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A search for longevity genes in *C. elegans* identifies a MARVEL domain-containing protein regulating innate immunity and lifespan

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A search for longevity genes in *C. elegans* identifies a MARVEL domain-containing protein regulating innate immunity and lifespan

by

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PREFACE

Looking back over my graduate career, I am struck by the tremendous support and encouragement I have received from my peers, teachers, and family. There are many without whom I would not have made it this far, and, though ineloquent and incomplete, I hope this acknowledgement will let them know how much I am grateful.

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A search for longevity genes in *C. elegans* identifies a MARVEL domain-containing protein regulating innate immunity and lifespan

Meredith Judy

Abstract

To date, many genes and pathways regulating lifespan have been identified in the nematode *Caenorhabditis elegans*. Over 20 years of research have uncovered conserved roles for insulin signaling, metabolism, dietary restriction, and reproductive signaling in the control of lifespan. Yet, despite this work, we still lack a complete picture of aging. To identify new components of lifespan regulation, we undertook two RNAi-based screens. In the first, we searched for genes which, when knocked down, accelerate aging in *C. elegans*. Using lifespan analysis as well as analysis of tissue morphology, we identified five potential "progeria regulators". These five genes, four of which were independently identified by other researchers, negatively regulate lifespan. Further work will determine their exact role in aging regulation.

A separate RNAi screen for components of dietary restriction-mediated longevity also identified a negative regulator of lifespan. *mrvl-1* encodes a MARVEL-domain containing membrane protein which is partially localized to the Golgi apparatus. RNAi inhibition of *mrvl-1* significantly shortens lifespan and accelerates aging while overexpression extends lifespan in *C. elegans*. Using transcriptional profiling to identify genes involved in *mrvl-1*-regulation of aging, we found significant overlap with the lifespan-regulating *daf-2*/insulin/IGF-1 signaling pathway as well as with the innate immune response in *C. elegans*. Investigations into the immune activity of *mrvl-1*

revealed that whereas RNAi treatment causes increased sensitivity to the bacterial pathogen *Pseudomonas aeruginosa*, overexpression of *mrvl-1* increases resistance. As bacterial food sources may potentially pose a late-life hazard to *C. elegans*, *mrvl-1* likely helps the worm cope with the detrimental effects of proliferating and pathogenic bacteria. Indeed, *mrvl-1* overexpression no longer extends the lifespan of animals fed non-proliferating, UV-killed bacteria. This work describes a novel lifespan gene that protects the worm from harmful bacteria, thus promoting survival and extending lifespan.

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Chapter 1: Introduction

Why we study aging

For many years, aging was thought to be a random, entropic process. Biologists hypothesized that damage accumulated in an unregulated manner in the cells of an organism, leading to its eventual death (Ljubuncic and Reznick, 2009). But if this is the case, why is it that animals such as mice, squirrels, and naked mole rats, all rodents of similar size, have very different lifespans (Gorbunova et al., 2008)? Within the past two decades, research from many labs has led to a new understanding of aging. This research utilized model organisms such as yeast, fruit flies, and the nematode *Caenorhabditis elegans* to show that even single gene mutations can dramatically affect lifespan. Aging, it turns out, is a genetically regulated process, which can be studied using experimental approaches in the laboratory. Furthermore, many of the genes found to influence lifespan in model organisms are conserved between evolutionarily diverse species. Results from laboratory animals may be extrapolated to "higher" organisms, leading to new treatments and a new understanding of the mechanisms of aging and age-related diseases.

Genes and pathways that influence aging

Insulin/IGF-1 signaling

In the early 1980s, researchers began to identify long-lived mutants in the nematode *C. elegans* (Friedman and Johnson, 1988; Klass, 1983). Despite their discovery of an aging gene (*age-1*), it was unclear how these mutations caused lifespan extension. Fortunately, other work revealed a role for the insulin-signaling pathway in

lifespan regulation; mutations in the *daf-2* gene, which encodes a receptor similar to the human insulin and IGF-1 receptors, can double the lifespan of *C. elegans* (Kenyon et al., 1993; Kimura et al., 1997). Further work then showed that the original aging mutation, *age-1*, is also part of this pathway and encodes a downstream phosphatidylinositol-3 kinase (Dorman et al., 1995; Morris et al., 1996).

