mutation

CF4540: *hsf-1(sy441); rsks-1(sv31)*

CF4542: *hsf-1(sy441); rsks-1(mu482);* svEx136[*unc-36(+) rsks-1(+) sur-5::gfp]*

CF4543: *hsf-1(sy441); T24F1.4(tm5213)*

*Development assay:*

Arrested L1 larvae were spotted onto plates preheated to 25.8°C. After spotting, plates were placed back at 25.8°C in an open Tupperware container for one hour to re-equilibrate temperature. After one hour, the container was closed (except for one corner) to prevent drying for four days. Each condition utilized four plates containing roughly 25 animals each. After 4 days, conditions were blinded and scored on a qualitative five-point scale for developmental stage. Animals with a score of three had passed the point of vulval eversion (transition to adulthood) but had not yet produced any eggs. Therefore, animals with a score of three or higher were classified as adults in the figures and tables shown here.

*EMS Mutagenesis and screening:*

Mutant *hsf-1(sy441)* worms grown at 20°C on OP50 were bleach-prepped, and the eggs were incubated overnight in M9. The next day, a total of 10,000 L1 larvae were divided among four 10 cm plates containing OP50 (~2500/plate) and incubated at 20°C. Once this $P_0$ population reached early L4, the worms were collected from the plates, washed 3 times, and re-suspended in 15mL of M9 buffer. In a separate 50mL conical tube, 100µL of EMS (Sigma #M-0880) was mixed into 5mL of M9. The worm suspension was then added to this new conical tube containing EMS, the top was parafilmed, and the tube was placed in a rotator at 20°C for 4 hours. The final concentration of EMS under these conditions was ~50mM, for an expected mutation rate of $5x10^{-4}$ mutations/gene/gamete (Brenner, 1974). Next, the worms were pelleted, the supernatant was removed, and the worms were washed thrice with 15mL of M9. Lastly, the worm pellet was split equally among four 10cm plates containing OP50, and recovered at 20°C for 24 hours.

At the end of this recovery period, the $P_0$ animals were collected from the plates and bleached using standard protocol. Assuming 5 viable eggs were obtained per $P_0$, this yielded a total of ~50,000

F1's, representing 50x genomic coverage. These eggs were plated evenly onto 20 separate 10cm plates containing OP50 (2500/plate), and grown to adulthood at 20°C. The gravid populations on each of these 20 plates were then collected individually and bleached, and each resulting batch of $F_2$ eggs was plated onto its own 15cm plate containing OP50 (25,000/plate). The $F_2$ worms were then grown at 25°C. On day 5, the plates were screened for any worms that had developed beyond the L3 arrest phase, and these candidates were collected onto individual 3cm OP50 plates and recovered at 20°C.

*Validation and Phenotypic Analysis of Screen Hits:*

All candidates recovered from the screen were validated by determining whether the suppression-of-*hsf-1*-arrest phenotype bred true to the next generation. Those that failed this test were discarded. The validated lines were then genotyped for reversal of the *hsf-1(sy441)* point mutation, with intent to discard true genetic revertants.

In order to narrow down the 17 remaining mutant lines, we further characterized them for penetrance and expressivity. First, we measured the percentage of suppression-of-*hsf-1*-arrest that each line displayed. This was done by picking 50 eggs onto a 3cm OP50 plate, incubating for 4 days at 25°C, and then counting the number of worms that developed past L3 arrest. Investigation was continued only for lines with 50% or higher population rescue. Next, we determined the furthest developmental stage each line could achieve at 25°C, using the same experimental set-up just described, and only pursued those that could produce gravid adults.

*Genetic Characterization of Screen Hits:*

We characterized the allelic nature of the 9 remaining lines. Males were generated for each, as well as for *hsf-1(sy441)* single mutants, and reciprocal crosses were carried out to identify any dominant or sex-specific mutations. Given that all suppressors were found to be recessive, the males generated

above were then used to perform complementation assays. To ensure independence, all lines isolated from the same plate (of the original 20) were interrogated with reciprocal crosses, looking for heterozygous progeny that maintained the suppression-of-*hsf-1*-arrest phenotype. Second, reciprocal complementation crosses were carried out between all remaining lines (across the original 20 populations), with the intent to again keep only one from each complementation group. This yielded a set of five lines with unique, recessive, and penetrant mutations that allowed *hsf-1(sy441)* worms to reach gravid adulthood at 25°C. Four of these five strains exhibited extended lifespan at 20°C, but this phenotype was lost upon backcrossing.

*Identification of candidate genes:*

These five suppressor strains were backcrossed to the control *hsf-1(sy441)* strain a total of six times before genomic sequencing. The suppressor strains were then backcrossed a total of nine times before use in all other experiments.

Genomic DNA from each mutant strain was isolated from roughly 300µl of pelleted worms from a mixed population. DNA was sheared using the Covaris M220 sonicator to an average length of 350 base pairs. After shearing, DNA was quantified using the Qubit dsDNA high sensitivity assay kit. DNA libraries were prepared from genomic DNA utilizing the Bioo Scientific NEXTflex Rapid DNA-Seq Library Prep Kit. Library quality was assessed utilizing the Agilent high-sensitivity DNA analysis kit. Libraries were sequenced by the UCSF Institute for Human Genetics Core Facility according to the manufacturer's protocol using an Illumina HiSeq 2500.

Genomic information from the Human Genetics Core Facility was analyzed using the Galaxy platform through Amazon Web Services. Genes with high-quality protein-coding point mutations were then examined for effects on the development arrest phenotype.

*Lifespan Analysis:*

Life span analysis was performed at 20°C as described previously (Apfeld and Kenyon 1998). Animals were grown on OP50 *E. coli* and transferred to fresh plates on the first day of adulthood ("day 1") and every 2 days thereafter. Every two days, starting on day 1, animals were scored. Animals that moved were scored as alive, animals that did not move, even after being prodded with a platinum wire, were scored as dead, and animals that could not be found or displayed phenotypes such rupturing or bagging were censored.

*Translation Inhibiting Drugs:*

Salubrinal (SML0951) and homoharringtonine (SML1091) were purchased from Sigma-Aldrich. Cycloheximide (#94271) was purchased from VWR Life Sciences. Drugs were diluted and spotted on plates with bacteria to a final DMSO concentration of 0.19% (unless otherwise noted) and allowed to diffuse into the agar for one day before animals were plated for development experiments.

*Real-time qPCR:*

RNA extraction, cDNA preparation, and real-time qPCR were performed as described previously (Van Gilst *et al.* 2005) from 3 biological replicates of each condition, with at least 500 animals included in each condition. For larval experiments, animals were grown from arrested L1 larvae at 25.8°C until >50% of the population had grown to the L1 lethargus stage between the L1 and L2 larval stages (approximately 13 hours for wild-type background and approximately 16 hours for *hsf-1(sy441)* background) before being washed and flash-frozen for RNA extraction. For heat-shock experiments, animals were grown from arrested L1 larvae at 20°C until >50% of the population had reached young adulthood, after which the animals were separated into heat-shock conditions, which were placed at 30°C, and non-heat-shock conditions, which were placed at 20°C, for one hour before being washed and

flash-frozen for RNA extraction. Data were standardized to three control primers: *tba-1*, *cdc-42*, and *pmp-3*. For *rps-6* dilution experiments, data were standardized to *tba-1* and *cdc-42* only. Primer sequences are listed in table 3.3.

*RNAi:*

HT-115 RNAi bacteria were obtained from existing Ahringer and Vidal libraries. The control for all RNAi experiments was L4440 vector. For each experiment, single colonies were grown overnight at 37°C in 3 mL LB with 100µg/mL carbenicillin and 10µg/mL tetracycline. The next day, the resulting stationary-phase cultures were diluted tenfold with LB containing 100µg/mL carbenicillin and grown for two hours at 37°C. 1M IPTG was then added to the culture to reach a final concentration of 2mM, after which 100µL of the resulting culture were spotted onto NGM plates. The bacteria were grown on these plates overnight at 30°C. Animals were placed on plates the following day in all experiments except those involving a drug condition, in which case drugs were added and left to diffuse into the plate for an additional day before the addition of experimental and control animals.

*Size Measurement:*

Arrested L1 larvae were spotted onto plates preheated to 20°C (for young-adult experiments) or 25.8°C (for larval experiments). For young-adult experiments, animals were grown for three days at 20°C, and for larval experiments, animals were grown until L1-L2 lethargus at 25.8°C. Animals were then washed off plates, immobilized in 10mM levamisole, and imaged at 80x magnification. Animal size was then quantified in ImageJ by measuring the area inside a polygon drawn around each animal.

*Statistical Analyses:*

On all dot plots, bars represent the mean, and error bars represent the SEM. For developmental assays of multiple genes in parallel, significance was measured across three independent experiments using Cochran-Mantel-Haenszel tests with Bonferroni corrections unless otherwise noted. For lifespan assays, log-rank tests with Bonferroni corrections were used. For RT-qPCR experiments and animal size experiments, one-way ANOVAs with Tukey post tests were used on three biological replicates.

**Results:**

*A genetic screen identifies mutations that rescue the* hsf-1(sy441) *developmental arrest*

To investigate how *hsf-1* regulates development, we conducted an unbiased forward mutagenesis screen in *C. elegans* to rescue the growth-arrest phenotype displayed by *hsf-1(sy441)* mutants grown at 25°C. Following EMS mutagenesis (Brenner 1973), we recovered seventeen independent mutants that, when shifted from 20°C to 25.8°C at the mid-L4 (final) larval stage, produced progeny that grew to adulthood. We picked five strains (see methods) to backcross and sequence to discover candidate genes. *hsf-1(sy441)* mutants carrying the suppressors reached adulthood exhibiting apparently-normal anatomy, but they displayed a reduced body size and could not be cultivated past a single generation at 25.8°C (Figure 3.1A, B). The progeny of some strains grown at 25.8°C produced eggs that did not hatch, whereas others produced progeny that arrested at L1. If adult animals were shifted back to 20°C, they produced offspring which developed normally.

When parents were shifted to 25.8°C at the L4 stage, 100% of *hsf-1(sy441)* progeny without suppressor mutations arrested at the L1-L3 stage, whereas 100% of progeny homozygous for the five suppressor mutations reached adulthood. If instead eggs were bleach-extracted from the gonads of the parental generation at 20°C, allowed to hatch into minimal medium (causing L1 developmental arrest), and then placed on 25.8°C plates, a small percentage of *hsf-1(sy441)* single mutant-animals could reach

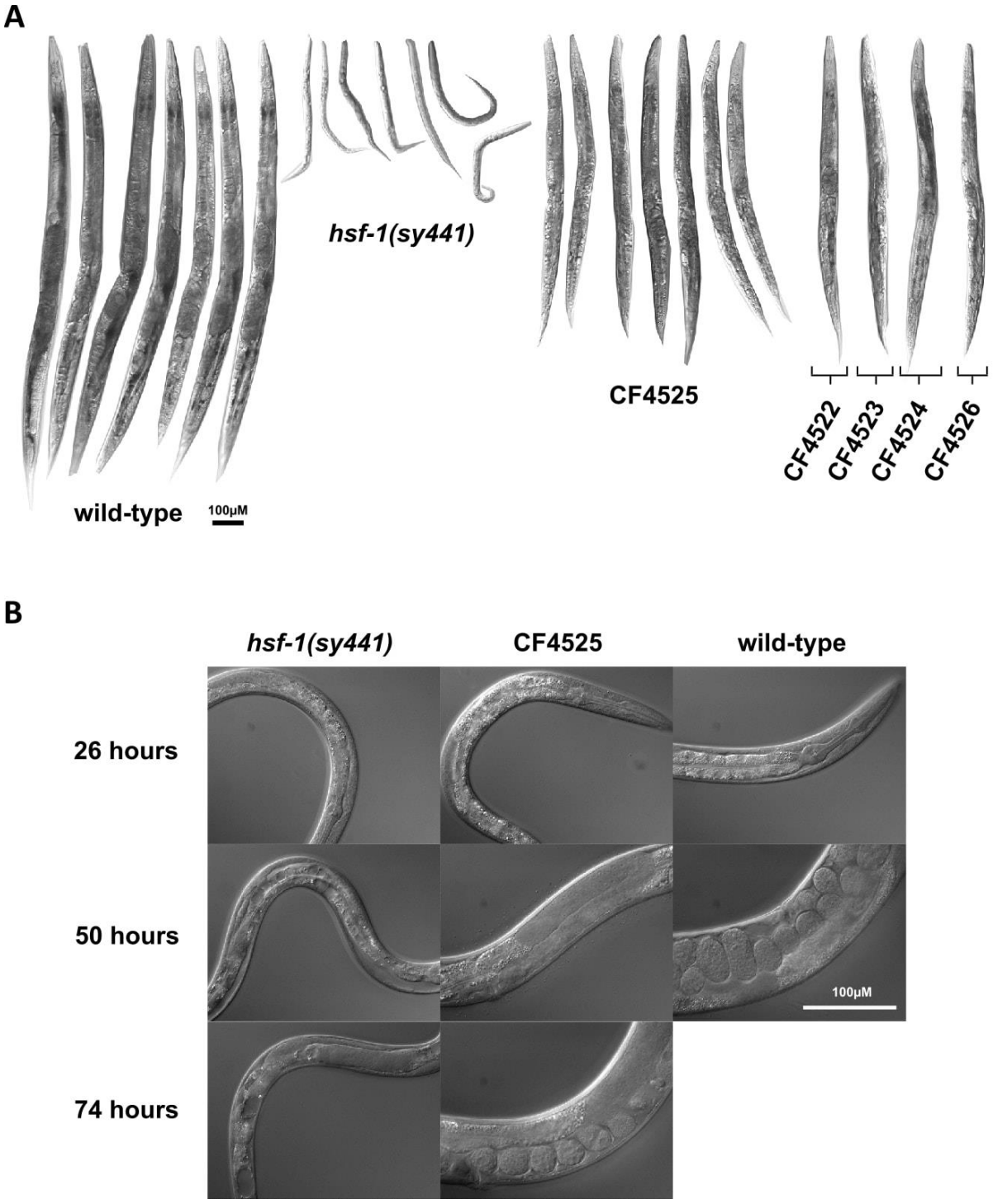



**Figure 3.1:** Mutants discovered in a mutagenesis screen for rescue of the developmental arrest of *hsf-1(sy441)* mutants. (A) Appearance of wild-type, *hsf-1(sy441)*, and multiple suppressor strains after 4 days at 25.8°C from arrested L1 larvae cultured at 20°C, imaged at 100x magnification. Strains CF4522, CF4523, CF4524 and CF4526 contain additional suppressors from the screen, but were not analyzed further in this study. (B) Appearance of wild-type, *hsf-1(sy441)*, and CF4525 suppressor strain grown at 25.8°C to various timepoints from arrested L1 larvae cultured at 20°C, imaged at 600x magnification.

early adulthood but rarely produced eggs, while still 100% of progeny homozygous for the suppressor mutations reached adulthood. In order to detect subtle shifts in the percentage of animals that reached adulthood, all of the assays described here utilized arrested L1 larvae hatched at 20°C and then shifted to the higher temperature.

Because our suppressor mutations rescued the developmental growth-arrest phenotype of *hsf-1(sy441)* animals, we tested whether they could also rescue another well-documented *hsf-1(sy441)* phenotype, shortened adult lifespan (Garigan *et al.* 2002; Hajdu-Cronint et al 2004). None of the mutants extended the shortened lifespan that *hsf-1(sy441)* mutant-animals exhibit at the temperatures permissive for growth (Figure 3.2). We also tested whether the suppressor strains affected resistance to heat stress. To our surprise, we found that our *hsf-1(sy441)* background was more heat resistant than wild-type, but this seemed unaffected by most of the suppressor mutations. CF4524 was a lone exception and showed more thermosensitivity(Figure 3.3).

*Loss of the gene* rsks-1 *rescues the* hsf-1(sy441) *developmental arrest*

Genomic sequencing revealed that one of the suppressor strains (CF4525) contained a premature stop codon in the gene *rsks-1* at the 295$^{th}$ base pair, *rsks-1(mu482)*. The *rsks-1* gene encodes *C. elegans'* ribosomal S6 Kinase ortholog, which promotes normal levels of translation. We determined that this mutation was the causative factor for the developmental rescue in two ways: first, we confirmed the developmental rescue with a previously-isolated null allele, *rsks-1(sv31)*, and with RNAi-mediated knockdown of *rsks-1* (Figure 3.4A). Second, we showed that transgenic re-expression of *rsks-1* in the suppressor strain blocked the rescue phenotype (Figure 3.4B, C). While there was variation in the baseline percentage of *hsf-1(sy441)* animals that reached adulthood (Tables 3.1-3.2), this variation did not obfuscate the rescue phenotype of *rsks-1* RNAi. In 44 out of 46 trials, we saw a significant difference

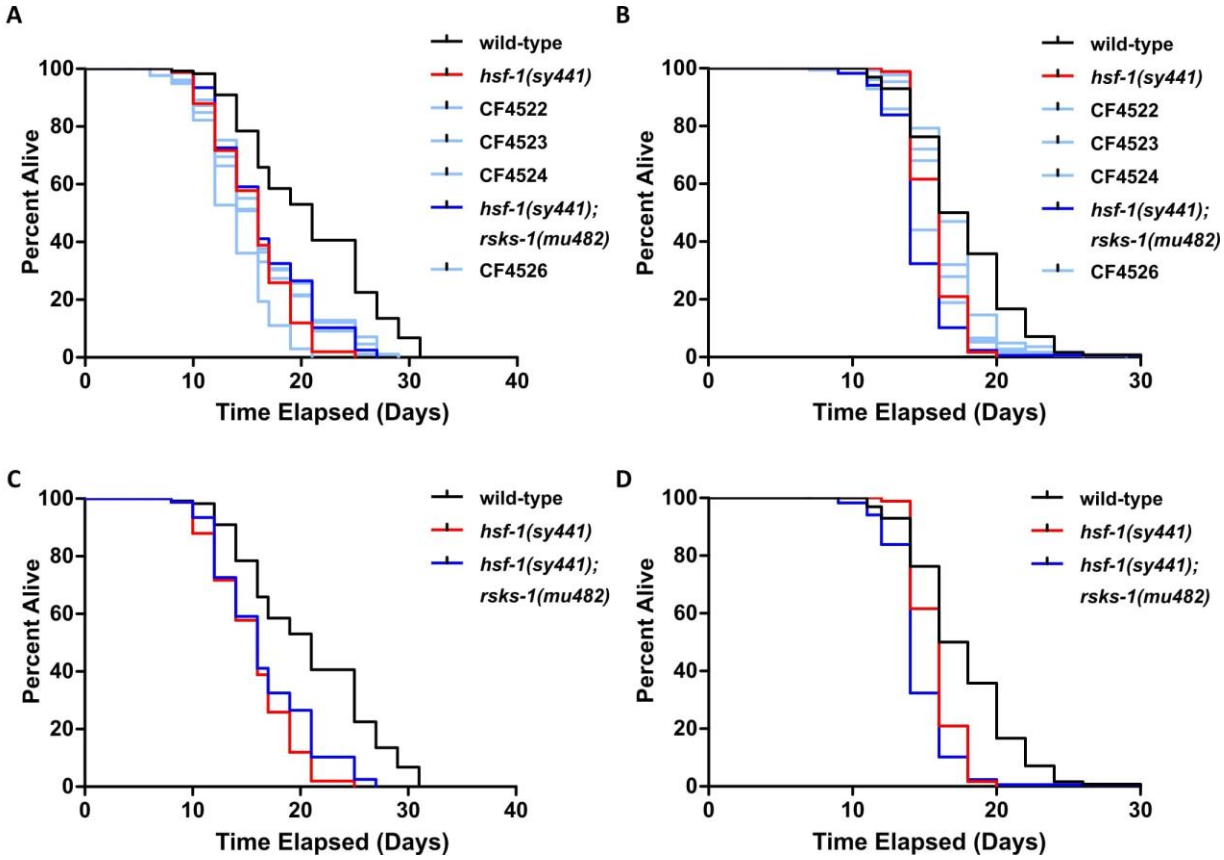



**Figure 3.2:** Strains carrying suppressors of the developmental arrest of *hsf-1(sy441)* mutants do not extend lifespan, either without (A) or with (B) the addition of 50μM FUDR to prevent progeny production. Bottom graphs display the same data as the graphs above them, showing only a subset of strains for clarity.