The DAF-2 receptor most likely responds to one or more of the ~37 insulin-like peptides expressed in the worm (Pierce et al., 2001). In fact, recent work has shown a negative feedback loop exists between INS-7 and the DAF-2 signaling pathway (Murphy et al., 2007). External environmental signals may also control the expression of these insulin-like peptides. Two studies from our lab demonstrate that sensory signals can regulate lifespan through the DAF-2 pathway; mutations in sensory signal-transduction or sensory cilia extend lifespan up to 50% (Apfeld and Kenyon, 1999) while removal of some sensory neurons by laser ablation can either positively or negatively regulate lifespan, evidence for a regulatory circuit in the nervous system of the worm (Alcedo and Kenyon, 2004). Downstream of the DAF-2 receptor lies the PI3-kinase *age-1*, as well as AKT and PDK homologs, mutations in all of which extend lifespan (Hertweck et al., 2004; Morris et al., 1996; Paradis et al., 1999; Paradis and Ruvkun, 1998). Thus, the DAF-2 receptor and the downstream PI 3-kinase/AKT/PDK components act to promote aging.

But how does the insulin/IGF-1 signaling pathway influence lifespan? It turns out that the lifespan extension mediated by this pathway requires the FOXO-family transcription factor DAF-16 (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997). DAF-16 drives or represses the transcription of a number of genes, including antioxidant,

antimicrobial, chaperone, and metabolic genes (McElwee et al., 2003; Murphy et al., 2003). DAF-16 itself is a longevity promoter; if we inhibit the activity of genes positively regulated by DAF-16, the lifespan of long-lived *daf-2* mutants is shortened. Likewise, turning down activity of negatively regulated genes lengthens lifespan in wild-type animals (Murphy et al., 2003). These effects seem to be additive or cumulative. For example, knockdown of a gene positively regulated by DAF-16 shortens lifespan in *daf-2* mutants, but does not shorten it as much as knockdown of *daf-16* itself. Thus, DAF-16 is the regulatory node in this lifespan-influencing pathway, controlling the expression of genes that then work in concert to influence lifespan.

Other work has shown that two more transcription factors are needed for lifespan extension by *daf-2* mutations, the heat shock factor HSF-1 (Hsu et al., 2003; Morley and Morimoto, 2004) and the Nrf-like stress response factor SKN-1 (Oliveira et al., 2009; Tullet et al., 2008). Both, like DAF-16, affect the transcription of genes necessary for the extension of *daf-2* mutant lifespan. These include antioxidants like glutathione S-transferases (Tullet et al., 2008) and molecular chaperones such as small heat shock proteins (Morley and Morimoto, 2004).

One striking feature of long-lived *daf-2* mutants is that all their tissues remain healthy and youthful looking even at very old ages (Garigan et al., 2002; Herndon et al., 2002). The apparent pervasiveness of *daf-16* activity could be due to cell autonomous actions. However, Apfeld and Kenyon used genetic mosaics to show that removing wild-type activity of *daf-2* in only some tissues, still causes all tissues to look *daf-2(-)* (Apfeld and Kenyon, 1998). Thus, *daf-2* mutant cells somehow signal to wild-type cells to act as if they were mutant as well. Cell non-autonomous actions were again demonstrated by

making tissue-specific expression constructs of *daf-2* and *daf-16*. This work revealed that *daf-2*/IIS activity in the intestine and nervous system is important for control of aging rate in *C. elegans* (Libina et al., 2003; Wolkow et al., 2000).

The effect of the *daf-2*/IIS signaling pathway on aging raises the question, why should inhibiting genes necessary for growth and nutrient uptake extend lifespan? In *C. elegans*, the *daf-2*/IIS signaling pathway not only affects aging, but also controls entry into the alternative larval stage called dauer (Gems et al., 1998). This stage allows animals to survive for long periods of time in harsh environments with little food (Cassada and Russell, 1975). In fact, some *daf-2* mutants display characteristics reminiscent of dauers, such as lethargy and altered fat storage (Ashrafi et al., 2003; Gaglia and Kenyon, 2009; Gems et al., 1998; Kimura et al., 1997). However, these phenotypes can be uncoupled; one study used a (13)C isotope assay to quantify fat absorption and synthesis in mutants carrying different alleles of *daf-2*. They find that while some long-lived mutants display a *daf-16*-dependent elevation of synthesized fats, other long-lived alleles did not (Perez and Van Gilst, 2008). Dauer formation and longevity can be uncoupled too. Dillin *et al.* used RNAi to turn down *daf-2* activity during specific time-points in the life cycle of *C. elegans*. This work shows that *daf-2* reduction during adulthood only, can extend lifespan to the same extent as *daf-2* RNAi treatment given from hatching. Furthermore, *daf-2* knockdown during development only, is insufficient to lengthen adult lifespan (Dillin et al., 2002a). Thus, it seems that *daf-2* has two functions: during development it controls entry into the dauer stage while during adulthood it regulates aging. Perhaps this lifespan module may have evolved not to

regulate aging *per se*, but to allow animals to survive harsh conditions, and survive until conditions become better suited for development and reproduction.

What about other organisms? Is this endocrine control of aging specific to *C. elegans*? As with many other biological processes, the insulin/IGF-1 signaling pathway and its regulation of aging are conserved across many species. In *Drosophila melanogaster*, mutations in either the insulin-like receptor (InR) or its receptor substrate (chico) extend lifespan by more than 50% (Clancy et al., 2001; Tatar et al., 2001). Furthermore, dFOXO, the *daf-16* fly homolog, significantly increases lifespan when specifically expressed in the *Drosophila* fat body (Giannakou et al., 2004; Hwangbo et al., 2004).

Mutations in the insulin and IGF-1 receptors can also lead to significant lifespan effects in mice. Although complete knockdown of the insulin receptor can cause diabetes, obesity, and shortened lifespan in mammals, fat-specific insulin receptor knockout mice have significantly increased longevity. And, not only is mean lifespan increased by 18%, these mice display a 50-70% reduction in fat mass (Bluher et al., 2003). Likewise, null IGF-1 knockout mice are not viable, but their heterozygous siblings live on average 26% longer than wild-type littermates (Holzenberger et al., 2003).

Finally, recent studies have found a link between insulin/IGF-1 signaling and extreme longevity in humans. Heterozygous mutations in the IGF-1 receptor are overrepresented in female Ashkenazi Jewish centenarians (Suh et al., 2008). Another study found that polymorphisms in AKT1 and FOXO3A were significantly associated with lifespan in three different cohorts (Pawlikowska et al., 2009). Furthermore, DNA

variants for FOXO3A and FOXO1 have been linked to centenarians in at least seven different cohorts from around the world (Anselmi et al., 2009; Flachsbart et al., 2009; Li et al., 2009; Lunetta et al., 2007; Willcox et al., 2008). Thus, a fundamental mechanism of aging, first discovered in *C. elegans*, is conserved and relevant in multiple species. Importantly, many of the mice, flies, and humans with altered insulin/IGF-1 signaling, exhibit not only increased longevity, but increased healthspan as well (Berryman et al., 2008), reinforcing the idea that changes in insulin/IGF-1 signaling can affect quality of life in addition to lengthening it.

Dietary restriction

As yet, caloric or dietary restriction (DR) is the only environmental manipulation shown to extend lifespan in both vertebrates and invertebrates (Mair and Dillin, 2008). A reduction in food intake not accompanied by malnutrition, it is also one of the oldest manipulations shown to extend lifespan. In 1935 McCay *et al.* first published results showing that a 25% or 50% reduction in calories could significantly extend the lifespan of rats relative to *ad libitum*-fed controls (McCay et al., 1989). Since then, DR has been extensively studied in yeast (Jiang et al., 2000; Lin et al., 2002), nematodes (Houthoofd et al., 2003; Kaeberlein et al., 2006; Klass, 1977; Lakowski and Hekimi, 1998; Lee et al., 2006), fruit flies (Magwere et al., 2004; Mair et al., 2003; Partridge et al., 2005), and rodents (Masoro, 2005; McCay et al., 1989; Weindruch and Walford, 1982) as well as less extensively in organisms as diverse as spiders (Austad, 1989), rotifers (Fanestil and Barrows, 1965) and dogs (Kealy et al., 2002). And, although it has yet to be a proven life

extender in humans, recent evidence suggests DR acts in non-human primates to extend both life and healthspan (Colman et al., 2009; Messaoudi et al., 2006).

Despite this extensive work, the mechanism of DR-mediated lifespan extension is unclear. Still, the past 10 years has seen considerable advances in our understanding of DR and revealed several genetic pathways important for DR-mediated lifespan extension. These include the transcription factors FoxA/*pha-4* (Panowski et al., 2007) and Nrf2/*skn-1* (Bishop and Guarente, 2007) and the NAD-dependent deacetylase Sir2/*sir-2.1*. Sir2 has been shown to be required for lifespan extension by glucose restriction in yeast (Lin et al., 2000) and overexpression of the gene can extend lifespan in both fruit flies and nematodes (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). However, Sir2/*sir-2.1* does not always appear to be needed for DR-mediated lifespan extension (Hansen et al., 2007; Kaeberlein et al., 2004; Kaeberlein et al., 2006; Tsuchiya et al., 2006).