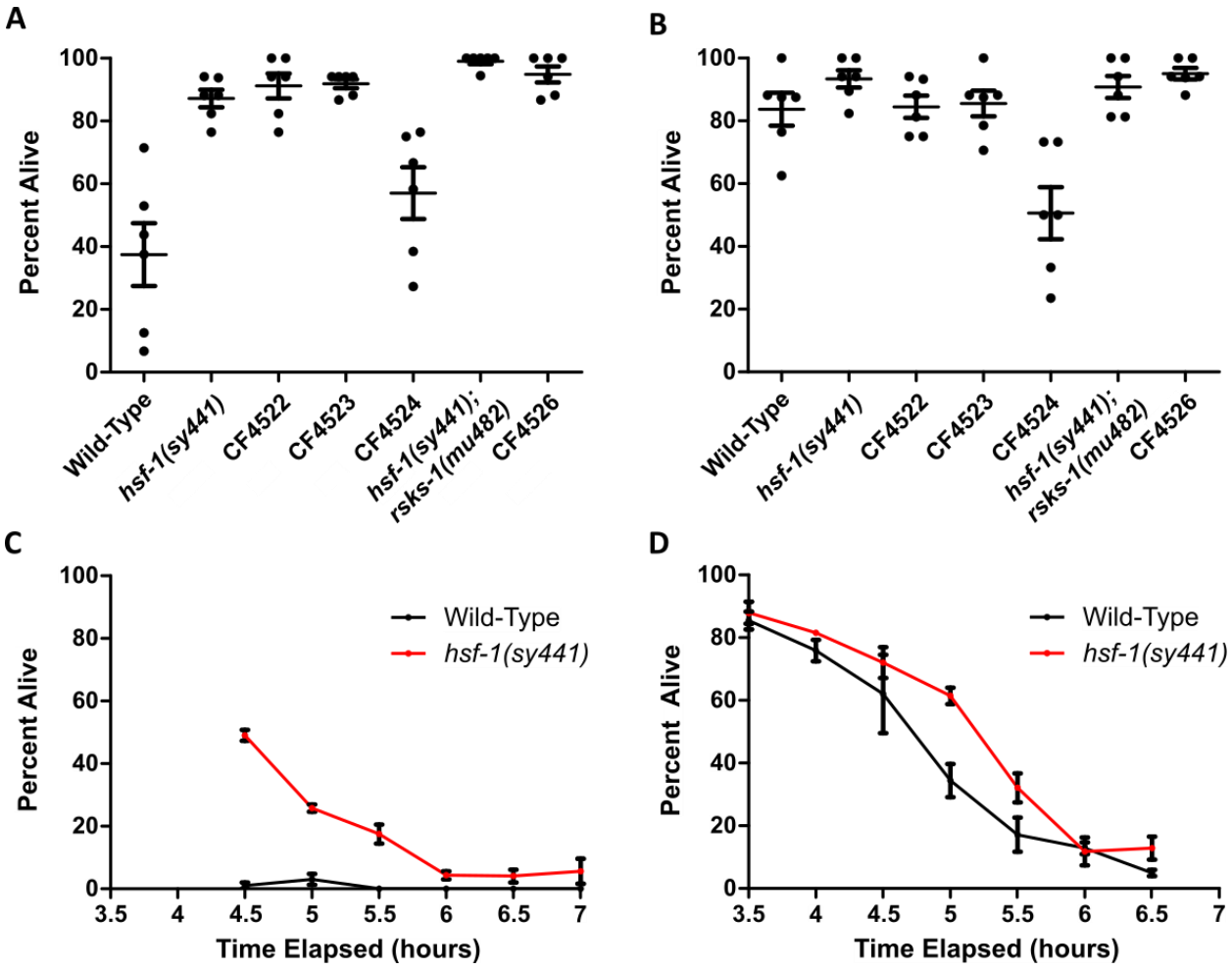



**Figure 3.3:** Thermotolerance of *hsf-1(sy441)* and suppressor strains. (A) Average survival after 4 hours in a 35°C incubator, data points are percent alive on six plates of approximately seventeen animals per condition. (B) Average survival after 4.5 hours a 35°C incubator, data points are percent alive on six plates of approximately seventeen animals per condition. (C,D) Average survival over time at 35°C. Data points are the average percent alive of three plates with approximately 40 animals scored quickly at room temperature before being placed back at 35°C.

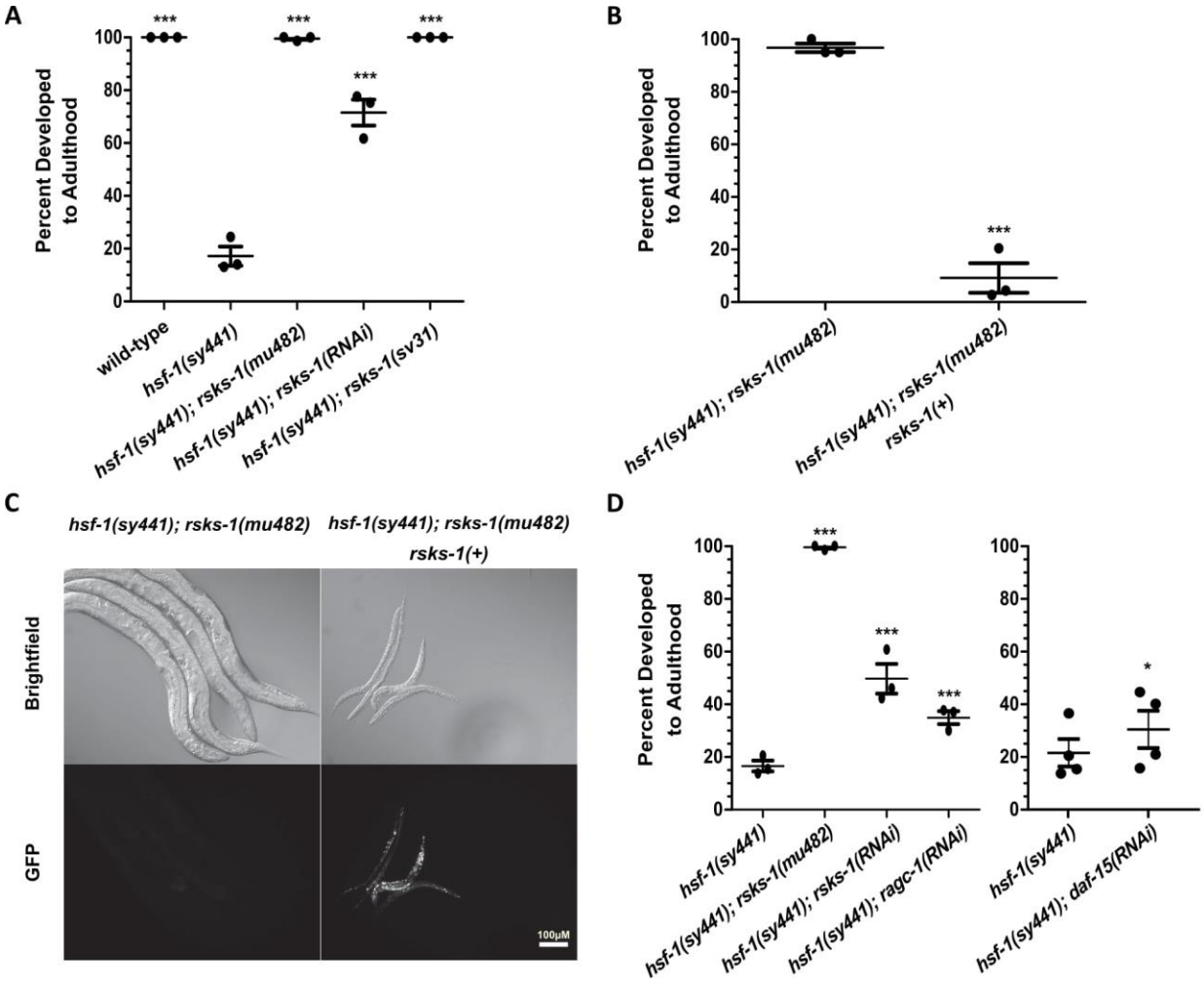



**Figure 3.4:** Loss of *rsks-1/*S6 kinase or reduction of mTOR function was sufficient to rescue the developmental arrest of *hsf-1(sy441)*. (A) Loss of *rsks-1* rescued the developmental arrest of *hsf-1(sy441),* ***p < 0.001 compared to *hsf-1(sy441)* using the CMH test with three replicates. (B,C) Transgenic overexpression of *rsks-1::GFP* in *hsf-1(sy441); rsks-1(mu482)* double mutants suppressed the rescue. All animals are *hsf-1(sy441); rsks-1(mu482)* double mutants with the stochastically-inherited transgene svEx136[*unc[36(+) rsks-1(+) sur-5::gfp*]. Strain identity was blinded and development was scored, and then groups were categorized based on whether GFP was visible, ***p<0.001 compared to animals without GFP using the CMH test with three experiments; animals were imaged at 100x magnification. (D) RNAi knockdown of genes in the mTOR pathway also rescued the developmental arrest of *hsf-1(sy441)*, *p<0.05, ***P<0.001 compared to *hsf-1(sy441)* using the CMH test with three replicates (four for *daf-15*).

in the percentage of *hsf-1(sy441)* animals that developed to adulthood between animals grown on vector-only control RNAi bacteria and those grown on *rsks-1* RNAi bacteria (Figure 3.5).

*Reduction in mTOR-pathway function also rescues the* hsf-1(sy441) *developmental arrest*

What is the mechanism by which *rsks-1* loss influences *hsf-1* mutants? S6 kinase is a key target of mTOR kinase. To determine if the developmental rescue phenotype could be produced by a reduction in mTOR activity more broadly, we used RNAi to knock down *daf-15*/RAPTOR, a component of the mTORC1 complex, as well as *ragc-1*/RAG GTPase, a positive regulator of mTORC1 (Fukuyama *et al.* 2012; Jia *et al.* 2004). Reduced expression of both of these genes significantly rescued the *hsf-1(sy441)* developmental arrest (Figure 3.3D), albeit to a lower degree than did RNAi knockdown of *rsks-1*.

rsks-1(-)-*mediated* hsf-1(sy441) *rescue was not mimicked by a reduction in translation*

Loss of *rsks-1* causes a reduction in translation (Hansen *et al.* 2006). One possible explanation for the mechanism of developmental rescue conferred by *rsks*-1 mutation is that *hsf-1(sy441)* animals, predicted to have reduced proteostatic capability, are overburdened by the amount of protein being translated during development at 25°C, and the reduced rate of translation incurred by mutating *rsks-1* reduces that burden to a manageable level in *hsf-1(sy441)* worms. To investigate this hypothesis, we reduced translation in other ways, by treatment with several chemical inhibitors of translation and via various RNAi treatments. If our hypothesis were correct, one would expect the following: at high concentrations these treatments would be lethal and at low concentrations they would have no effect, but at some range of intermediate concentrations they would lower the translation rate to a level permissible for developmental rescue.

We first used RNAi to knockdown *rps*-6, encoding a small ribosomal protein that is phosphorylated by S6 kinase. Because a reduction in translation is known to result in reduced body size

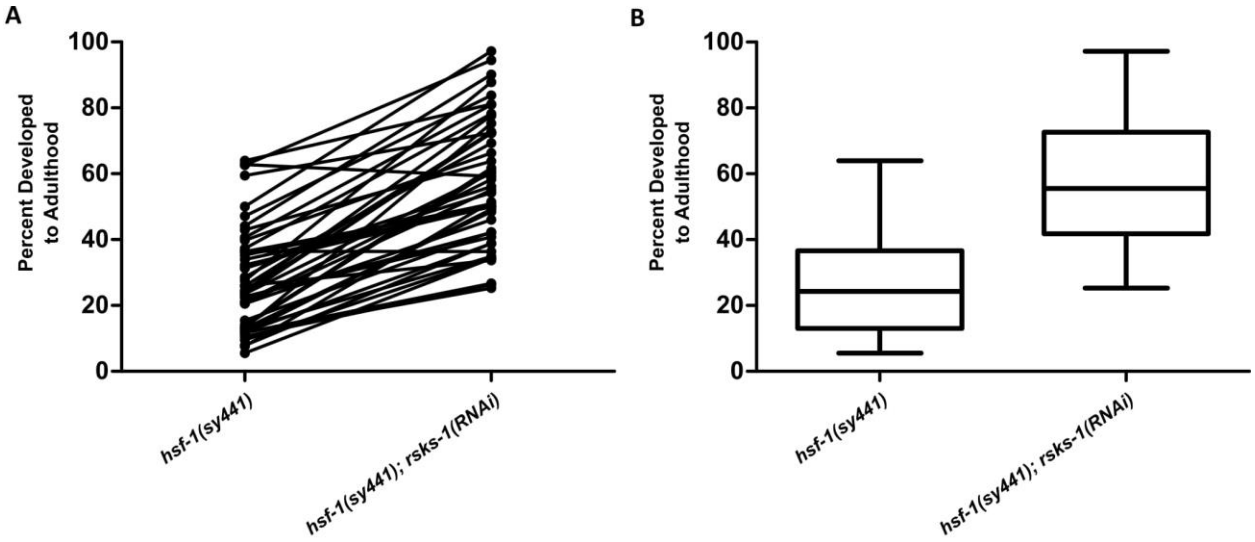



**Figure 3.5:** The percentage of *hsf-1(sy441)* animals which reach adulthood at 25.5°C is variable, but *rsks-1* knockdown robustly increases this percentage. (A) *rsks-1* RNAi significantly increases the percentage of *hsf-1(sy441)* animals which develop to adulthood in 44 out of 46 trials, data points from the same experiment are connected by a line. (B) Box and whisker plots of the percentage of *hsf-1(sy441)* animals which developed to adulthood across 46 replicates.

(Pan *et al.* 2007), we assessed the efficacy of translational knockdown indirectly by measuring the size of the young-adult animals grown at 20°C on *rps-6* RNAi diluted with vector control to achieve multiple levels of knockdown (Figure 3.6A). At concentrations that significantly reduced animal size close to that of *rsks-1(mu482)* mutants, we found that *rps-6* RNAi reduced the number of *hsf-1(sy441)* animals that reached adulthood (Figure 3.6B-C), showing that a reduction in translation may actually be harmful to *hsf-1(sy441)* development. To see whether *rps-6* RNAi treatment on its own was perhaps stressful or toxic to the animals, we measured expression of levels of canonical genes from the ER unfolded-protein response (UPR), mito-UPR, or HSR pathways using RT-qPCR, and found that none were significantly altered (Figure 3.6D, Figure 3.7A). This was despite seeing a significant, if smaller, effect of *rps-6* inhibition on animal size at this time point (Figure 3.6B).

We also tested the effects of other translation-inhibiting conditions, including RNAi of *ifg-1*, a gene which promotes translation additively with S6 kinase (Pan *et al.* 2007), salubrinal, a chemical inhibitor or translation initiation, and homoharringtonine, a chemical inhibitor of elongation (Figure 3.7C-F, Table 3.1). Similar to knockdown of *rps-6,* treatment with homoharringtonine caused a reduction in the percent of animals that developed to adulthood at concentrations that also significantly reduced animal size. Animals treated with higher concentrations of salubrinal and *ifg-1* RNAi showed a similar reduction. Only *hsf-1(sy441)* animals grown on 45nM salubrinal showed a marginally-significant increase in development to adulthood. Thus, while multiple distinct methods for reducing translation could cause reductions in animal size, no translation-inhibiting conditions mimicked the rescuing effect of *rsks-1* inactivation in terms of either penetrance or expressivity on development of *hsf-1(sy441)* animals. Together, these data suggest that reduced translation alone cannot explain the *rsks-1(mu482)* mutation's rescue of *hsf-1(sy441)'s* development.