The kinase TOR (target of rapamycin) has also been linked to lifespan control. Reductions in TOR signaling in several different organisms cause defects in growth, indicating a potential role in nutrient sensing (Kapahi and Zid, 2004). As DR-mediated longevity is often thought of as a response to lowered nutritional conditions, it seems probable that a nutrient sensing pathway would be involved. Reductions in TOR can increase lifespan in nematodes, fruit flies, and yeast (Hansen et al., 2007; Jia et al., 2004; Kaeberlein et al., 2005; Kapahi et al., 2004; Luong et al., 2006; Meissner et al., 2004; Vellai et al., 2003), and this lifespan extension is dependent on nutritional status in both *C. elegans* and *Drosophila* (Hansen et al., 2007; Kapahi et al., 2004). Downstream effectors of TOR may include autophagy, protein synthesis, and amino acid transport

(Hansen et al., 2008; Kapahi and Zid, 2004). Indeed, Zid *et al.* show that translational modification of mitochondrial gene expression by the TOR-regulated 4E-BP (eukaryotic initiation factor eIF4E binding protein), likely plays a role in DR-mediated lifespan extension (Zid et al., 2009).

The insulin/IGF-1 signaling pathway, which has possible interactions with TOR, has also been implicated in DR-mediated lifespan extension. Long-lived growth hormone receptor gene-disrupted (GHR-KO) mice fail to respond to dietary restriction and intermittent fasting (Arum et al., 2009; Bonkowski et al., 2006). Furthermore, long-lived *Drosophila chico*^{1} homozygotes have an altered response to DR (Clancy et al., 2002). Interestingly, dFOXO is not required for lifespan extension by at least two different DR regimes in *Drosophila*, but does seem to modulate the flies' response to DR; *Drosophila* overexpressing dFOXO in the fat bodies has an altered response to DR compared to controls (Giannakou et al., 2008). The authors of the study hypothesize that this may be due to modification of expression of nutrient responsive genes (Gershman et al., 2007).

The importance of the insulin/IGF-1 signaling pathway as well as the FoxO regulating-AMPK/*aak-2* pathway to dietary restriction in *C. elegans* appears more complicated. For instance, one study finds that *aak-2* is necessary for longevity induced by a solid media DR (Greer et al., 2007), and acts in part via DAF-16 (Greer et al., 2009). However, DAF-16 is not needed for lifespan extension in the genetic *eat-2* model of DR (Lakowski and Hekimi, 1998). Likewise, although the increased lifespan of *daf-2* mutants is dependent on AAK-2, that of *eat-2* mutants is not (Curtis et al., 2006). Adding

to, or perhaps elucidating this puzzle, new evidence suggests that different methods of DR employ different mechanisms of lifespan extension (Greer and Brunet, 2009).

Other Pathways

Aside from the insulin/IGF-1 and dietary restriction pathways, there are several other biological processes that have been implicated in aging regulation. Though not a focus of my thesis, I will briefly mention and describe what is known about two of these pathways.

Two RNAi screens for clones that increase longevity in *C. elegans* identified multiple components of the mitochondrial electron transport chain (Dillin et al., 2002b; Lee et al., 2003). At first this seems counterintuitive, as defects in mitochondria are hypothesized to increase production of reactive oxygen species – molecules which are sometimes thought to cause aging. However, further studies show that inhibition of respiration can extend lifespan across multiple species including yeast, flies, and mice (Copeland et al., 2009; Dell'agnello et al., 2007; Jazwinski, 2005; Liu et al., 2005). The mechanism of lifespan extension by mitochondrial dysfunction seems to function at least partially through the "retrograde response", a conserved transcriptional response in which information flows from the mitochondria to the nucleus (Cristina et al., 2009; Kirchman et al., 1999). This pathway upregulates the expression of various genes, including those involved in cell protection and alternative metabolic pathways (Cristina et al., 2009).

Evolutionary theory predicts that the reproductive system might also regulate aging. One theory of aging is that longevity evolved as a trade-off for progeny production. In other words, if resources are limited, most of an organism's

energy/resources will be invested in producing progeny, not in maintenance of older post-reproductive individuals (Partridge and Gems, 2006). In fact, discoveries from our lab show that the reproductive system of *C. elegans* does regulate aging; removing the germ cells either by genetic or laser ablation can increase lifespan by ~60% (Arantes-Oliveira et al., 2002; Hsin and Kenyon, 1999). However, ablation of the entire gonad (germline as well as somatic gonad) blocks this lifespan extension (Hsin and Kenyon, 1999). This contradicts the reproductive/somatic maintenance trade-off theory since animals lacking the entire gonad are obviously sterile, but are not long-lived.