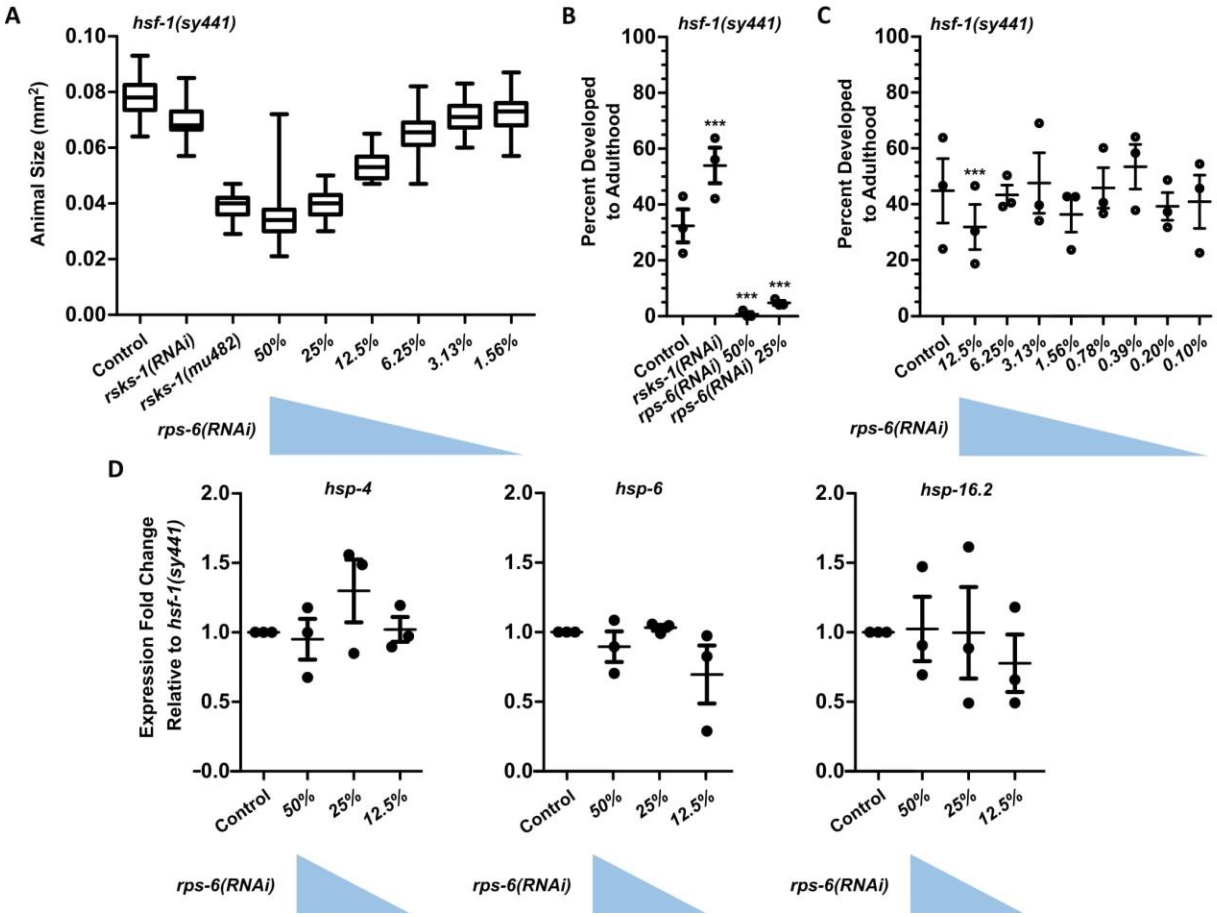



**Figure 3.6:** The ability of *rsks-1(-)* to rescue of the development of *hsf-1(sy441)* mutants is not explained by a reduction in translation. (A) *rps-6* RNAi reduces the size of *hsf-1(sy441)* young-adult animals grown at 20°C. Serial 2-fold dilution of *rps-6* RNAi produced incrementally smaller effects (50% denotes 1:1 *rps-6*:vector-control RNAi-bacteria). (B) Dilutions of *rps-6* RNAi-bacteria with vector-control RNAi-bacteria that show size reduction similar to *rsks-1(mu482)* mutants significantly reduce the percent of *hsf-1(sy441)* animals which develop to adulthood at 25.8°C (50% denotes 1:1 *rps-6*:vector-control RNAi), ***p<0.001 compared to control *hsf-1(sy441)* by CMH test with three replicates. (C) None of multiple dilutions of *rps-6* RNAi-bacteria with vector-control RNAi-bacteria rescued developmental arrest at 25.8°C (50% denotes 1:1 *rps-6*:vector-control RNAi), ***p<0.001 compared to control *hsf-1(sy441)* by CMH test with three replicates. (D) Concentrations of *rps-6* RNAi-bacteria that reduce baseline development to adulthood of *hsf-1(sy441)* animals do not activate stress-response ER-UPR, mito-UPR, or HSR pathways in animals grown at 25.8°C and harvested at the L1-L2 lethargus stage, by one-way ANOVA with Tukey post-test on three biological replicates.

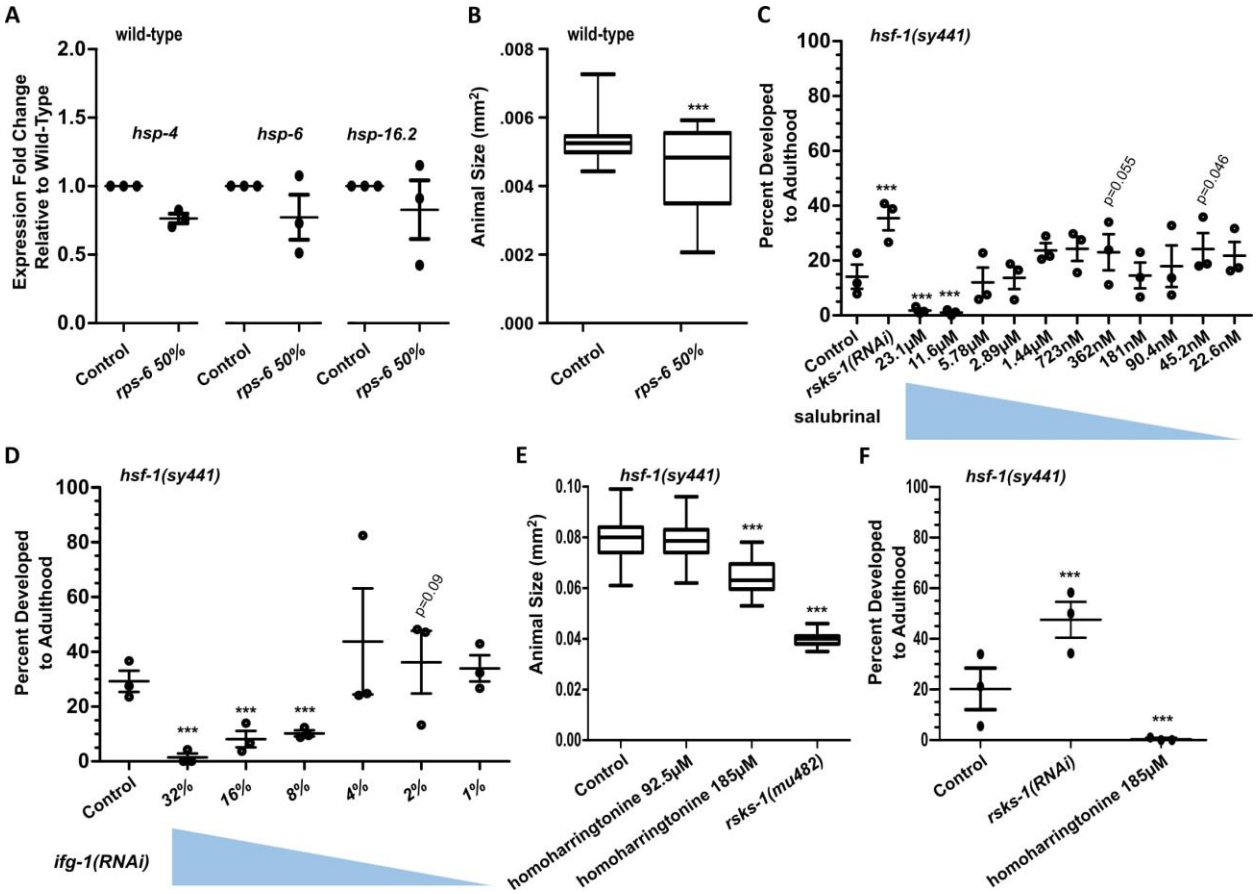



**Figure 3.7:** The ability of *rsks-1(-)* to rescue of the development of *hsf-1(sy441)* mutants is not explained by a reduction in translation. (A) *rps-6* RNAi inhibition does not activate stress-response ER-UPR, Mito-UPR, or HSR pathways in wild-type animals grown at 25.8°C and harvested at the L1-L2 lethargus stage (50% denotes 1:1 *rps-6*:vector-control RNAi-bacteria). (B) *rps-6* RNAi inhibition significantly reduces the size of animals grown at 25.8°C at the L1-L2 lethargus stage (in 2/3 replicates, ***p<0.001 by Student's t-test). (C) Two concentrations of the translation initiation inhibitor salubrinal showed a small amount of rescue near p=0.05 level of significance, ***p<0.001 compared to control *hsf-1(sy441)* by CMH test with three replicates. (D) None of multiple concentrations of *ifg-1* RNAi rescued developmental arrest (1% denotes 1:99 *ifg-1*:vector-control RNAi-bacteria), ***p<0.001 compared to control *hsf-1(sy441)* by CMH test with three replicates. (E) 185µM translation elongation inhibitor homoharringtonine significantly reduces the size of *hsf-1(sy441)* young-adult animals grown at 20°C in 2/3 replicates, ***p<0.001 compared to control by one-way ANOVA with Tukey post-test. (F) 185µM translation elongation inhibitor homoharringtonine significantly reduces the percentage of *hsf-1(sy441)* animals which develop to adulthood, ***p<0.001 compared to control by CMH test with three replicates.

rsks-*1(-) mutants do not activate canonical unfolded-protein response pathways*

Because we could not attribute the rescuing effects of *rsks-1* mutation to a simple lowering of the translational burden, we hypothesized that it might be increasing the animal's capability to manage proteotoxic stress through non-heat-shock response pathways such as the mitochondrial or ER UPR pathways. Like the heat-shock response, these pathways upregulate the expression of protein chaperones such as BIP/HSP-70 which facilitate protein folding (Shen *et al.* 2001).

The development of *hsf-1(sy441); rsks-1(mu482)* double mutants was partially inhibited by knockdown of the ER-UPR pathway gene *xbp-1* (Figure 3.8A), an intervention that has no effect on wild type, showing that the animals are sensitive to disruption of additional proteostatic systems beyond the heat-shock response. This suggests the possibility that *rsks-1* loss up-regulates *xbp-1* transcriptional-target genes, such as *hsp-4* (the *C. elegans* BIP ortholog). However, when assayed using RT-qPCR, *hsp-4* did not appear to be upregulated by loss of *rsks-1* in *hsf-1(sy441)* mutants (Figure 3.8B). Likewise, RT-qPCR of the mitochondrial-UPR target gene *hsp-6* revealed that the mitochondrial-UPR was not upregulated. These findings suggest that an increase in UPR function is not the mechanism by which *rsks-1* loss rescues development.

*The developmental rescue of* hsf-1(sy441) *mutants is mTOR-specific but does not act through canonical lifespan pathways that interact with mTOR*

mTOR and heat-shock factor are intimately tied to lifespan regulation and stress resistance. We wondered whether activating other pathways known to increase lifespan and proteostasis could also rescue its development. Both *daf-2* (insulin/IGF-1 receptor) and *cco-1/cox-5B* (mitochondrial electron transport gene) RNAi extend lifespan (Dillin *et al.* 2002; Kenyon *et al.* 1993), but neither rescued the block in *hsf-1(sy441)* development (Figure 3.8C). In fact, *daf-2* RNAi seemed to have a negative effect on development. To further investigate, we used RNAi to inhibit the transcription factors *daf-16, pha-4* and

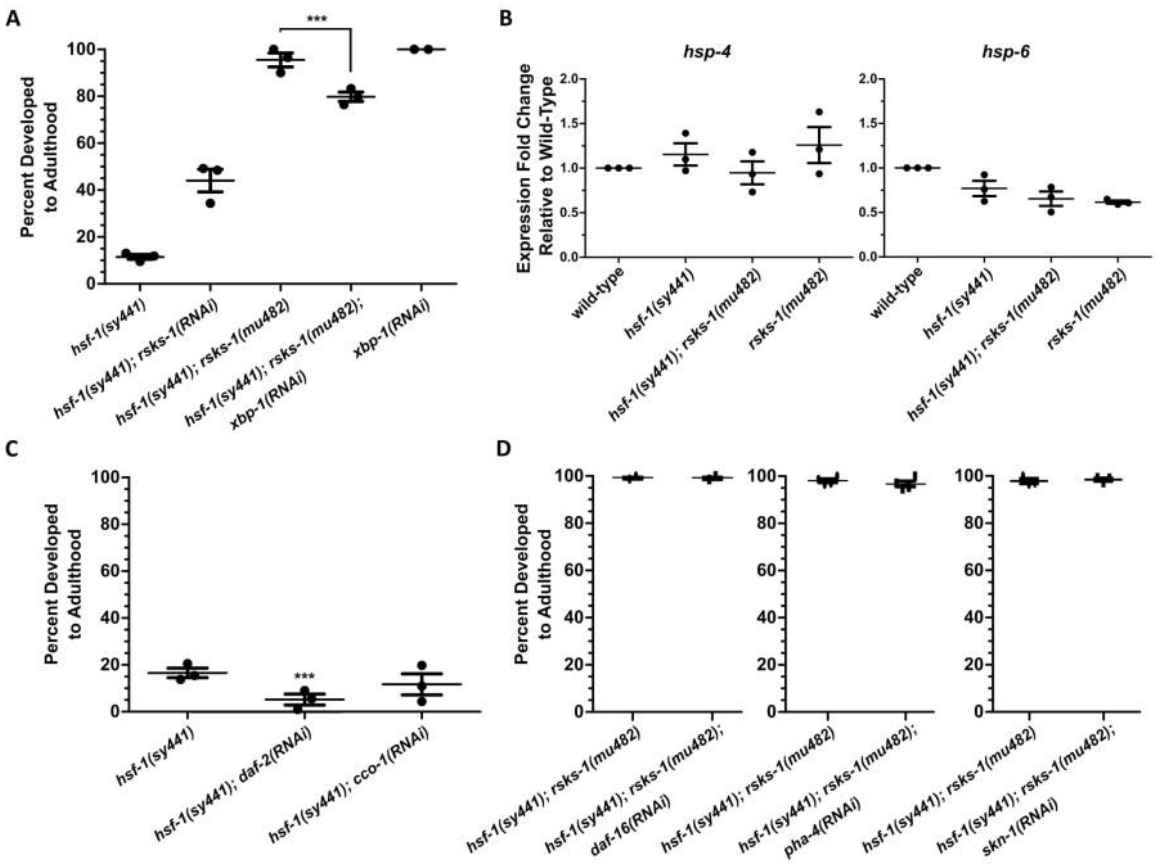



**Figure 3.8:** Stress-responses in *hsf-1(sy441)* animals carrying the *rsks-1(mu482)* suppressor. (A) *hsf-1(sy441); rsks-1(mu482)* double mutants are more sensitive to RNAi of *xbp-1*, an activator of the ER UPR, than are wild-type animals, ***p<0.001 using CMH test with three replicates. (B) Canonical ER UPR and mitochondrial UPR chaperones were not upregulated in *hsf-1(sy441); rsks-1(mu482)* double mutants, using one-way ANOVA with Tukey post-test on three biological replicates. (C) Inhibition of the *daf-2* insulin/IGF-1 receptor or the mitochondrial electron transport chain, which extend the lifespan of wild type, failed to rescue the developmental arrest of *hsf-1(sy441)* mutants, ***p<0.001 using CMH test with three replicates. (D) Transcription factors required for mTOR reduction-of-function to extend lifespan are not required for *rsks-1* mutation to rescue *hsf-1(sy441)* developmental arrest, using CMH test with three replicates.

*skn-1.* Each of these genes promotes *C. elegans'* stress resistance and lifespan extension in mTOR reduction-of-function and/or other long-lived mutants (Robida-Stubbs *et al.* 2012; Seo *et al.* 2013; Sheaffer et al. 2008), but none blocked the ability of *rsks-1* loss to rescue the *hsf-1(sy441)* developmental arrest (Figure 3.8D). We then tested a subset of genes in the published literature that have been shown to be a part of the genetic networks associated with *rsks-1* or *hsf-1*, including those from microarray datasets (Baird *et al.* 2014; Chen *et al.* 2013; Magnuson *et al.* 2012). Of the 65 additional genes tested, we saw no evidence of RNAi knockdown either rescuing the development of *hsf-1(sy441)* mutants or having a more severe reduction-of-development effect on *hsf-1(sy441); rsks-1(mu482)* double mutants vs. wild-type (Table 3.2).