The reproductive system influences longevity by a complex pathway involving steroid signaling through the nuclear hormone receptor DAF-12 (Antebi et al., 2000; Hsin and Kenyon, 1999). However, to live long, animals without a germline require *daf-16* (Hsin and Kenyon, 1999). Furthermore, in response to germ line removal, DAF-16 accumulates in intestinal nuclei (Lin et al., 2001). The somatic gonad, however, may use a distinct mechanism to regulate lifespan. Unlike the germline, it is not required for DAF-16 nuclear accumulation in the intestine but is required for expression of specific DAF-16 target genes (Yamawaki et al., 2008). How the somatic gonad signals other tissues to regulate lifespan is not known, but recent work from our lab suggests that it promotes longevity through steroid hormone signaling to DAF-12 which then activates a distinct subset of genes (Yamawaki, personal communication). Additional work from our lab has focused on how the germline signals other tissues to influence lifespan. These studies show that the ankyrin repeat protein KRI-1 and the transcription elongation/splicing factor TCER-1 are needed for germline loss to extend lifespan. Both link the reproductive system to DAF-16 activity (Berman et al., 2006; Ghazi et al., 2009).

Although not as extensively studied as in *C. elegans*, regulation of aging by the reproductive system may be conserved in other species as well. For instance, elimination of the germ cells in *Drosophila* increases lifespan and modulates insulin sensitivity (Flatt et al., 2008). Another intriguing study reveals potential mediation of lifespan by the reproductive tissues in mice; transplanting ovaries from young mice into older recipients significantly increases lifespan (Cargill et al., 2003; Mason et al., 2009).

Progeria

Human progeroid syndromes

In discussing aging research, we tend to think in terms of longevity and lifespan extension. However, important information can be gleaned from aging's other extreme: progeria or accelerated aging. In fact, one of the reasons why we know aging is genetically regulated stems from our knowledge of human progeroid diseases. These syndromes are caused by single gene mutations that lead to precocious aging, development of age-related diseases such as cancer and heart disease, and subsequent early death (Ding and Shen, 2008; Kudlow et al., 2007; Navarro et al., 2006). One classic example is Werner's syndrome. Patients with this disease have mutations in a member of the RecQ helicase family, WRN (Yu et al., 1996). WRN functions in both DNA repair and transcription and its loss causes genome instability (Bachrati and Hickson, 2003). Patients usually develop age-associated traits in their nervous, immune, connective tissue and endocrine-metabolic systems during early adulthood and die by their mid-40s (Goto, 1997). Another progeroid disorder, Hutchinson-Gilford syndrome, affects children. These patients appear normal at birth, but within a year develop signs of

aging such as cessation of growth, hair loss, degeneration of the skin, muscles and bone, and atherosclerosis, which often leads to death by ~13 years of age (Lans and Hoeijmakers, 2006). This disease is caused by a mutation in a gene encoding the nuclear envelope protein lamin A (Eriksson et al., 2003), a protein which influences not only maintenance of nuclear structure, but also chromatin structure, gene expression, and possibly even protein degradation (Ding and Shen, 2008; Musich and Zou, 2009).

Some animal models of progeria

Although much is known about these human progerias, there are many unanswered questions. For instance, it is unclear exactly how the disruption of DNA repair or nuclear structure can lead to organismal aging. Better understanding of the mechanisms and molecular bases of these diseases may increase our comprehension of normal human aging. Recently, animal models of progeria have been used to gain insight into the mechanisms of disease. There are at least three mouse models of Werner syndrome, including one that has implicated telomere loss in the pathogenesis of the disease (Chang et al., 2004; Kudlow et al., 2007).

And what about the role of nuclear structure in Hutchinson-Gilford syndrome? Studies done with *C. elegans* reveal that wild-type animals show age-specific alterations in the nuclear architecture of most non-neuronal cells types. Furthermore, knockdown of the *C. elegans* lamin A homolog leads to shortened lifespan (Haithcock et al., 2005). It is unclear how known aging pathways may influence the progression of progeric diseases. Perhaps work in model organisms may help reveal the answer.

The study of progeria in C. elegans

C. elegans can be a powerful tool in the study of progeria. Aging itself is readily studied with the lifespan assay; death is scored when an animal fails to respond to gentle prodding. In this assay, lengthened lifespan is a clear indication of changes in the regulation of aging. A shortened lifespan, however, is less easily interpreted. After all, manipulations causing sickness or abnormal development could likely lead to shortened lifespan as well as changes in aging regulation. Indicators of aging are thus needed to positively identify manipulations that cause progeria. In an attempt to classify aging phenotypes in *C. elegans*, Garigan *et al.* used Nomarski microscopy to show that several changes occur with age. These include appearance of necrotic cavities, accumulation of yolk protein and intestinal lipofuscin autofluorescence, deterioration of the germline tissues, and bacterial packing in the intestine and pharynx (Garigan et al., 2002).

Since this publication, other studies have further defined biomarkers of aging. For instance, different lifespan manipulations cause distinct spectral shifts in autofluorescent lipofuscin in *C. elegans* (Gerstbrein et al., 2005). Cheng *et al.*, measured pharyngeal pumping and body movement over time and show that age-related declines in these processes correlate with lifespan as well as with each other (Huang et al., 2004). Another group developed a pattern recognition computation model to track complex morphological changes in the *C. elegans* pharynx over time. They found that the pattern of pharynx morphology could be correlated with the future rate of functional decline in that organ (Johnston et al., 2008). Finally, Herndon *et al.* used GFP markers and electron microscopy to analyze changes with age. Interestingly, this study reveals progressive

deterioration in the muscle of *C. elegans*, with relative preservation of the nervous system (Herndon et al., 2002).

Innate immunity and pathogen infection

Immunosenescence and pathogen infection in C. elegans

Another parameter that changes with age is immune function. As organisms age, their immune system declines and deteriorates. The elderly are often more susceptible and take longer to recover from infections, and are less responsive to vaccination. Although this phenomenon is well known, the causes of immunosenescence remain poorly understood (Panda et al., 2009). *C. elegans* provide a simple model for studying the connection between immunity and aging. Many of the conserved regulatory pathways influencing aging also play a role in the control of the *C. elegans* immune system (discussed below). And, like aging, research into the mechanism of immune function has seen an abundance of new knowledge within the last 20 years or so (Nicholas and Hodgkin, 2004).

As a free-living soil-dwelling nematode, *C. elegans* likely encounter many microbial pathogens in its environment. It stands to reason that *C. elegans* would have developed defensive mechanisms to deal with these pathogens. Indeed, studies of the worm's response to microbial pathogens, has revealed an evolutionarily conserved innate immune system (Nicholas and Hodgkin, 2004). Although no viral pathogens have been found, a variety of bacteria and several fungi have been shown to infect and sometimes kill *C. elegans* (Darby, 2005). They do so with a variety of methods; for instance, the plague bacterium *Yersinia pestis* attacks the worm cuticle and forms a biofilm around its

head, preventing it from eating (Darby et al., 2002). *Microbacterium nematophilum* produces a debilitating rather than lethal infection by adhering to the rectum and post-anal cuticle and causing swelling (Hodgkin et al., 2000). The most common method, however, appears to be infection through the intestine. When *C. elegans* feed, bacteria are broken up in the pharyngeal grinder, thus ensuring that no live bacteria are found in the gut. However, bacteria sometimes pass through to the intestine intact. The Gram-negative bacteria *Pseudomonas aeruginosa* (Tan et al., 1999), *Salmonella typhimurium* (Labrousse et al., 2000), and *Serratia marcescens* (Mallo et al., 2002), as well as the fungus *Cryptococcus neoformans* (Mylonakis et al., 2002) and the Gram-positive bacteria *Enterococcus faecalis* (Garsin et al., 2001) are only a few of the pathogens that accumulate in the intestine in this way. Once in the gut, bacterial pathogens can cause distension of the intestine, damage to the intestinal epithelium, and eventually death.

Pathogen defense in C. elegans

To cope with these attacks, *C. elegans* mount an evolutionarily conserved innate immune response. Innate immunity is the first line of defense in response to pathogen attack and includes the detection of pathogen-associated molecular patterns, the upregulation of antimicrobial genes, and, in the case of vertebrates, the activation of more specific responses via the adaptive immune system (Akira et al., 2006). Though *C. elegans* lack this latter portion of the immune response, recent evidence suggests that their innate immune system does contain pathogen-specific protection; distinct pathogens can cause unique transcriptional responses as well as common, pathogen-shared responses (Wong et al., 2007). Other studies have applied this method of transcriptional

profiling as well and have helped elucidate the components of *C. elegans* immune signaling.