*Developmental rescue requires residual hsf-1 function but does not act through canonical heat-shock-regulated genes*

Despite the lack of its transactivation domain, the fact that the *hsf-1(sy441)* allele does not cause arrest at 20°C, as the null mutation does (Li *et al.* 2016), shows that the HSF-1(sy441) mutant protein retains some functionality. To test whether endogenous levels of the mutant protein are required for *rsks-1* null mutations to rescue development at 25.8°C, we knocked down *hsf-1* using RNAi in the *hsf-1(sy441); rsks-1(mu482)* double mutant. RNAi knockdown of residual HSF-1(sy441) function blocked the *rsks-1* rescue phenotype (Figure 3.9A). In addition, we found that transgenically increasing the *hsf-1(sy441)* gene dosage was sufficient to rescue the developmental arrest of *hsf-1(sy441)* mutants (Figure 3.9B). However, despite the fact that residual levels of HSF-1(sy441) are necessary for developmental rescue and overexpression of HSF-1(sy441) can be sufficient for developmental rescue, we saw no evidence through RT-qPCR that *rsks-1* mutation elevated the expression levels of the *hsf-1* gene nor genes known to be regulated by HSF-1 in heat-shock or developmental contexts (Figure 3.9C) (Li *et al.* 2016). Thus, the hypothesis that, directly or indirectly, loss of S6 kinase promotes growth to

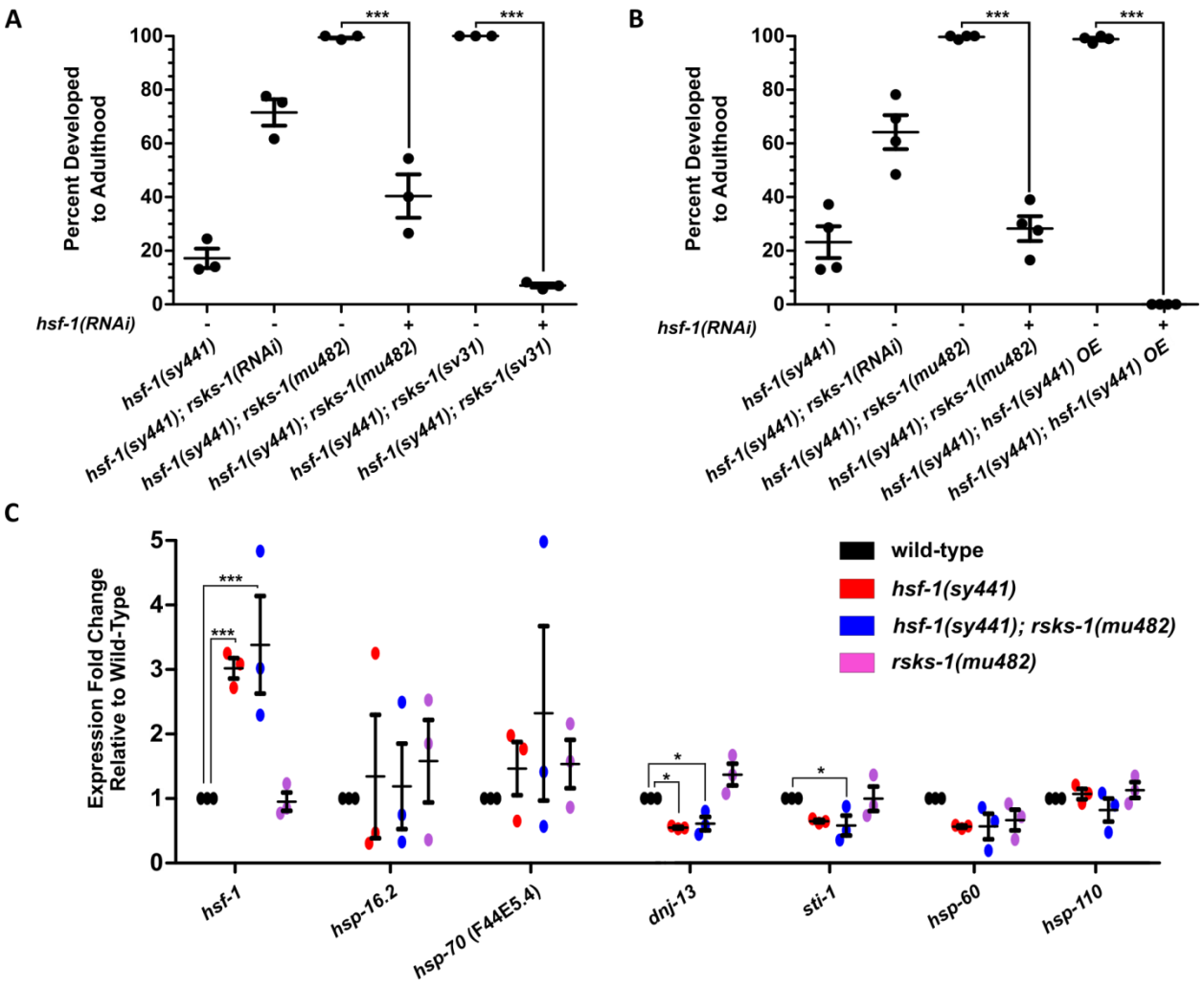



**Figure 3.9:** *hsf-1(sy441)* activity is necessary and can be sufficient to rescue developmental arrest, but *rsks-1(mu482)* does not appear to affect expression of *hsf-1* or its canonical targets. (A) *hsf-1* RNAi treatment prevented two *rsks-1*/S6 kinase null mutations from rescuing the developmental arrest of *hsf-1(sy441)* mutants, ***p<0.001 using the CMH test with three replicates. (B) Overexpression of the *hsf-1(sy441)* allele rescued the developmental arrest of *hsf-1(sy441)* mutants, ***p<0.001 using the CMH test with four replicates. (C) Expression levels of canonical heat-shock genes and developmentally-regulated *hsf-1*-response genes are unaffected by the *rsks-1(mu482)* mutation, as measured by RT-qPCR, *p<0.05 **p<0.01 ***p<0.001 by one-way ANOVA with Tukey multiple comparison post-test measured for each gene independently with three biological replicates.

adulthood by enhancing the effectiveness of HSF-1(sy441) remains an attractive, though unproven, model.

Three findings from our RT-qPCR experiments were surprising. First, we found that *hsf-1(sy441)* animals exhibited a higher level of *hsf-1* expression compared to wildtype. Second, we found that *rsks-1* mutation did not increase expression of *hsp-16.2* or the *hsp-70* gene F44E5.4, in contrast to a previous report (Seo *et al.* 2013). Third, one might suspect *hsp-16.2* and *hsp-70* (F44E5.4) expression levels to be higher in wild type than in *hsf-1(sy441)* mutants because 25.8°C is higher than the ambient temperature for *C. elegans*, but this was not the case. To more closely replicate previously published experiments, we conducted new RT-qPCR experiments on young-adult animals which were grown at 20°C and then heat shocked at 30°C or left at ambient temperature for one hour. Under these conditions, as shown previously, the increase in *hsp-16.2* expression upon heat shock was severely blunted in the *hsf-1(sy441)* background (Figure 3.10A). Curiously, *hsp-70* (F44E5.4) expression was unaffected (Figure 3.10B), suggesting that the *sy441* allele affects only a subset of heat-shock target genes. Contrary to Seo *et al.*, we did not observe an increase in the expression of *hsp-16.2* or *hsp-70* (F44E5.4) in *rsks-1* mutant animals compared to wild-type (Figure 3.9C), but we did see a possible increase in *hsp-70* (F44E5.4) expression after heat shock in *rsks-1* mutant animals. This suggests to us that loss of *rsks-1* can affect the regulation of heat-shock genes, but the mechanism may be complicated by factors such as genetic background or differences between *rsks-1* mutant alleles. Finally, we found that the increase in *hsf-1* expression seen in *hsf-1(sy441)* mutant larvae was no longer significant in adulthood (Figure 3.10D).

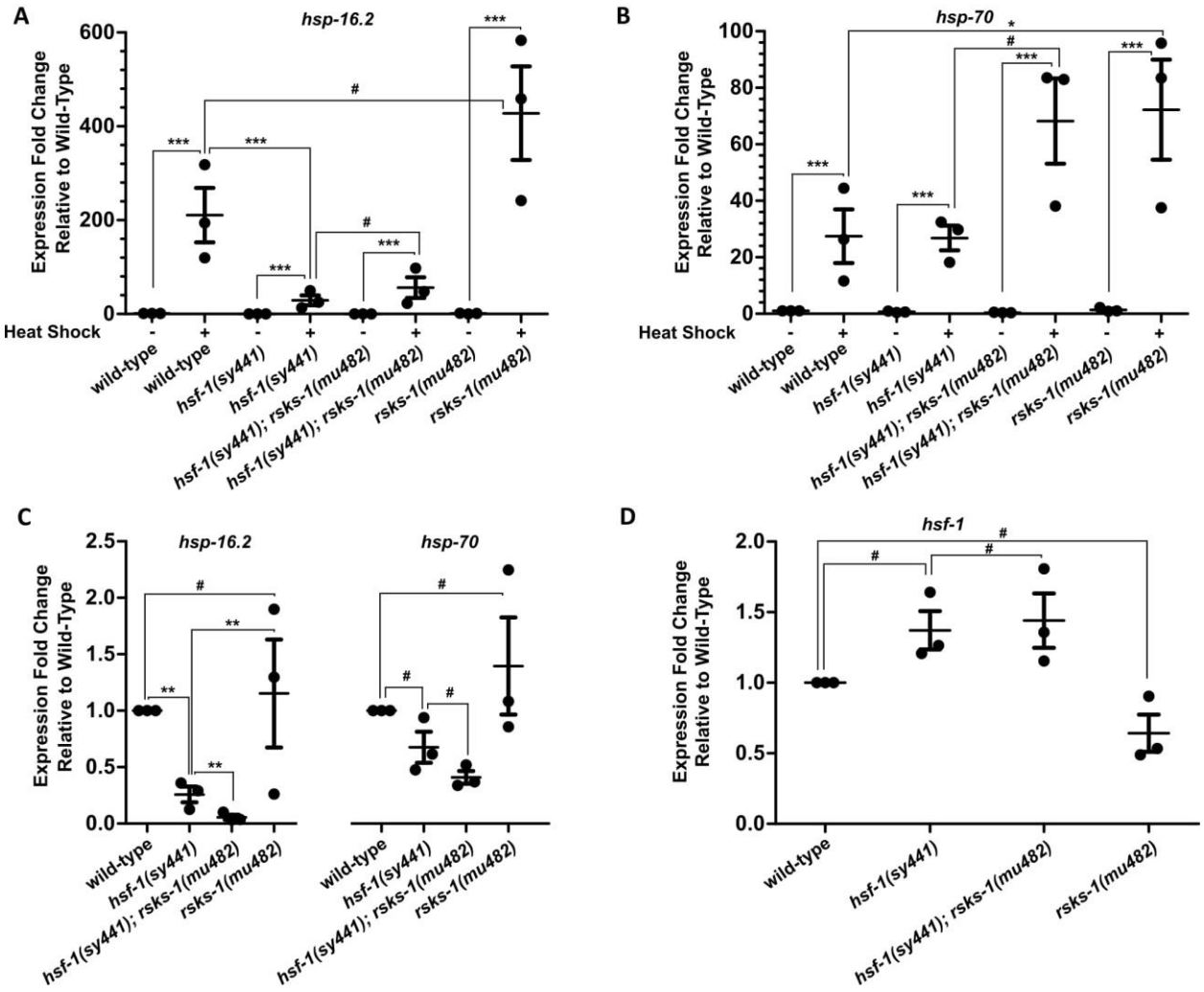



**Figure 3.10:** Effect of genetic background and 30°C heat shock on change in expression of *hsf-1, hsp-16.2,* and *hsp-70.* (A) Heat shock increases expression of *hsp-16.2* significantly more in wild-type young-adult animals compared to *hsf-1(sy441)* mutant animals, #p>0.05, ***p<0.001 by one-way ANOVA with Tukey post-test on three biological replicates. (B) Heat shock increases the expression of *hsp-70(f44e5.5)* equally in wild-type and *hsf-1(sy441)* animals, and *rsks-1(mu482)* mutation may increase the expression of *hsp-70(f44e5.5)* after heat shock, #p>0.05, *p<0.05, ***p<0.001 by one-way ANOVA with Tukey post-test on three biological replicates. (C) *hsf-1(sy441)* young-adult animals have significantly reduced *hsp-16.2* expression but not *hsp-70(f44e5.5)* expression compared to wild-type at 20°C, #p>0.05, **p<0.01, by one-way ANOVA with Tukey post-test on three biological replicates. (D) Young-adult *hsf-1(sy441)* animals grown at 20°C show a trend, but no significant change in *hsf-1* expression levels, unlike that seen in animals grown at 25.8°C and harvested at L1-L2 lethargus, #p>0.05, by one-way ANOVA with Tukey post-test on three biological replicates.

**Discussion:**

*hsf-1* and its orthologs have been studied extensively in the contexts of stress, aging, and human pathologies such as cancer and neurodegenerative diseases (Li *et al.* 2017). Despite characterization of the requirement for a functioning heat-shock factor during development, the specific molecular roles it plays in development remain largely unknown. In this paper we show that the mTOR pathway, and more specifically the downstream mTOR target ribosomal S6 kinase, acts to truncate development in *hsf-1(sy441)* reduction-of-function mutants.

Interestingly, lifespan extension caused by inhibiting S6-kinase or mTOR activity is known to be blocked by the *hsf-1(sy441)* mutation (Seo *et al.* 2013). Consistent with this finding, our S6-kinase suppressor mutation failed to extend the lifespan of *hsf-1(sy441)* mutants. While this finding shows that HSF-1 is required for *rsks-1* loss to extend lifespan, here we find another reciprocal relationship; namely, that *rsks-1* loss can rescue the developmental arrest caused by reduction of HSF-1 function. In one case, low levels of S6K cause endogenous levels of HSF-1 to extend lifespan, whereas in the other case, low levels of HSF-1 cause endogenous levels of S6K to arrest development. The details of these reciprocal interactions remain elusive; nevertheless, our findings reveal a recurring, but potentially complex, relationship between these two important regulators of development, stress resistance, and lifespan.

It is interesting to note that when *hsf-1(sy441)* homozygotes are shifted to 25°C at the L4 larval stage, they lay eggs that hatch but fail to reach adulthood. However, when L1 larvae are starvation-arrested at 20°C and then fed and shifted to 25.8°C, some of those larvae reach adulthood. In other words, the general “arrest point” in the life cycle is different under these two conditions. This finding indicates that *hsf-1* loss does not cause a growth blockade at one specific point in the life cycle. Instead, this finding suggests the model that loss of HSF-1 function impacts a time-dependent accumulation of proteostatic damage rather than a specific developmental requirement. The opposite situation occurs in *Drosophila*, where HSF-1 activity is dispensable specifically after passing the first two larval stages

(Jedlicka *et al.* 1997). Because no *hsf-1(sy441); rsks-1(mu482)* double-mutant animals arrest in the first generation but do produce progeny which then arrest, we hypothesize that loss of *rsks-1* does not bypass HSF-1 function but rather delays or reduces the damage caused by the reduction of HSF-1 function.

It is surprising that we were unable to phenocopy the effects of *rsks-1* mutation through any mechanism other than mTOR knockdown, a condition predicted to reduce *rsks-1* activity. While we cannot know for certain whether a very specific amount of translation inhibition could rescue the developmental arrest, we have established that multiple methods of reducing translation at multiple concentrations are insufficient to rescue development, and indeed at levels of translation inhibition that significantly reduce animal size, we tended to find a harmful effect on development. If reduced translation were the mechanism of growth arrest suppression, then the situation we found in this study would contrast dramatically with the effects that a similar scan of translation-RNAi knockdowns produced on another phenotype, lifespan extension. In that case, a wide variety of RNAi knockdowns scored positively, even without careful dose-response analysis (Hansen *et al.* 2006).

Another potential way that translation could be involved in this phenotype is if inhibiting S6K fails to inhibit, or even promotes, the translation of a specific subset of genes. Many stress responses, such as the heat-shock response and integrated stress response, activate a subset of genes while inhibiting translation generally. If S6K inhibition causes a similar effect, then chemical or RNAi-mediated inhibition of translation alone, without activation of specific targets, would not be enough to rescue development.

Other than the small inhibition of rescue caused by *xbp-1* knockdown and the rescue caused by knockdown of *daf-15* and *ragc-1*, we did not identify other genes known to interact with *rsks-1* that are related to this phenotype. The finding that *daf-2* and *cco-1* knockdown do not rescue development makes it unlikely that the rescue is mediated by some general increase in stress resistance or lifespan-

increasing pathways. Even so, it was surprising to us that knockdown of transcription factors *daf-16, pha-4, skn-1* failed to prevent the rescue phenotype. These transcription factors are essential for stress resistance and lifespan extension produced by inhibiting components of the mTOR pathway. In addition, we saw no evidence across 65, either predicted to be targets of S6K or proteins known to interact functionally with S6K, of RNAi inhibition rescuing development of *hsf-1(sy441)* mutants, or having a more severe effect on the development of *hsf-1(sy441); rsks-1* double mutants vs. wild-type. While we cannot be sure from these experiments of the efficacy of each individual RNAi clone, in each experiment positive controls were present, and in some cases a visible phenotype (other than rescue) was produced by the RNAi clone, such as loss of eggs in animals with inhibited *pha-4*.

Despite the *rsks-1* mutants requiring some level of *hsf-1* function to rescue the *hsf-1(sy441)* arrest phenotype, we saw no evidence of an activation of known *hsf-1*-activated genes or other stress-response pathways. In yeast, only two heat-shock proteins are required to rescue the loss of *hsf-1* for development (Solís *et al.* 2016), therefore it is possible that one or more unmeasured or non-canonical heat-shock proteins are independently regulated by *hsf-1* and *rsks-1*, and it is through this unknown gene(s) that development is being rescued. For instance, Baird *et al.* previously showed that overexpression of the *sy441* allele improves cytoskeletal integrity through up-regulation of *pat-10.* We did not test the effects on *rsks-1* mutation on cytoskeletal integrity, so it remains a possible explanation for the rescue phenotype caused by *rsks-1* mutation.

It was surprising to note that the *hsf-1(sy441)* mutant animals showed more thermotolerance than wild-type controls. *hsf-1(sy441)* is considered an *hsf-1* hypomorph primarily based on the finding that it significantly reduces the induction of *hsp-16.2* in response to heat shock (Hajdu-Cronin *et al.* 2004) and it reduces lifespan (if to a slightly smaller magnitude) similar to *hsf-1* RNAi (Seo *et al.* 2013). However, I have shown that while *hsf-1(sy441)* does show less expression of *hsp-16.2*, the mutation has no effect on *hsp-70* (F44E5.4) expression, and the *hsf-1(sy441)* mutants also seem to have increased

thermotolerance. In another publication, McColl *et al.* showed that *hsf-1(sy441)* had the same level of thermotolerance as wild-type. I speculate that *hsf-1(sy441)* may be hypomorphic for only a subset of HSF-1 targets and that the organismal response to an extreme and deadly heat stress may be regulated by input from other pathways that differ between backgrounds, leading to presence or absence of thermotolerance in *hsf-1(sy441)* in different laboratories. It is also possible that differing methods of toxic heat stress differ in an unknown but crucial way that alters results between labs. The main focus of my study was development rather than thermotolerance, but this observation remains an interesting avenue for investigation.

Most of the experiments in this study involved either modulating the expression levels of genes with RNAi or measuring expression levels through RT-qPCR. As a kinase, it is possible that protein S6K modulates the activity of proteins directly without affecting expression levels. If such an activity increase activated the heat-shock response, the ER UPR, or the mito-UPR broadly, we would expect to measure that through an increased expression of canonical target genes, but it is possible that S6 kinase phosphorylates specific proteins in these pathways to mediate developmental arrest. This would be interesting to investigate in the future.

In conclusion, a genetic screen for suppressors of the developmental growth arrest of *hsf-1(sy441)* partial loss-of-function mutants revealed a previously unknown relationship between heat-shock factor and mTOR/S6 kinase activity. Reducing S6 kinase activity allows animals with insufficient HSF-1 activity to progress much further through the life cycle than would otherwise be possible. Because heat-shock factor is known to enhance proteostasis under conditions of heat stress, we propose that loss of S6 kinase postpones arrest either by reducing the levels of damaged macromolecules produced in the cell, or by increasing the cell's ability to remove or repair them.