Mallo *et al.* were the first group to compare mRNA transcripts from worms infected with *S. marcescens* with those from uninfected animals. They found that infection induces expression of a variety of effector molecules that provide recognition and defense against invading pathogens. These molecules include lysozymes that cleave bacterial cell walls, a lipase that can act directly against invading microorganisms, and C-type lectins which may recognize and bind to carbohydrates on pathogenic microorganisms (Mallo et al., 2002). Other studies have identified additional anti-pathogen molecules; DAF-16 positively regulates expression of saposin-like protein genes (Murphy et al., 2003). These resemble amoebapores, peptides that form ion-channels in the membranes of target cell membranes. DAF-16 also regulates thaumatin genes. These encode proteins that may have antifungal activity mediated by membrane permeabilisation (Murphy et al., 2003). Finally, Troemel *et al.* and others report upregulation of CUB-like genes upon infection. CUB domains are thought to be involved in protein-protein interactions and have been found in proteases such as the complement components of the mammalian immune system (Shivers et al., 2008).

Immune signaling in C. elegans

Besides identifying pathogen recognition and host defense molecules, transcriptional profiling and genetic epistasis experiments have also led to the identification of multiple signal transduction pathways that may regulate immune response in *C. elegans*. One of the first identified is the TGF-β pathway. Already

identified as regulators of dauer entry, male tail morphology, axon guidance, and body size (Patterson and Padgett, 2000), a number of genes that are part of this pathway are also found to be upregulated by infection with *S. marcescens* (Mallo et al., 2002). These authors also found that loss of the TGF-β ligand DBL-1 increases susceptibility of *C. elegans* to *S. marcescens* (Mallo et al., 2002). This may not be altogether surprising in view of the fact that both *Drosophila* and vertebrate TGF-β components appear to be involved in immune function (Irving et al., 2001; Magor and Magor, 2001).

One pathway conspicuously absent from immune signaling in *C. elegans* is the Toll-like signaling system found in both *Drosophila* and mammals (Akira et al., 2006). While Toll-Nf-κB is a central control axis for innate immunity in other organisms, many of its components seem to be completely lacking in *C. elegans*. Only one member, the *C. elegans* Toll homolog *tol-1*, appears to be involved; *tol-1* mutants are more susceptible to infection by *Salmonella enterica* (Tenor and Aballay, 2008). However, activity of this gene may be more important for pathogen avoidance rather than expression of antimicrobial effectors (Pujol et al., 2001).

MAP kinase pathways are also involved in the regulation of immune function in *C. elegans*. A mutagenesis screen for enhanced susceptibility to *P. aeruginosa* isolated *nsy-1* and *sek-1*, two genes in the p38 MAPK pathway (Kim et al., 2002). Later, Troemel *et al.* used microarray analysis to show that ~25% of genes known to be part of this pathway were also regulated by exposure to *P. aeruginosa* (Troemel et al., 2006). The p38 MAPK pathway is not specific to *Pseudomonas* infection; it is also involved in the *C. elegans* response to infection from *S. enterica* and the fungus *Drechmeria coniospora* (Aballay et al., 2003; Ziegler et al., 2009).

The GATA transcription factor *elt-2* also regulates the expression of immune effector molecules in *C. elegans*. Shapira *et al.* used gene expression and RNAi experiments to show that ELT-2 is required for immune response to *P. aeruginosa* infection but makes no contribution to cadmium, heat or oxidative stress resistance (Shapira et al., 2006). Kerry *et al.* found that ELT-2 is required not just for *P. aeruginosa* resistance, but also for immunity to *S. enterica*, *Enterococcus faecalis*, and *Cryptococcus neoformans* (Kerry et al., 2006). Furthermore, these authors show that ELT-2 controls expression of *clec-67*, a C-type lectin consistently upregulated by pathogen infection (Kerry et al., 2006).

Overlap between immune signaling and aging regulation

One interesting feature of the biology of many long-lived mutants is their resistance to environmental stresses (Barsyte et al., 2001; Garsin et al., 2003; Honda and Honda, 1999; Larsen, 1993; Lithgow et al., 1995; Scott et al., 2002). This raises the question: are these animals long-lived because they are resistant to stress or is stress resistance a consequence of longevity? Studies of aging and immune regulation have found some overlap between the mechanisms employed for each process. However, it is still unclear whether these mechanisms are shared or whether they work in parallel.