## Chapter 4: Conclusion

Aging is a complex, whole-organism phenotype that changes over time as the result of many interacting genetic and cellular processes. Understanding aging is not only an engaging scientific challenge, but it is also an avenue by which we might ameliorate broad swaths of human suffering. For my thesis project at UCSF I have worked to elucidate two specific aspects of aging: regulation by the nervous system, and the nature of a transcription factor integral to the aging process.

In Chapter 2 I investigated the mechanism by which a single gustatory neuron pair, ASI, regulates lifespan. I showed that, similar to ablation of the neuron pair, silencing the chemical signaling of ASI with ASI-specific expression of tetanus toxin extends lifespan and likely does so through reducing secretion of DAF-7/ TGFβ, inhibiting the IIS pathway, and activating DAF-16. This simple explanation, however, belies some interesting but confusing findings. While *daf-16* is entirely required for *daf-7* mutants to increase lifespan it is only mostly required for silencing-ASI to do so. In addition, while almost 100% of *daf-7* mutants form dauers at 25°C, animals with silenced-ASI form no dauers and ablated-ASI animals form an amount somewhere in between. A difference between ablating and silencing is also seen with respect to the gustatory neuron pair ASJ, in which I saw remarkably different lifespan effects in the silenced strain than has been previously reported in the case of ablation. These results together hint at a more complex regulation of lifespan than ASI as a simple nutrient detector which raises or lowers the secretion of DAF-7 and thereby affects lifespan. The intra-neuron communications and eventual chemical signals other than DAF-7 that alter lifespan remain to be discovered. It would be interesting to see how more dynamic perturbations to the signaling of a single neuron affect both the neuronal activity of other neurons as well as their transcriptomes to eventually affect lifespan. Such an approach is made possible through technologies such as optogenetics, calcium indicators, single-neuron RNA-seq, and single-neuron RNAi. Unlike genetic deletions of important signaling molecules or wide-scale disruption of the nervous system, single neuron perturbation might

better reflect endogenous signals from the worm nervous system that regulate its lifespan in a natural context.

After investigating how primary sensory neurons, arguably one of the most upstream effectors of lifespan, regulate the process of aging, I investigated in Chapter 3 how two fundamental cellular processes, the heat shock response and the mTOR pathway, communicate with one another. I found that the arrest phenotype caused by loss of *hsf-1* function can be greatly ameliorated, but not completely rescued, by mutation of ribosomal S6 kinase and reduced-mTOR signaling more generally. This rescue is not due to a reduction in translation alone, and it is also not mediated through a variety of transcription factors and other genes known to interact with the mTOR pathway or play a role in lifespan. A mutation in S6 kinase normally extends lifespan but fails to do so in the *hsf-1(sy441)* mutant background, which, along with my studies, reveals a previously unknown reciprocal relationship between *rsks-1* and *hsf-1*: in one case, low levels of S6 kinase cause endogenous levels of HSF-1 to extend lifespan, whereas in the other case, low levels of HSF-1 cause endogenous levels of S6 kinase to arrest development.

I see two primary paths forward to further investigate this phenomenon of *hsf-1(sy441)* developmental arrest. The first is to collect large data sets to compare the single and double mutants. While I was able to find micro array and rna-seq datasets for *rsks-1* and *hsf-1(sy441)* mutants, there exists no such data set for animals carrying both mutations. Furthermore, because RSKS-1 is a kinase which regulates translation, proteomic and phosphoproteomic datasets may be even more helpful in determining how the developmental rescue phenotype is mediated. The second path is to focus not on the link between loss of *rsks-1* and developmental rescue but rather on the developmental arrest phenotype itself. What is the relationship between higher temperature, time exposed to higher temperature, and percentage of animals which arrest? Perplexingly, five different complementary suppressor mutations all produced animals with a similarly reduced size. Could it be that some aspect of

animal size or cell size rather than translation rate causes toxicity? A better understanding of precisely what deficit is causing arrest would inform a model of how *rsks-1* mutation rescues that deficit.

Modulation of both the mTOR pathway and the heat shock pathway are avenues being explored to improve human health, and while both may be able to improve cellular function over the lifespan, alterations in function may have implications for unwanted side effects such as reduced immune function or increased cancer risk. Communication between the two pathways may therefore prove important for effective optimization of pharmacological or other interventions. While I was not able to discover a molecular intermediate that facilitates communication between *rsks-1* and *hsf-1*, the discovery of such an intermediate remains an exciting direction for future research.

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## T**ables:**

**Table 2.1:** List of all lifespans conducted

| **Experiment #** | **Neuronal condition** | **Genetic condition** | **Median Lifespan** | **Sample Size** | **Automated?** | **integrated construct or extrachromosomal** |
|---|---|---|---|---|---|---|
| 1 | Silenced-ASI | | 17.5 | 71 | yes | integrated |
| 1 | Wild-Type | | 16.9 | 66 | yes | integrated |
| 1 | Silenced-ASI | *daf-3(RNAi)* | 15.6 | 166 | yes | integrated |
| 1 | Wild-Type | *daf-3(RNAi)* | 16.1 | 110 | yes | integrated |
| 1 | Silenced-ASI | *hif-1(RNAi)* | 15.6 | 97 | yes | integrated |
| 1 | Wild-Type | *hif-1(RNAi)* | 15 | 62 | yes | integrated |
| 1 | Silenced-ASI | *hsf-1(RNAi)* | 17.3 | 153 | yes | integrated |
| 1 | Wild-Type | *hsf-1(RNAi)* | 13.1 | 114 | yes | integrated |
| 2 | Silenced-ASI | | 17.1 | 84 | yes | integrated |
| 2 | Wild-Type | | 15.6 | 87 | yes | integrated |
| 2 | Ablated-ASI | | 17.8 | 69 | yes | integrated |
| 2 | Silenced-ASI | *daf-16(RNAi)* | 12.4 | 77 | yes | integrated |
| 2 | Wild-Type | *daf-16(RNAi)* | 12.5 | 92 | yes | integrated |
| 2 | Ablated-ASI | *daf-16(RNAi)* | 12.5 | 69 | yes | integrated |
| 3 | Silenced-ASI | | 18 | 47 | yes | integrated |
| 3 | Wild-Type | | 16.3 | 70 | yes | integrated |
| 3 | Ablated-ASI | | 18.2 | 41 | yes | integrated |
| 3 | Silenced-ASI | *daf-16(RNAi)* | 12.2 | 108 | yes | integrated |
| 3 | Ablated-ASI | *daf-16(RNAi)* | 12.1 | 89 | yes | integrated |
| 3 | Wild-Type | *daf-16(RNAi)* | 11.6 | 29 | yes | integrated |
| 4 | Silenced-ASI | | 18.7 | 159 | yes | integrated |
| 4 | Wild-Type | | 18.1 | 79 | yes | integrated |
| 4 | Ablated-ASI | | 19.6 | 145 | yes | integrated |
| 4 | Silenced-ASJ | | 14.7 | 88 | yes | integrated |
| 4 | Silenced-ASI; Silenced-ASI | | 17.3 | 157 | yes | integrated |
| 4 | Silenced-ASJ; Ablated-ASI | | 19.3 | 121 | yes | integrated |
| 5 | Wild-Type | | 11.2 | 153 | yes | integrated |
| 5 | Wild-Type | *daf-3(mgdf90)* | 10.5 | 46 | yes | integrated |
| 5 | Ablated-ASI | | 13.4 | 102 | yes | integrated |
| 5 | Ablated-ASI | *daf-3(mgdf90)* | 10.1 | 35 | yes | integrated |
| 6 | Wild-Type | *daf-3(mgdf90)* | 19 | 63 | no | integrated |
| 6 | Silenced-ASI | *daf-3(mgdf90)* | 19 | 49 | no | integrated |
| 7 | Wild-Type | | 16 | 94 | no | integrated |
| 7 | Wild-Type | *hsf-1(RNAi)* | 11 | 86 | no | integrated |

| Experiment # | Neuronal condition | Genetic condition | Median Lifespan | Sample Size | Automated? | integrated construct or extrachromosomal |
|---|---|---|---|---|---|---|
| 7 | Silenced-ASI | *hsf-1(RNAi)* | 16 | 87 | no | integrated |
| 7 | Ablated-ASI | *hsf-1(RNAi)* | 9 | 79 | no | integrated |
| 8 | Wild-Type | | 18 | 46 | no | integrated |
| 8 | Wild-Type | *hsf-1(RNAi)* | 15 | 82 | no | integrated |
| 8 | Silenced-ASI | | 22 | 82 | no | integrated |
| 8 | Silenced-ASI | *hsf-1(RNAi)* | 20 | 90 | no | integrated |
| 8 | Ablated-ASI | | 20 | 49 | no | integrated |
| 8 | Ablated-ASI | *hsf-1(RNAi)* | 15 | 91 | no | integrated |
| 9 | Silenced-ASI | | 16.3 | 156 | yes | extrachromosomal |
| 9 | Wild-Type | | 14.1 | 86 | yes | extrachromosomal |
| 9 | Silenced-ASI | *daf-3(mgdf90)* | 14.4 | 167 | yes | extrachromosomal |
| 9 | Wild-Type | *daf-3(mgdf90)* | 14.6 | 165 | yes | extrachromosomal |
| 9 | Silenced-ASI | *kri-1(ok1251)* | 21.3 | 134 | yes | extrachromosomal |
| 9 | Wild-Type | *kri-1(ok1251)* | 20.4 | 59 | yes | extrachromosomal |
| 10 | Silenced-ASI | | 17 | 66 | no | extrachromosomal |
| 10 | Wild-Type | | 15 | 55 | no | extrachromosomal |
| 10 | Silenced-ASI | | 15.9 | 141 | yes | extrachromosomal |
| 10 | Wild-Type | | 13.4 | 93 | yes | extrachromosomal |
| 10 | Silenced-ASI | *daf-16(RNAi)* | 11.3 | 84 | yes | extrachromosomal |
| 10 | Wild-Type | *daf-16(RNAi)* | 10.8 | 119 | yes | extrachromosomal |
| 11 | Silenced-ASI | | 16.9 | 118 | yes | extrachromosomal |
| 11 | Wild-Type | | 14.4 | 110 | yes | extrachromosomal |
| 12 | Ablated-ASI (ablated with a laser) | | 23 | 18 | no | extrachromosomal |
| 12 | Wild-Type | | 21 | 28 | no | extrachromosomal |
| 13 | Silenced-ASI | *daf-16(mu86)* | 13.1 | 36 | yes | extrachromosomal |
| 13 | Wild-Type | *daf-16(mu86)* | 10.9 | 51 | yes | extrachromosomal |
| 14 | Wild-Type | *daf-16(mu86)* | 15 | 130 | no | extrachromosomal |
| 14 | Silenced-ASI | *daf-16(mu86)* | 17 | 98 | no | extrachromosomal |
| 15 | Wild-Type | | 21 | 68 | no | extrachromosomal |
| 15 | Wild-Type | *hsf-1(RNAi)* | 14 | 117 | no | extrachromosomal |
| 15 | Silenced-ASI | | 23 | 98 | no | extrachromosomal |
| 15 | Silenced-ASI | *hsf-1(RNAi)* | 14 | 125 | no | extrachromosomal |
| 16 | Wild-Type | *daf-16(RNAi)* | 13 | 145 | no | extrachromosomal |
| 16 | Ablated-ASI | *daf-16(RNAi)* | 14 | 124 | no | integrated |
| 16 | Silenced-ASI | *daf-16(RNAi)* | 13 | 167 | no | extrachromosomal |
| 16 | Wild-Type | *daf-16(RNAi)* | 13 | 172 | no | extrachromosomal |

| **Experiment #** | **Neuronal condition** | **Genetic condition** | **Median Lifespan** | **Sample Size** | **Automated?** | **integrated construct or extrachromosomal** |
|---|---|---|---|---|---|---|
| 16 | Ablated-ASI crossed with Silenced-ASI expressing background | *daf-16(RNAi)* | 15 | 154 | no | extrachromosomal |
| 16 | Ablated ASI crossed with non-expresssing Silenced-ASI background | *daf-16(RNAi)* | 14 | 149 | no | extrachromosomal |

**Table 3.1:** Table of conditions that reduced translation.

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 1 | *hsf-1(sy441)* | daf-15 | | 52 | 42 | 44.7 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 1% | | 65 | 31 | 32.3 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 16% | | 68 | 11 | 13.9 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 2% | | 66 | 59 | 47.2 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 32% | | 102 | 0 | 0.0 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 4% | | 79 | 26 | 24.8 |
| Experiment 1 | *hsf-1(sy441)* | ifg-1 8% | | 86 | 9 | 9.5 |
| Experiment 1 | *hsf-1(sy441)* | Vector Control | | 76 | 44 | 36.7 |
| Experiment 1 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 0 | 136 | 100.0 |
| Experiment 1 | *hsf-1(sy441); rsks-1(sv31)* | Vector Control | | 0 | 63 | 100.0 |
| Experiment 1 | *wild-type* | ifg-1 16% | | 1 | 76 | 98.7 |
| Experiment 1 | *wild-type* | ifg-1 32% | | 6 | 70 | 92.1 |
| Experiment 1 | *wild-type* | ifg-1 4% | | 0 | 67 | 100.0 |
| Experiment 1 | *wild-type* | ifg-1 8% | | 1 | 71 | 98.6 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 1% | | 44 | 16 | 26.7 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 100% | | 98 | 0 | 0.0 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 16% | | 83 | 6 | 6.7 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 2% | | 78 | 12 | 13.3 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 32% | | 76 | 0 | 0.0 |
| Experiment 2 | *hsf-1(sy441)* | ifg-1 4% | | 22 | 7 | 24.1 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 2 | *hsf-1(sy441)* | ifg-1 8% | | 83 | 8 | 8.8 |
| Experiment 2 | *hsf-1(sy441)* | rsks-1 | | 41 | 62 | 60.2 |
| Experiment 2 | *hsf-1(sy441)* | Vector Control | | 94 | 29 | 23.6 |
| Experiment 2 | *hsf-1(sy441); rsks-1(mu482)* | ifg-1 32% | | 8 | 30 | 78.9 |
| Experiment 2 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 1 | 37 | 97.4 |
| Experiment 2 | *wild-type* | ifg-1 100% | | 55 | 1 | 1.8 |
| Experiment 2 | *wild-type* | ifg-1 16% | | 9 | 46 | 83.6 |
| Experiment 2 | *wild-type* | ifg-1 32% | | 17 | 43 | 71.7 |
| Experiment 3 | *hsf-1(sy441)* | rps-6 1.5% | | 73 | 4 | 5.2 |
| Experiment 3 | *hsf-1(sy441)* | rps-6 100% | | 157 | 0 | 0.0 |
| Experiment 3 | *hsf-1(sy441)* | rps-6 12.5% | | 23 | 0 | 0.0 |
| Experiment 3 | *hsf-1(sy441)* | rps-6 25% | | 105 | 2 | 1.9 |
| Experiment 3 | *hsf-1(sy441)* | rps-6 50% | | 126 | 0 | 0.0 |
| Experiment 3 | *hsf-1(sy441)* | rsks-1 | | 55 | 65 | 54.2 |
| Experiment 3 | *hsf-1(sy441)* | Vector Control | | 103 | 47 | 31.3 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | rps-6 100% | | 134 | 8 | 5.6 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 0 | 138 | 100.0 |
| Experiment 3 | *wild-type* | rps-6 1.5% | | 0 | 87 | 100.0 |
| Experiment 3 | *wild-type* | rps-6 100% | | 76 | 0 | 0.0 |
| Experiment 3 | *wild-type* | rps-6 12.5% | | 3 | 58 | 95.1 |
| Experiment 3 | *wild-type* | rps-6 25% | | 15 | 57 | 79.2 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 3 | *wild-type* | rps-6 3.125% | | 0 | 103 | 100.0 |
| Experiment 3 | *wild-type* | rps-6 50% | | 58 | 11 | 15.9 |
| Experiment 3 | *wild-type* | rps-6 6.25% | | 0 | 97 | 100.0 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 1% | | 77 | 58 | 43.0 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 100% | | 138 | 4 | 2.8 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 16% | | 103 | 4 | 3.7 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 2% | | 57 | 53 | 48.2 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 32% | | 112 | 5 | 4.3 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 4% | | 87 | 39 | 31.0 |
| Experiment 4 | *hsf-1(sy441)* | ifg-1 8% | | 120 | 17 | 12.4 |
| Experiment 4 | *hsf-1(sy441)* | rsks-1 | | 22 | 158 | 87.8 |
| Experiment 4 | *hsf-1(sy441)* | Vector Control | | 158 | 60 | 27.5 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | ifg-1 100% | | 78 | 42 | 35.0 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | ifg-1 16% | | 0 | 110 | 100.0 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | ifg-1 32% | | 25 | 71 | 74.0 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | ifg-1 8% | | 6 | 137 | 95.8 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 0 | 142 | 100.0 |
| Experiment 4 | *wild-type* | ifg-1 16% | | 8 | 133 | 94.3 |
| Experiment 4 | *wild-type* | ifg-1 32% | | 6 | 127 | 95.5 |
| Experiment 4 | *wild-type* | ifg-1 8% | | 0 | 113 | 100.0 |
| Experiment 5 | *wild-type* | Vector Control | 0.37% DMSO | 0 | 110 | 100.0 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 5 | *wild-type* | Vector Control | 2.9uM salubrinal | 2 | 90 | 97.8 |
| Experiment 5 | *wild-type* | Vector Control | 185.2uM salubrinal | 10 | 84 | 89.4 |
| Experiment 5 | *wild-type* | Vector Control | 23.1uM salubrinal | 6 | 84 | 93.3 |
| Experiment 5 | *wild-type* | Vector Control | 362nM salubrinal | 0 | 98 | 100.0 |
| Experiment 5 | *wild-type* | Vector Control | 46.3uM salubrinal | 2 | 110 | 98.2 |
| Experiment 5 | *wild-type* | Vector Control | 5.8uM salubrinal | 1 | 106 | 99.1 |
| Experiment 5 | *wild-type* | Vector Control | 723nM salubrinal | 2 | 128 | 98.5 |
| Experiment 5 | *wild-type* | Vector Control | 92.6uM salubrinal | 2 | 100 | 98.0 |
| Experiment 5 | *wild-type* | Vector Control | 11.6uM salubrinal | 8 | 72 | 90.0 |
| Experiment 5 | *wild-type* | Vector Control | 1.4uM salubrinal | 0 | 107 | 100.0 |
| Experiment 6 | *wild-type* | Vector Control | 0.1% DMSO | 0 | 107 | 100.0 |
| Experiment 6 | *wild-type* | Vector Control | 2.9uM harringtonine | 0 | 108 | 100.0 |
| Experiment 6 | *wild-type* | Vector Control | 185.2uM harringtonine | 87 | 20 | 18.7 |
| Experiment 6 | *wild-type* | Vector Control | 23.1uM harringtonine | 1 | 97 | 99.0 |
| Experiment 6 | *wild-type* | Vector Control | 362nM harringtonine | 2 | 102 | 98.1 |
| Experiment 6 | *wild-type* | Vector Control | 370.4uM harringtonine | 40 | 55 | 57.9 |
| Experiment 6 | *wild-type* | Vector Control | 46.3uM harringtonine | 1 | 101 | 99.0 |
| Experiment 6 | *wild-type* | Vector Control | 5.8uM harringtonine | 0 | 78 | 100.0 |
| Experiment 6 | *wild-type* | Vector Control | 723nM harringtonine | 0 | 77 | 100.0 |
| Experiment 6 | *wild-type* | Vector Control | 92.6uM harringtonine | 29 | 60 | 67.4 |
| Experiment 6 | *wild-type* | Vector Control | 11.6uM harringtonine | 0 | 94 | 100.0 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 6 | *wild-type* | Vector Control | 1.4uM harringtonine | 0 | 85 | 100.0 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 0.09% | | 92 | 27 | 22.7 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 0.18% | | 87 | 52 | 37.4 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 0.37% | | 44 | 79 | 64.2 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 0.75% | | 80 | 55 | 40.7 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 1.5% | | 107 | 80 | 42.8 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 12.5% | | 105 | 46 | 30.5 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 3.125% | | 75 | 39 | 34.2 |
| Experiment 7 | *hsf-1(sy441)* | rps-6 6.25% | | 68 | 44 | 39.3 |
| Experiment 7 | *hsf-1(sy441)* | Vector Control | | 82 | 72 | 46.8 |
| Experiment 7 | *wild-type* | rps-6 100% | | 76 | 16 | 17.4 |
| Experiment 7 | *wild-type* | rps-6 12.5% | | 0 | 128 | 100.0 |
| Experiment 7 | *wild-type* | rps-6 25% | | 33 | 127 | 79.4 |
| Experiment 7 | *wild-type* | rps-6 50% | | 56 | 45 | 44.6 |
| Experiment 7 | *wild-type* | rps-6 6.5% | | 0 | 103 | 100.0 |
| Experiment 7 | *wild-type* | Vector Control | | 0 | 101 | 100.0 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 1 | 158 | 99.4 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 0.045% | | 76 | 77 | 50.3 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 0.09% | | 60 | 72 | 54.5 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 0.18% | | 82 | 78 | 48.8 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 0.37% | | 27 | 38 | 58.5 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 8 | *hsf-1(sy441)* | rps-6 0.75% | | 52 | 79 | 60.3 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 1.5% | | 52 | 39 | 42.9 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 12.5% | | 80 | 70 | 46.7 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 25% | | 72 | 48 | 40.0 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 3.125% | | 38 | 85 | 69.1 |
| Experiment 8 | *hsf-1(sy441)* | rps-6 6.25% | | 107 | 109 | 50.5 |
| Experiment 8 | *hsf-1(sy441)* | rsks-1 | | 27 | 116 | 81.1 |
| Experiment 8 | *hsf-1(sy441)* | Vector Control | | 62 | 110 | 64.0 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | | 1 | 158 | 99.4 |
| Experiment 8 | *wild-type* | rps-6 100% | | 150 | 0 | 0.0 |
| Experiment 8 | *wild-type* | rps-6 12.5% | | 16 | 89 | 84.8 |
| Experiment 8 | *wild-type* | rps-6 25% | | 21 | 117 | 84.8 |
| Experiment 8 | *wild-type* | rps-6 50% | | 90 | 9 | 9.1 |
| Experiment 8 | *wild-type* | Vector Control | | 0 | 127 | 100.0 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 0.045% | | 82 | 48 | 36.9 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 0.09% | | 64 | 54 | 45.8 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 0.18% | | 94 | 44 | 31.9 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 0.37% | | 67 | 41 | 38.0 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 0.75% | | 67 | 39 | 36.8 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 1.5% | | 80 | 25 | 23.8 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 12.5% | | 82 | 19 | 18.8 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 9 | *hsf-1(sy441)* | rps-6 25% | | 84 | 20 | 19.2 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 3.125% | | 71 | 47 | 39.8 |
| Experiment 9 | *hsf-1(sy441)* | rps-6 6.25% | | 57 | 39 | 40.6 |
| Experiment 9 | *hsf-1(sy441)* | rsks-1 | | 36 | 94 | 72.3 |
| Experiment 9 | *hsf-1(sy441)* | Vector Control | | 107 | 34 | 24.1 |
| Experiment 9 | *wild-type* | rps-6 100% | | 89 | 0 | 0.0 |
| Experiment 9 | *wild-type* | rps-6 12.5% | | 0 | 139 | 100.0 |
| Experiment 9 | *wild-type* | rps-6 25% | | 13 | 116 | 89.9 |
| Experiment 9 | *wild-type* | rps-6 50% | | 68 | 49 | 41.9 |
| Experiment 9 | *wild-type* | Vector Control | | 0 | 105 | 100.0 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 23nM salubrinal | 57 | 12 | 17.4 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 45nM salubrinal | 91 | 20 | 18.0 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 90nM salubrinal | 87 | 7 | 7.4 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 181nM salubrinal | 97 | 7 | 6.7 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 0.37% DMSO | 97 | 13 | 11.8 |
| Experiment 10 | *hsf-1(sy441)* | rsks-1 | 0.37%DMSO | 55 | 20 | 26.7 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 67 | 21 | 23.9 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 66 | 28 | 29.8 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 1.4uM salubrinal | 54 | 22 | 28.9 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 1.56uM salubrinal | 82 | 5 | 5.7 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 23.1uM salubrinal | 68 | 1 | 1.4 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 5.8uM salubrinal | 96 | 6 | 5.9 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 78 | 0 | 0.0 |
| Experiment 10 | *hsf-1(sy441)* | Vector Control | | 79 | 39 | 33.1 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | 23.1uM salubrinal | 68 | 10 | 12.8 |
| Experiment 10 | *wild-type* | Vector Control | 0.37% DMSO | 0 | 101 | 100.0 |
| Experiment 10 | *wild-type* | Vector Control | 5.8uM salubrinal | 0 | 83 | 100.0 |
| Experiment 10 | *wild-type* | Vector Control | 11.6uM salubrinal | 0 | 97 | 100.0 |
| Experiment 10 | *wild-type* | Vector Control | | 0 | 67 | 100.0 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 181nM salubrinal | 82 | 16 | 16.3 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 45nM salubrinal | 82 | 19 | 18.8 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 90nM salubrinal | 82 | 13 | 13.7 |
| Experiment 11 | *hsf-1(sy441)* | rsks-1 | 0.19%DMSO | 11 | 7 | 38.9 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 0.19%DMSO | 47 | 4 | 7.8 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 181nM salubrinal | 99 | 16 | 13.9 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 0.37% DMSO | 83 | 7 | 7.8 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 95 | 12 | 11.2 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 87 | 33 | 27.5 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 1.4uM salubrinal | 69 | 19 | 21.6 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 2.9uM salubrinal | 96 | 19 | 16.5 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 23.1uM salubrinal | 90 | 3 | 3.2 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 5.8uM salubrinal | 74 | 6 | 7.5 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 11 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 81 | 1 | 1.2 |
| Experiment 11 | *hsf-1(sy441)* | Vector Control | | 48 | 27 | 36.0 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | 5.8uM salubrinal | 65 | 59 | 47.6 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | 11.6uM salubrinal | 51 | 21 | 29.2 |
| Experiment 11 | *wild-type* | Vector Control | 5.8uM salubrinal | 0 | 101 | 100.0 |
| Experiment 11 | *wild-type* | Vector Control | 11.6uM salubrinal | 0 | 100 | 100.0 |
| Experiment 12 | *hsf-1(sy441)* | rsks-1 | 0.19%DMSO | 40 | 58 | 59.2 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 0.19%DMSO | 90 | 40 | 30.8 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 137 | 49 | 26.3 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 362nM harringtonine | 126 | 37 | 22.7 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 723nM harringtonine | 111 | 37 | 25.0 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 1.4uM harringtonine | 110 | 21 | 16.0 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 2.9uM harringtonine | 135 | 23 | 14.6 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 185.2uM harringtonine | 102 | 0 | 0.0 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 23.1uM harringtonine | 129 | 18 | 12.2 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 46.3uM harringtonine | 98 | 16 | 14.0 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 130 | 15 | 10.3 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 92.6uM harringtonine | 112 | 14 | 11.1 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | 11.6uM harringtonine | 101 | 14 | 12.2 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | | 54 | 91 | 62.8 |
| Experiment 12 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | 92.6uM harringtonine | 20 | 66 | 76.7 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 12 | *wild-type* | Vector Control | 0.19% DMSO | 0 | 66 | 100.0 |
| Experiment 12 | *wild-type* | Vector Control | 185.2uM harringtonine | 2 | 110 | 98.2 |
| Experiment 12 | *wild-type* | Vector Control | 46.3uM harringtonine | 0 | 91 | 100.0 |
| Experiment 12 | *wild-type* | Vector Control | 92.6uM harringtonine | 0 | 69 | 100.0 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.012% DMSO | 83 | 4 | 4.6 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 65 | 14 | 17.7 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.046% DMSO | 96 | 18 | 15.8 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.092% DMSO | 77 | 15 | 16.3 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 97 | 13 | 11.8 |
| Experiment 13 | *hsf-1(sy441)* | rsks-1 | 0.19%DMSO | 68 | 23 | 25.3 |
| Experiment 13 | *hsf-1(sy441)* | Vector Control | 0.37% DMSO | 70 | 9 | 11.4 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 83 | 14 | 14.4 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 6nM harringtonine | 94 | 19 | 16.8 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 11nM harringtonine | 80 | 20 | 20.0 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 77 | 27 | 26.0 |
| Experiment 14 | *hsf-1(sy441)* | rsks-1 | 0.023%DMSO | 63 | 32 | 33.7 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 23nM harringtonine | 78 | 11 | 12.4 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 45nM harringtonine | 51 | 17 | 25.0 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 84 | 28 | 25.0 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 0.092% DMSO | 99 | 22 | 18.2 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 55 | 25 | 31.3 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 57 | 27 | 32.1 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 362nM harringtonine | 64 | 21 | 24.7 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 723nM harringtonine | 73 | 9 | 11.0 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 1.4uM harringtonine | 70 | 17 | 19.5 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | 2.9uM harringtonine | 58 | 9 | 13.4 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | H20 | 62 | 36 | 36.7 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 23nM salubrinal | 71 | 33 | 31.7 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 0.046% DMSO | 77 | 27 | 26.0 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 45nM salubrinal | 68 | 38 | 35.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 0.092% DMSO | 72 | 25 | 25.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 90nM salubrinal | 78 | 38 | 32.8 |
| Experiment 15 | *hsf-1(sy441)* | rsks-1 | 0.19%DMSO | 58 | 40 | 40.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 0.19%DMSO | 85 | 25 | 22.7 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 181nM salubrinal | 80 | 24 | 23.1 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 97 | 50 | 34.0 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 108 | 20 | 15.6 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 1.4uM salubrinal | 58 | 15 | 20.5 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 2.9uM salubrinal | 78 | 18 | 18.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 23.1uM salubrinal | 127 | 1 | 0.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 5.8uM salubrinal | 71 | 21 | 22.8 |
| Experiment 15 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 97 | 2 | 2.0 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | Vector Control | H2O | 88 | 63 | 41.7 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 69 | 49 | 41.5 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 6nM harringtonine | 73 | 33 | 31.1 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 11nM harringtonine | 50 | 26 | 34.2 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 82 | 25 | 23.4 |
| Experiment 16 | *hsf-1(sy441)* | rsks-1 | 0.023%DMSO | 24 | 64 | 72.7 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 23nM harringtonine | 87 | 29 | 25.0 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 45nM harringtonine | 82 | 47 | 36.4 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 47 | 31 | 39.7 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 0.092% DMSO | 98 | 36 | 26.9 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 43 | 31 | 41.9 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 61 | 25 | 29.1 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 362nM harringtonine | 77 | 27 | 26.0 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 723nM harringtonine | 52 | 24 | 31.6 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 1.4uM harringtonine | 73 | 20 | 21.5 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | 2.9uM harringtonine | 76 | 22 | 22.4 |
| Experiment 16 | *hsf-1(sy441)* | Vector Control | H20 | 64 | 49 | 43.4 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 6nM salubrinal | 122 | 47 | 27.8 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 6nM salubrinal | 95 | 22 | 18.8 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 23nM salubrinal | 83 | 28 | 25.2 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 23nM salubrinal | 85 | 18 | 17.5 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 23nM salubrinal | 94 | 22 | 19.0 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 120 | 17 | 12.4 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 118 | 47 | 28.5 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 107 | 26 | 19.5 |
| Experiment 17 | *hsf-1(sy441)* | rsks-1 | 0.19%DMSO | 54 | 66 | 55.0 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 82 | 18 | 18.0 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 106 | 43 | 28.9 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 130 | 6 | 4.4 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 96 | 39 | 28.9 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 112 | 29 | 20.6 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 131 | 20 | 13.2 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 110 | 1 | 0.9 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 70 | 1 | 1.4 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 51 | 8 | 13.6 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 36 | 10 | 21.7 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 43 | 11 | 20.4 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 52 | 6 | 10.3 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 39 | 8 | 17.0 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 60 | 4 | 6.3 |
| Experiment 18 | *hsf-1(sy441)* | rsks-1 | 0.023%DMSO | 29 | 10 | 25.6 |
| Experiment 18 | *hsf-1(sy441)* | rsks-1 | 0.023%DMSO | 30 | 14 | 31.8 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 18 | *hsf-1(sy441)* | rsks-1 | 0.023%DMSO | 36 | 10 | 21.7 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 52 | 8 | 13.3 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 42 | 7 | 14.3 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 53 | 0 | 0.0 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 43 | 5 | 10.4 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 54 | 2 | 3.6 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 40 | 9 | 18.4 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 51 | 1 | 1.9 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 56 | 2 | 3.4 |
| Experiment 18 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 40 | 2 | 4.8 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 73 | 27 | 27.0 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 103 | 12 | 10.4 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 3nM harringtonine | 113 | 10 | 8.1 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 103 | 8 | 7.2 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 68 | 24 | 26.1 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 0.023% DMSO | 107 | 9 | 7.8 |
| Experiment 19 | *hsf-1(sy441)* | rsks-1 | 0.023% DMSO | 82 | 53 | 39.3 |
| Experiment 19 | *hsf-1(sy441)* | rsks-1 | 0.023% DMSO | 58 | 55 | 48.7 |
| Experiment 19 | *hsf-1(sy441)* | rsks-1 | 0.023% DMSO | 80 | 10 | 11.1 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 107 | 15 | 12.3 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 107 | 15 | 12.3 |

| Experiment # | *Genotype* | RNAi | Drug Treatment | # Animals that did not reach adulthood | # Adults | Percentage of Animals that Developed to Adulthood |
|---|---|---|---|---|---|---|
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 90nM harringtonine | 99 | 20 | 16.8 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 96 | 8 | 7.7 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 90 | 6 | 6.3 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 181nM harringtonine | 93 | 13 | 12.3 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 81 | 6 | 6.9 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 94 | 4 | 4.1 |
| Experiment 19 | *hsf-1(sy441)* | Vector Control | 5.8uM harringtonine | 127 | 7 | 5.2 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 6nM salubrinal | 109 | 20 | 15.5 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 6nM salubrinal | 88 | 23 | 20.7 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 6nM salubrinal | 87 | 7 | 7.4 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 72 | 10 | 12.2 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 95 | 12 | 11.2 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 142 | 20 | 12.3 |
| Experiment 20 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 46 | 27 | 37.0 |
| Experiment 20 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 80 | 34 | 29.8 |
| Experiment 20 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 60 | 57 | 48.7 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 81 | 14 | 14.7 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 54 | 10 | 15.6 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 362nM salubrinal | 33 | 6 | 15.4 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 32 | 4 | 11.1 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 80 | 8 | 9.1 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 723nM salubrinal | 128 | 16 | 11.1 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 123 | 1 | 0.8 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 74 | 2 | 2.6 |
| Experiment 20 | *hsf-1(sy441)* | Vector Control | 11.6uM salubrinal | 139 | 5 | 3.5 |
| Experiment 21 | *hsf-1(sy441)* | Vector Control | | 79 | 23 | 22.5 |
| Experiment 21 | *hsf-1(sy441)* | rsks-1 | | 44 | 32 | 42.1 |
| Experiment 21 | *hsf-1(sy441)* | rps-6 50% | | 63 | 0 | 0.0 |
| Experiment 21 | *hsf-1(sy441)* | rps-6 25% | | 95 | 4 | 4.0 |
| Experiment 21 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 85 | 5 | 5.6 |
| Experiment 21 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 71 | 37 | 34.3 |
| Experiment 21 | *hsf-1(sy441)* | Vector Control | 185.2uM harringtonine | 92 | 1 | 1.1 |
| Experiment 22 | *hsf-1(sy441)* | Vector Control | | 65 | 49 | 43.0 |
| Experiment 22 | *hsf-1(sy441)* | rsks-1 | | 46 | 81 | 63.8 |
| Experiment 22 | *hsf-1(sy441)* | rps-6 50% | | 98 | 0 | 0.0 |
| Experiment 22 | *hsf-1(sy441)* | rps-6 25% | | 93 | 4 | 4.1 |
| Experiment 22 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 67 | 18 | 21.2 |
| Experiment 22 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 30 | 42 | 58.3 |
| Experiment 23 | *hsf-1(sy441)* | Vector Control | 185.2uM harringtonine | 64 | 0 | 0.0 |
| Experiment 23 | *hsf-1(sy441)* | Vector Control | | 50 | 23 | 31.5 |
| Experiment 23 | *hsf-1(sy441)* | rsks-1 | | 36 | 46 | 56.1 |
| Experiment 23 | *hsf-1(sy441)* | rps-6 50% | | 91 | 2 | 2.2 |

| **Experiment #** | ***Genotype*** | **RNAi** | **Drug Treatment** | **# Animals that did not reach adulthood** | **# Adults** | **Percentage of Animals that Developed to Adulthood** |
|---|---|---|---|---|---|---|
| Experiment 23 | *hsf-1(sy441)* | rps-6 25% | | 91 | 6 | 6.2 |
| Experiment 23 | *hsf-1(sy441)* | Vector Control | 0.19% DMSO | 37 | 19 | 33.9 |
| Experiment 23 | *hsf-1(sy441)* | rsks-1 | 0.19% DMSO | 28 | 28 | 50.0 |
| Experiment 23 | *hsf-1(sy441)* | Vector Control | 185.2uM harringtonine | 61 | 0 | 0.0 |

**Table 3.2:** RNAi knockdown of many genes associated with mTOR, *rsks-1*, and *hsf-1* failed to rescue the *hsf-1(sy441)* developmental arrest or failed to prevent the *rsks-1(mu482)* mutation from rescuing the *hsf-1(sy441)* developmental arrest.

| **Experiment #** | **Genotype** | **RNAi** | **RNAi Clone Seq-uenced?** | **Comments** | **# Animals that did not reach adulthood** | **# Adults** | **% Animals Developed to Adulthood** |
|---|---|---|---|---|---|---|---|
| Experiment 1 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 70 | 0 | 0.0 |
| Experiment 1 | *hsf-1(sy441); rsks-1(mu482)* | *rsks-1* | yes | | 5 | 102 | 95.3 |
| Experiment 1 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 1 | 120 | 99.2 |
| Experiment 1 | *hsf-1(sy441)* | *hsf-1* | yes | | 76 | 0 | 0.0 |
| Experiment 1 | *hsf-1(sy441)* | Vector Control | yes | | 73 | 6 | 7.6 |
| Experiment 1 | *hsf-1(sy441)* | *rsks-1* | yes | | 49 | 52 | 51.5 |
| Experiment 1 | wild-type | *hsf-1* | yes | | 8 | 70 | 89.7 |
| Experiment 1 | wild-type | Vector Control | yes | | 0 | 64 | 100.0 |
| Experiment 1 | wild-type | *rsks-1* | yes | | 0 | 138 | 100.0 |
| Experiment 2 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 1 | 69 | 98.6 |
| Experiment 2 | *hsf-1(sy441)* | *cco-1* | yes | | 85 | 21 | 19.8 |
| Experiment 2 | *hsf-1(sy441)* | *daf-15* | yes | | 61 | 41 | 40.2 |
| Experiment 2 | *hsf-1(sy441)* | *daf-2* | yes | | 91 | 9 | 9.0 |
| Experiment 2 | *hsf-1(sy441)* | *hsb-1* | yes | | 85 | 22 | 20.6 |
| Experiment 2 | *hsf-1(sy441)* | Vector Control | yes | | 62 | 16 | 20.5 |
| Experiment 2 | *hsf-1(sy441)* | *ragc-1* | yes | | 63 | 37 | 37.0 |
| Experiment 2 | *hsf-1(sy441)* | *rsks-1* | yes | | 54 | 46 | 46.0 |
| Experiment 2 | wild-type | *cco-1* | yes | | 6 | 39 | 86.7 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 2 | wild-type | *daf-15* | yes | | 0 | 62 | 100.0 |
| Experiment 2 | wild-type | *daf-2* | yes | | 0 | 68 | 100.0 |
| Experiment 2 | wild-type | *hsb-1* | yes | | 0 | 64 | 100.0 |
| Experiment 2 | wild-type | Vector Control | yes | | 0 | 65 | 100.0 |
| Experiment 2 | wild-type | *ragc-1* | yes | | 0 | 122 | 100.0 |
| Experiment 2 | wild-type | *rsks-1* | yes | | 0 | 71 | 100.0 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | *daf-16* | yes | | 0 | 75 | 100.0 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 41 | 42 | 50.6 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-4* | yes | | 0 | 87 | 100.0 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 85 | 100.0 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 6 | 105 | 94.6 |
| Experiment 3 | *hsf-1(sy441); rsks-1(mu482)* | *stc-1* | yes | | 10 | 114 | 91.9 |
| Experiment 3 | *hsf-1(sy441)* | Vector Control | yes | | 32 | 32 | 50.0 |
| Experiment 3 | *hsf-1(sy441)* | *rsks-1* | yes | | 2 | 69 | 97.2 |
| Experiment 3 | wild-type | *hsf-1* | yes | | 1 | 75 | 98.7 |
| Experiment 3 | wild-type | *hsp-4* | yes | | 22 | 79 | 78.2 |
| Experiment 3 | wild-type | *pha-4* | yes | | 2 | 63 | 96.9 |
| Experiment 3 | wild-type | *stc-1* | yes | | 0 | 33 | 100.0 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *daf-16* | yes | | 1 | 66 | 98.5 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *hif-1* | yes | | 0 | 70 | 100.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 105 | 40 | 27.6 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-4* | yes | | 8 | 70 | 89.7 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 2 | 155 | 98.7 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 0 | 23 | 100.0 |
| Experiment 4 | *hsf-1(sy441); rsks-1(mu482)* | *rsks-1* | yes | | 2 | 81 | 97.6 |
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *daf-16* | yes | | 4 | 60 | 93.8 |
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsf-1* | yes | | 71 | 0 | 0.0 |
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsp-4* | yes | | 30 | 21 | 41.2 |
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | Vector Control | yes | | 3 | 109 | 97.3 |
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *pha-4* | yes | | 3 | 44 | 93.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 4 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *rsks-1* | yes | | 3 | 53 | 94.6 |
| Experiment 4 | *hsf-1(sy441)* | *hif-1* | yes | | 100 | 52 | 34.2 |
| Experiment 4 | *hsf-1(sy441)* | *hsf-1* | yes | | 145 | 0 | 0.0 |
| Experiment 4 | *hsf-1(sy441)* | Vector Control | yes | | 74 | 44 | 37.3 |
| Experiment 4 | *hsf-1(sy441)* | *rsks-1* | yes | | 36 | 129 | 78.2 |
| Experiment 4 | wild-type | *hif-1* | yes | | 0 | 93 | 100.0 |
| Experiment 5 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 61 | 39 | 39.0 |
| Experiment 5 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 95 | 100.0 |
| Experiment 5 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsf-1* | yes | | 120 | 0 | 0.0 |
| Experiment 5 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | Vector Control | yes | | 1 | 105 | 99.1 |
| Experiment 5 | *hsf-1(sy441)* | *cco-1* | yes | | 163 | 20 | 10.9 |
| Experiment 5 | *hsf-1(sy441)* | *daf-15* | yes | | 90 | 24 | 21.1 |
| Experiment 5 | *hsf-1(sy441)* | *daf-2* | yes | | 102 | 1 | 1.0 |
| Experiment 5 | *hsf-1(sy441)* | *hsb-1* | yes | | 103 | 11 | 9.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 5 | *hsf-1(sy441)* | *hsf-1* | yes | | 107 | 0 | 0.0 |
| Experiment 5 | *hsf-1(sy441)* | Vector Control | yes | | 94 | 15 | 13.8 |
| Experiment 5 | *hsf-1(sy441)* | *ragc-1* | yes | | 81 | 49 | 37.7 |
| Experiment 5 | *hsf-1(sy441)* | *rsks-1* | yes | | 51 | 79 | 60.8 |
| Experiment 5 | wild-type | *cco-1* | yes | | 2 | 99 | 98.0 |
| Experiment 5 | wild-type | *hsf-1* | yes | | 0 | 52 | 100.0 |
| Experiment 5 | wild-type | Vector Control | yes | | 0 | 69 | 100.0 |
| Experiment 6 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 35 | 15 | 30.0 |
| Experiment 6 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 37 | 100.0 |
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *daf-16* | yes | | 0 | 125 | 100.0 |
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hif-1* | yes | | 0 | 105 | 100.0 |
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsf-1* | yes | | 89 | 0 | 0.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsp-4* | yes | | 24 | 24 | 50.0 |
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | Vector Control | yes | | 0 | 119 | 100.0 |
| Experiment 6 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *pha-4* | yes | | 0 | 86 | 100.0 |
| Experiment 6 | *hsf-1(sy441)* | *hsf-1* | yes | | 231 | 1 | 0.4 |
| Experiment 6 | *hsf-1(sy441)* | Vector Control | yes | | 127 | 51 | 28.7 |
| Experiment 6 | *hsf-1(sy441)* | *rsks-1* | yes | | 47 | 106 | 69.3 |
| Experiment 6 | wild-type | *hif-1* | yes | | 0 | 144 | 100.0 |
| Experiment 7 | *hsf-1(sy441); rsks-1(mu482)* | *cco-1* | yes | | 6 | 173 | 96.6 |
| Experiment 7 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 78 | 81 | 50.9 |
| Experiment 7 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 178 | 100.0 |
| Experiment 7 | *hsf-1(sy441)* | *cco-1* | yes | | 87 | 4 | 4.4 |
| Experiment 7 | *hsf-1(sy441)* | *daf-15* | yes | | 80 | 15 | 15.8 |
| Experiment 7 | *hsf-1(sy441)* | *daf-2* | yes | | 51 | 3 | 5.6 |
| Experiment 7 | *hsf-1(sy441)* | *hsb-1* | yes | | 72 | 29 | 28.7 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 7 | *hsf-1(sy441)* | *hsf-1* | yes | | 85 | 1 | 1.2 |
| Experiment 7 | *hsf-1(sy441)* | Vector Control | yes | | 93 | 17 | 15.5 |
| Experiment 7 | *hsf-1(sy441)* | *ragc-1* | yes | | 49 | 21 | 30.0 |
| Experiment 7 | *hsf-1(sy441)* | *rsks-1* | yes | | 41 | 30 | 42.3 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 101 | 20 | 16.5 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-4* | yes | | 2 | 113 | 98.3 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-6* | yes | | 117 | 0 | 0.0 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 105 | 100.0 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *pat-10* | yes | | 17 | 100 | 85.5 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *W04b5.5* | yes | | 3 | 114 | 97.4 |
| Experiment 8 | *hsf-1(sy441); rsks-1(mu482)* | *xbp-1* | yes | | 29 | 94 | 76.4 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsf-1* | yes | | 103 | 0 | 0.0 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsp-4* | yes | | 22 | 98 | 81.7 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *hsp-6* | yes | | 152 | 0 | 0.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | Vector Control | yes | | 1 | 133 | 99.3 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *pat-10* | yes | | 81 | 33 | 28.9 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *W04b5.5* | yes | | 0 | 141 | 100.0 |
| Experiment 8 | *hsf-1(sy441); uthIs225[sur-5p::hsf-1(sy441); myo2p::tdTomato]* | *xbp-1* | yes | | 2 | 106 | 98.1 |
| Experiment 8 | *hsf-1(sy441)* | Vector Control | yes | | 180 | 27 | 13.0 |
| Experiment 8 | *hsf-1(sy441)* | *rsks-1* | yes | | 137 | 129 | 48.5 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 75 | 11 | 12.8 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-4* | yes | | 7 | 102 | 93.6 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-6* | yes | | 82 | 3 | 3.5 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 4 | 111 | 96.5 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *pat-10* | yes | | 27 | 70 | 72.2 |
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *W04b5.5* | yes | | 4 | 94 | 95.9 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 9 | *hsf-1(sy441); rsks-1(mu482)* | *xbp-1* | yes | | 20 | 78 | 79.6 |
| Experiment 9 | *hsf-1(sy441)* | Vector Control | yes | | 114 | 12 | 9.5 |
| Experiment 9 | *hsf-1(sy441)* | *rsks-1* | yes | | 84 | 44 | 34.4 |
| Experiment 9 | wild-type | *hsp-4* | yes | | 0 | 133 | 100.0 |
| Experiment 9 | wild-type | *hsp-6* | yes | | 42 | 2 | 4.5 |
| Experiment 9 | wild-type | Vector Control | yes | | 0 | 114 | 100.0 |
| Experiment 9 | wild-type | *pat-10* | yes | | 96 | 19 | 16.5 |
| Experiment 9 | wild-type | *w04b5.5* | yes | | 0 | 130 | 100.0 |
| Experiment 9 | wild-type | *xbp-1* | yes | | 0 | 95 | 100.0 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 38 | 9 | 19.1 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-4* | yes | | 1 | 51 | 98.1 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-6* | yes | | 42 | 0 | 0.0 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 6 | 54 | 90.0 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *pat-10* | yes | | 27 | 22 | 44.9 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *w04b5.5* | yes | | 1 | 53 | 98.1 |
| Experiment 10 | *hsf-1(sy441); rsks-1(mu482)* | *xbp-1* | yes | | 5 | 25 | 83.3 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 10 | *hsf-1(sy441)* | Vector Control | yes | | 111 | 15 | 11.9 |
| Experiment 10 | *hsf-1(sy441)* | *rsks-1* | yes | | 66 | 64 | 49.2 |
| Experiment 10 | wild-type | *hsp-6* | yes | | 66 | 0 | 0.0 |
| Experiment 10 | wild-type | Vector Control | yes | | 0 | 104 | 100.0 |
| Experiment 10 | wild-type | *pat-10* | yes | | 89 | 30 | 25.2 |
| Experiment 10 | wild-type | *w04b5.5* | yes | | 0 | 93 | 100.0 |
| Experiment 10 | wild-type | *xbp-1* | yes | | 0 | 79 | 100.0 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *ftt-2* | yes | | 9 | 120 | 93.0 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *zipt-7.1* | yes | | 1 | 134 | 99.3 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 42 | 55 | 56.7 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *hsp-60* | yes | | 19 | 114 | 85.7 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 3 | 84 | 96.6 |
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *lin-12* | | | 0 | 91 | 100.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 11 | *hsf-1(sy441); rsks-1(mu482)* | *pek-1* | yes | | 0 | 91 | 100.0 |
| Experiment 11 | wild-type | *ftt-2* | yes | | 0 | 117 | 100.0 |
| Experiment 11 | wild-type | *zipt-7.1* | yes | | 0 | 85 | 100.0 |
| Experiment 11 | wild-type | *hsf-1* | yes | | 0 | 78 | 100.0 |
| Experiment 11 | wild-type | *hsp-60* | yes | | 20 | 142 | 87.7 |
| Experiment 11 | wild-type | Vector Control | yes | | 0 | 105 | 100.0 |
| Experiment 11 | wild-type | *pek-1* | yes | | 0 | 85 | 100.0 |
| Experiment 12 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 31 | 37 | 54.4 |
| Experiment 12 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 1 | 78 | 98.7 |
| Experiment 12 | *hsf-1(sy441); rsks-1(mu482)* | *rsks-1* | yes | | 0 | 82 | 100.0 |
| Experiment 12 | *hsf-1(sy441)* | *hsf-1* | yes | | 99 | 2 | 2.0 |
| Experiment 12 | *hsf-1(sy441)* | Vector Control | yes | | 73 | 11 | 13.1 |
| Experiment 12 | *hsf-1(sy441)* | *rsks-1* | yes | | 21 | 64 | 75.3 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 12 | wild-type | *hsf-1* | yes | | 0 | 156 | 100.0 |
| Experiment 12 | wild-type | Vector Control | yes | | 0 | 101 | 100.0 |
| Experiment 12 | wild-type | *rsks-1* | yes | | 1 | 114 | 99.1 |
| Experiment 12 | *rsks-1(sv31)xhsf-1(sy441)* | *hsf-1* | yes | | 121 | 11 | 8.3 |
| Experiment 12 | *rsks-1(sv31)xhsf-1(sy441)* | Vector Control | yes | | 0 | 120 | 100.0 |
| Experiment 12 | *rsks-1(sv31)xhsf-1(sy441)* | *rsks-1* | yes | | 0 | 127 | 100.0 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *hsf-1* | yes | expressing extrachromosomal array | 41 | 10 | 19.6 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *hsf-1* | yes | not expressing extrachromsomal array | 51 | 17 | 25.0 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | expressing extrachromosomal array | 13 | 6 | 31.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | expressing extrachromosomal array | 22 | 3 | 12.0 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | not expressing extrachromsomal array | 6 | 103 | 94.5 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | not expressing extrachromsomal array | 1 | 34 | 97.1 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *rsks-1* | yes | expressing extrachromosomal array | 4 | 2 | 33.3 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *rsks-1* | yes | not expressing extrachromsomal array | 1 | 74 | 98.7 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 61 | 22 | 26.5 |
| Experiment 13 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 118 | 100.0 |
| Experiment 13 | *hsf-1(sy441)* | *hsf-1* | yes | | 76 | 0 | 0.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 13 | *hsf-1(sy441)* | Vector Control | yes | | 86 | 14 | 14.0 |
| Experiment 13 | *hsf-1(sy441)* | *rsks-1* | yes | | 44 | 71 | 61.7 |
| Experiment 13 | *wild-type* | *hsf-1* | yes | | 0 | 40 | 100.0 |
| Experiment 13 | *wild-type* | Vector Control | yes | | 0 | 61 | 100.0 |
| Experiment 13 | *rsks-1(sv31)xhsf-1(sy441)* | *hsf-1* | yes | | 82 | 5 | 5.7 |
| Experiment 13 | *rsks-1(sv31)xhsf-1(sy441)* | Vector Control | yes | | 0 | 82 | 100.0 |
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | expressing extrachromosomal array, animals hatched from egg lay | 22 | 1 | 4.3 |
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | not expressing extrachromsomal array, animals hatched from egg lay | 2 | 39 | 95.1 |
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *rsks-1* | yes | expressing extrachromosomal array, animals hatched from egg lay | 9 | 13 | 59.1 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | *rsks-1* | yes | not expressing extrachromsomal array, animals hatched from egg lay | 1 | 38 | 97.4 |
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 73 | 49 | 40.2 |
| Experiment 14 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 125 | 100.0 |
| Experiment 14 | *hsf-1(sy441)* | *hsf-1* | yes | | 197 | 3 | 1.5 |
| Experiment 14 | *hsf-1(sy441)* | Vector Control | yes | | 136 | 44 | 24.4 |
| Experiment 14 | *hsf-1(sy441)* | *rsks-1* | yes | | 41 | 142 | 77.6 |
| Experiment 14 | *wild-type* | *hsf-1* | yes | | 0 | 80 | 100.0 |
| Experiment 14 | *wild-type* | Vector Control | yes | | 0 | 98 | 100.0 |
| Experiment 14 | *rsks-1(sv31)xhsf-1(sy441)* | *hsf-1* | yes | | 94 | 7 | 6.9 |
| Experiment 14 | *rsks-1(sv31)xhsf-1(sy441)* | Vector Control | yes | | 0 | 95 | 100.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *C45H4.13* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 84 | 14 | 14.3 |
| Experiment 15 | *hsf-1(sy441)* | *c54e4.9* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 85 | 12 | 12.4 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *ckr-2* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 45 | 9 | 16.7 |
| Experiment 15 | *hsf-1(sy441)* | *F21D9.8* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 71 | 5 | 6.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *F33h12.6* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 70 | 22 | 23.9 |
| Experiment 15 | *hsf-1(sy441)* | *F43D9.1* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 90 | 13 | 12.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *H09603.2b* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 57 | 16 | 21.9 |
| Experiment 15 | *hsf-1(sy441)* | *rsks-1* | yes | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 41 | 85 | 67.5 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *srg-34* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 64 | 12 | 15.8 |
| Experiment 15 | *hsf-1(sy441)* | *sri-43* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 68 | 8 | 10.5 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *T07D10.1* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 75 | 8 | 9.6 |
| Experiment 15 | *hsf-1(sy441)* | *T24A6.4* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 106 | 13 | 10.9 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *T24F1.4* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 66 | 28 | 29.8 |
| Experiment 15 | *hsf-1(sy441)* | *W09D6.4* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 71 | 19 | 21.1 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *Y4358A.2* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 88 | 22 | 20.0 |
| Experiment 15 | *hsf-1(sy441)* | *y43f8b.10* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 81 | 14 | 14.7 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 15 | *hsf-1(sy441)* | *Y49E10.10* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 114 | 33 | 22.4 |
| Experiment 15 | *hsf-1(sy441)* | *ZK1010.5* | | preliminary screen using RNAi to knockdown genes that were downregulated in rsks-1 mutant in hsf-1 to see if any rescued development. Only 2 plates per condition and no control strain (Vector Control was contam) | 50 | 24 | 32.4 |
| Experiment 16 | *hsf-1(sy441)* | *F33h12.6* | | followup of preliminary screen with 4 plates each | 72 | 54 | 42.9 |
| Experiment 16 | *hsf-1(sy441)* | *H09603.2b* | | followup of preliminary screen with 4 plates each | 66 | 67 | 50.4 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 16 | *hsf-1(sy441)* | Vector Control | yes | followup of preliminary screen with 4 plates each | 71 | 56 | 44.1 |
| Experiment 16 | *hsf-1(sy441)* | *rsks-1* | yes | followup of preliminary screen with 4 plates each | 11 | 100 | 90.1 |
| Experiment 16 | *hsf-1(sy441)* | *T24F1.4* | | followup of preliminary screen with 4 plates each | 26 | 75 | 74.3 |
| Experiment 16 | *hsf-1(sy441)* | *W09D6.4* | | followup of preliminary screen with 4 plates each | 46 | 64 | 58.2 |
| Experiment 16 | *hsf-1(sy441)* | *Y4358A.2* | | followup of preliminary screen with 4 plates each | 56 | 54 | 49.1 |
| Experiment 16 | *hsf-1(sy441)* | *Y49E10.10* | | followup of preliminary screen with 4 plates each | 56 | 50 | 47.2 |
| Experiment 16 | *hsf-1(sy441)* | *ZK1010.5* | | followup of preliminary screen with 4 plates each | 59 | 78 | 56.9 |
| Experiment 17 | *hsf-1(sy441)* | *F33h12.6* | | repeat of followup, 4 plates each | 95 | 44 | 31.7 |
| Experiment 17 | *hsf-1(sy441)* | Vector Control | yes | repeat of followup, 4 plates each | 54 | 37 | 40.7 |
| Experiment 17 | *hsf-1(sy441)* | *rsks-1* | yes | repeat of followup, 4 plates each | 24 | 102 | 81.0 |
| Experiment 17 | *hsf-1(sy441)* | *T24F1.4* | | repeat of followup, 4 plates each | 77 | 49 | 38.9 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 17 | *hsf-1(sy441)* | *T24F1.4* | | repeat of followup, 4 plates each | 53 | 42 | 44.2 |
| Experiment 17 | *hsf-1(sy441)* | *Y4358A.2* | | repeat of followup, 4 plates each | 91 | 45 | 33.1 |
| Experiment 17 | *hsf-1(sy441)* | *ZK1010.5* | | repeat of followup, 4 plates each | 78 | 57 | 42.2 |
| Experiment 18 | *hsf-1(sy441)* | *aak-2* | yes | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 20 | 46 | 69.7 |
| Experiment 18 | *hsf-1(sy441)* | *age-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 26 | 5 | 16.1 |
| Experiment 18 | *hsf-1(sy441)* | *crb-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 29 | 23 | 44.2 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 18 | *hsf-1(sy441)* | *hke-4.2* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 19 | 11 | 36.7 |
| Experiment 18 | *hsf-1(sy441)* | *hsp-70* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 37 | 10 | 21.3 |
| Experiment 18 | *hsf-1(sy441)* | *jnk-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 19 | 30 | 61.2 |
| Experiment 18 | *hsf-1(sy441)* | *k02b12* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 43 | 34 | 44.2 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 18 | *hsf-1(sy441)* | Vector Control | yes | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 52 | 87 | 62.6 |
| Experiment 18 | *hsf-1(sy441)* | *lgg-1* | yes | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 42 | 17 | 28.8 |
| Experiment 18 | *hsf-1(sy441)* | *mnk-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 26 | 51 | 66.2 |
| Experiment 18 | *hsf-1(sy441)* | *par-5* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 52 | 10 | 16.1 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 18 | *hsf-1(sy441)* | *phb-2* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 42 | 26 | 38.2 |
| Experiment 18 | *hsf-1(sy441)* | *rheb-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 25 | 50 | 66.7 |
| Experiment 18 | *hsf-1(sy441)* | *rsks-1* | yes | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 7 | 120 | 94.5 |
| Experiment 18 | *hsf-1(sy441)* | *sek-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 35 | 31 | 47.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 18 | *hsf-1(sy441)* | *sgk-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 30 | 25 | 45.5 |
| Experiment 18 | *hsf-1(sy441)* | *sma-4* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 19 | 48 | 71.6 |
| Experiment 18 | *hsf-1(sy441)* | *stdh-1* | | preliminary screen using RNAi to knockdown genes from the rsks-1 interactors of wormbase. Only 2 plates per condition | 34 | 38 | 52.8 |
| Experiment 19 | *hsf-1(sy441); rsks-1(mu482)* | *hif-1* | yes | | 0 | 76 | 100.0 |
| Experiment 19 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 48 | 52 | 52.0 |
| Experiment 19 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 138 | 100.0 |
| Experiment 19 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 1 | 136 | 99.3 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 19 | *hsf-1(sy441)* | Vector Control | yes | | 100 | 35 | 25.9 |
| Experiment 19 | *hsf-1(sy441)* | *OP50* | | op50 grown for a few days from stationary phase | 46 | 69 | 60.0 |
| Experiment 19 | *hsf-1(sy441)* | *OP50* | | op50 grown by RNAi bacteria protocol | 60 | 45 | 42.9 |
| Experiment 19 | *hsf-1(sy441)* | *rsks-1* | yes | | 45 | 119 | 72.6 |
| Experiment 19 | *hsf-1(sy441)* | *T24F1.4* | | | 96 | 38 | 28.4 |
| Experiment 19 | *hsf-1(sy441)xT24F 1.4(tm5213)* | Vector Control | yes | | 125 | 36 | 22.4 |
| Experiment 19 | *hsf-1(sy441)xT24F 1.4(tm5213)* | *OP50* | | | 45 | 5 | 10.0 |
| Experiment 19 | *hsf-1(sy441)xT24F 1.4(tm5213)* | *rsks-1* | yes | | 58 | 57 | 49.6 |
| Experiment 19 | *hsf-1(sy441)xT24F 1.4(tm5397)* | Vector Control | yes | | 101 | 10 | 9.0 |
| Experiment 19 | wild-type | *hif-1* | yes | | 0 | 124 | 100.0 |
| Experiment 19 | wild-type | Vector Control | yes | | 0 | 90 | 100.0 |
| Experiment 19 | wild-type | *skn-1* | yes | | 0 | 147 | 100.0 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 20 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | expressing extrachromosomal array | 36 | 1 | 2.7 |
| Experiment 20 | *hsf-1(sy441); rsks-1(mu482); svEx136[unc-36(+) rsks-1(+) sur-5::gfp]* | Vector Control | yes | not expressing extrachromsomal array | 0 | 133 | 100.0 |
| Experiment 21 | *hsf-1(sy441); rsks-1(mu482)* | *gcs-1* | | | 2 | 90 | 97.8 |
| Experiment 21 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 85 | 100.0 |
| Experiment 21 | *hsf-1(sy441)* | *hif-1* | yes | | 68 | 29 | 29.9 |
| Experiment 21 | *hsf-1(sy441)* | Vector Control | yes | | 62 | 41 | 39.8 |
| Experiment 21 | *hsf-1(sy441)* | *rsks-1* | yes | | 29 | 57 | 66.3 |
| Experiment 21 | *hsf-1(sy441)* | *skn-1* | yes | | 59 | 37 | 38.5 |
| Experiment 21 | wild-type | *gcs-1* | | | 0 | 145 | 100.0 |
| Experiment 21 | wild-type | Vector Control | yes | | 0 | 135 | 100.0 |
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | *cct-2* | yes | | 27 | 126 | 82.4 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | *ced-1* | | | 0 | 127 | 100.0 |
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | *gsk-3* | yes | | 3 | 115 | 97.5 |
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 37 | 77 | 67.5 |
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | 0% developed is not normal. Could vector have been contaminated? | 105 | 0 | 0.0 |
| Experiment 22 | *hsf-1(sy441); rsks-1(mu482)* | *pfs-2* | yes | | 74 | 56 | 43.1 |
| Experiment 22 | *hsf-1(sy441)* | *cct-2* | yes | | 85 | 0 | 0.0 |
| Experiment 22 | *hsf-1(sy441)* | *crh-1* | yes | | 117 | 32 | 21.5 |
| Experiment 22 | *hsf-1(sy441)* | *hrp-2* | yes | | 57 | 47 | 45.2 |
| Experiment 22 | *hsf-1(sy441)* | Vector Control | yes | 0% developed is not normal. Could vector have been contaminated? | 95 | 0 | 0.0 |
| Experiment 22 | *hsf-1(sy441)* | *nhr-14* | yes | | 85 | 47 | 35.6 |
| Experiment 22 | *hsf-1(sy441)* | *nhr-91* | yes | | 32 | 58 | 64.4 |
| Experiment 22 | *hsf-1(sy441)* | *rsks-1* | yes | | 22 | 81 | 78.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 22 | *hsf-1(sy441)* | *tiar-1* | yes | | 56 | 52 | 48.1 |
| Experiment 22 | *hsf-1(sy441)* | *Y55F3AM.3* | yes | | 77 | 73 | 48.7 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | *cct-2* | yes | | 15 | 55 | 78.6 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | *crh-1* | yes | | 2 | 109 | 98.2 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 49 | 50 | 50.5 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 7 | 111 | 94.1 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | *nhr-14* | yes | | 0 | 116 | 100.0 |
| Experiment 23 | *hsf-1(sy441); rsks-1(mu482)* | *pfs-2* | yes | | 68 | 31 | 31.3 |
| Experiment 23 | *hsf-1(sy441)* | Vector Control | yes | | 66 | 36 | 35.3 |
| Experiment 23 | *hsf-1(sy441)* | *nhr-10* | yes | | 44 | 22 | 33.3 |
| Experiment 23 | *hsf-1(sy441)* | *nhr-91* | yes | | 45 | 20 | 30.8 |
| Experiment 23 | *hsf-1(sy441)* | *rsks-1* | yes | | 27 | 28 | 50.9 |
| Experiment 23 | *hsf-1(sy441)* | *tiar-1* | yes | | 77 | 34 | 30.6 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 23 | wild-type | *cct-2* | yes | | 35 | 97 | 73.5 |
| Experiment 23 | wild-type | Vector Control | yes | | 0 | 108 | 100.0 |
| Experiment 23 | wild-type | *pfs-2* | yes | | 4 | 104 | 96.3 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 135 | 185 | 57.8 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 100 | 214 | 68.2 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 71 | 137 | 65.9 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 6 | 341 | 98.3 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 17 | 309 | 94.8 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 8 | 163 | 95.3 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 4 | 244 | 98.4 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 9 | 195 | 95.6 |
| Experiment 24 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 10 | 351 | 97.2 |
| Experiment 24 | *hsf-1(sy441)* | Vector Control | yes | | 70 | 40 | 36.4 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 24 | *hsf-1(sy441)* | *rsks-1* | yes | | 47 | 47 | 50.0 |
| Experiment 24 | *hsf-1(sy441)* | *skn-1* | yes | | 44 | 55 | 55.6 |
| Experiment 24 | Wild Type | *pha-4* | yes | | 39 | 96 | 71.1 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 40 | 55 | 57.9 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 32 | 49 | 60.5 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *hsf-1* | yes | | 42 | 66 | 61.1 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 7 | 147 | 95.5 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 88 | 100.0 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 7 | 95 | 93.1 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 0 | 135 | 100.0 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 3 | 98 | 97.0 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 0 | 107 | 100.0 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *pha-4* | yes | | 3 | 115 | 97.5 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 1 | 105 | 99.1 |
| Experiment 25 | *hsf-1(sy441); rsks-1(mu482)* | *skn-1* | yes | | 3 | 111 | 97.4 |
| Experiment 25 | *hsf-1(sy441)* | Vector Control | yes | | 58 | 83 | 58.9 |
| Experiment 25 | *hsf-1(sy441)* | Vector Control | yes | | 45 | 51 | 53.1 |
| Experiment 25 | *hsf-1(sy441)* | Vector Control | yes | | 24 | 52 | 68.4 |
| Experiment 25 | *hsf-1(sy441)* | *rsks-1* | yes | | 32 | 96 | 75.0 |
| Experiment 25 | *hsf-1(sy441)* | *rsks-1* | yes | | 39 | 99 | 71.7 |
| Experiment 25 | *hsf-1(sy441)* | *rsks-1* | yes | | 22 | 49 | 69.0 |
| Experiment 25 | wild-type | *pha-4* | yes | | 3 | 118 | 97.5 |
| Experiment 26 | *rsks-1(mu482)* | Vector Control | yes | | 4 | 130 | 97.0 |
| Experiment 26 | *hsf-1(sy441)* | *rict-1* | yes | | 26 | 26 | 50 |
| Experiment 26 | *rsks-1(mu482)* | *atg-18* | yes | | 0 | 75 | 100 |
| Experiment 26 | *hsf-1(sy441); rsks-1(mu482)* | Vector Control | yes | | 3 | 31 | 91.2 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 26 | *hsf-1(sy441); rsks-1(mu482)* | *lgg-1* | yes | | 4 | 48 | 92.3 |
| Experiment 26 | *hsf-1(sy441)* | *rsks-1* | yes | | 12 | 62 | 83.78 |
| Experiment 26 | *rsks-1(mu482)* | *hsf-1* | yes | | 7 | 81 | 92.04 |
| Experiment 26 | *rsks-1(mu482)* | *lgg-1* | yes | | 4 | 134 | 97.10 |
| Experiment 26 | *hsf-1(sy441); rsks-1(mu482)* | *aak-2* | yes | | 0 | 40 | 100 |
| Experiment 26 | *rsks-1(mu482)* | *argk-1* | yes | | 4 | 104 | 96.3 |
| Experiment 26 | *hsf-1(sy441); rsks-1(mu482)* | *argk-1* | yes | | 4 | 55 | 93.2 |
| Experiment 26 | *rsks-1(mu482)* | *aak-2* | yes | | 1 | 94 | 98.9 |
| Experiment 26 | *hsf-1(sy441)* | Vector Control | yes | | 46 | 41 | 47.1 |
| Experiment 27 | *hsf-1(sy441)* | Vector Control | yes | | 109 | 52 | 32.3 |
| Experiment 27 | *hsf-1(sy441)* | *rict-1* | yes | | 119 | 63 | 34.6 |
| Experiment 27 | *hsf-1(sy441)* | *rsks-1* | yes | | 63 | 63 | 50.0 |
| Experiment 28 | *hsf-1(sy441)* | Vector Control | yes | | 61 | 35 | 36.5 |

| Experiment # | Genotype | RNAi | RNAi Clone Seq-uenced? | Comments | # Animals that did not reach adulthood | # Adults | % Animals Developed to Adulthood |
|---|---|---|---|---|---|---|---|
| Experiment 28 | *hsf-1(sy441)* | *rict-1* | yes | | 105 | 28 | 21.1 |
| Experiment 28 | *hsf-1(sy441)* | *rsks-1* | yes | | 49 | 28 | 36.4 |

**Table 3.3:** RT-qPCR primer sequences

| Gene Name | Forward Primer | Reverse Primer | Reference |
|---|---|---|---|
| *tba-1* | GTACACTCCACTGATCTCTGCTGACAAG | CTCTGTACAAGAGGCAAACAGCCATG | Hoogewijs *et al.* 2008 |
| *cdc-42* | CTGCTGGACAGGAAGATTACG | CTCGGACATTCTCGAATGAAG | Hoogewijs *et al.* 2008 |
| *pmp-3* | GTTCCCGTGTTCATCACTCAT | ACACCGTCGAGAAGCTGTAGA | Hoogewijs *et al.* 2008 |
| *Hsp-4* | AGATGCCGCCAAGAATCAGC | TCGACGATCTTGAACGGCCAG | |
| *Hsp-6* | CGTGGTGTTCCACAAGTCGAG | TCCTCAGCGGCATTCTTTTCG | |
| *hsf-1* | TTTGCATTTTCTCGTCTCTGTC | TCTATTTCCAGCACACCTCGT | Baird *et al.* 2014 |
| *hsp-16.2* | TCCATCTGAGTCTTCTGAGATTGTTA | TGGTTTAAACTGTGAGACGTTGA | Baird *et al.* 2014 |
| F44E5.4 *(hsp-70)* | TGCACCAATCTGGACAATCT | TCCAGCAGTTCCAGGATTTC | Baird *et al.* 2014 |
| *dnj-13* | ACGATGTCATTAAGCCGGG | GAGTTGGATTGAGTTGTGATGG | Li *et al.* 2016 |
| *sti-1* | CATGTTCACTACGACAAAGCG | TCCAACTTCGACGGCTTTC | Li *et al.* 2016 |
| *hsp-60* | CGCCGTCTCTGTCACTATG | AGGTTCTGGTATTTGTCCTTGAG | Li *et al.* 2016 |
| *hsp-110* | ACTATTCTCTGCATGCCACTC | GGATCACTGAATTTTCGTCCAATC | Li *et al.* 2016 |

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