As mentioned previously, the *C. elegans* germline regulates aging in a DAF-16 dependent manner. Sterility affects resistance to pathogen infection as well (Kim et al., 2002). Animals without progeny are better able to cope with the bacterial pathogen *P. aeruginosa*, and, similar to lifespan extension, this resistance seems to be dependent on the transcription factor DAF-16 (Evans et al., 2008a; Miyata et al., 2008). However, a

recent study finds that germline removal increases resistance to two different Gram-negative pathogens, *P. aeruginosa* and *S. marcescens*, independently of DAF-16 activity (Alper et al., 2009). This discrepancy may be due to differences in bacterial culture conditions. Indeed, Alper *et al.* find that when *P. aeruginosa* are grown at higher temperatures, germline-mediated pathogen resistance is now partially dependent on DAF-16 (Alper et al., 2009). Thus, pathogens may have different virulence factors based on culture conditions, and these virulence factors may determine which transcriptional cascade *C. elegans* use to combat infection. It remains to be seen exactly where germline regulation fits into the *C. elegans* armamentarium against pathogen infection. However, germline signaling seems to act at least in parallel to the p38 MAPK pathway to regulate immune function, as loss of germline does not change expression of *nsy-1*-regulated genes (Alper et al., 2009).

The *daf-2*/IIS pathway is another that regulates both aging and immunity in *C. elegans*. Transcriptional profiling studies have found overlap between genes upregulated by pathogen infection and those regulated by the *daf-2*/IIS pathway (McElwee et al., 2003; Murphy et al., 2003; Shapira et al., 2006; Troemel et al., 2006). These genes include the saposins, *lys-7*, and the thaumatin genes (Ewbank, 2006). Not surprisingly, long-lived *daf-2* and *age-1* mutants are resistant to a variety of pathogens and this resistance is suppressed by a *daf-16* mutation (Garsin et al., 2003). It is puzzling, therefore, that *daf-16* mutants themselves are not more susceptible to infection (Garsin et al., 2003). Perhaps, as in germline regulation, other pathways such as the p38 MAPK pathway, might complement DAF-16 activity; indeed, immune effectors such as *lys-1* and *nlp-29* which are up-regulated upon infection, are not regulated by DAF-16 (Mallo et

al., 2002; Murphy et al., 2003). Complicating matters, one study found that some of the genes positively regulated by the p38 MAPK pathway are in fact negatively regulated by DAF-16 (Troemel et al., 2006). DAF-16 may regulate only some genes and maybe only under some infection conditions. One intriguing possibility is that DAF-16 regulates genes for a basal or constitutive response to microbial pathogens.

Further insight into the role of insulin/IGF-1 signaling in innate immunity derives from two recent studies. The first of these shows that intestinal DAF-16 expression is required for resistance to *P. aeruginosa* and that *P. aeruginosa*-mediated suppression of immune defense genes is dependent upon DAF-2 and DAF-16. Furthermore, this suppression is mediated by the insulin-like peptide, INS-7 (Evans et al., 2008b), a likely DAF-2 agonist (Murphy et al., 2007). The second study elucidates the connection between *daf-2*/IIS regulation of aging and immunity. It explores the possibility that the long-life and pathogen resistance of *daf-2*/IIS mutants are consequences of the same underlying mechanism. The authors examine the roles of four serine threonine kinases that act downstream of DAF-2. They find that regulation of pathogen resistance and lifespan is decoupled; while lifespan extension requires the PI3 K-dependent kinase homolog PDK-1 and the serum- and glucocorticoid-inducible kinase homolog SGK-1 (Hertweck et al., 2004; Paradis et al., 1999), pathogen resistance requires the Akt/PKB homologs AKT-1 and AKT-2 (Evans et al., 2008a). Furthermore, mutations in these genes as well as in *daf-2*, reduce bacterial colonization in the gut (Evans et al., 2008a). It will be interesting to discover if other upstream components regulate *akt-1* and *akt-2* activity. In any case, insulin/IGF-1 signaling seems to regulate pathogen resistance and lifespan by convergent signals acting on DAF-16.

As in humans, susceptibility to pathogens increases with age in *C. elegans*. Young animals appear to be more resistant to infections compared to adults (Kurz et al., 2003; Laws et al., 2004). Whether this is due to decreased immune function or tissue decline is unknown. Interestingly, though, the *C. elegans* intestine in older animals is different from that in young animals. As mentioned before, most bacteria are pumped through the pharynx and destroyed by the grinder so that no live bacteria are found in the intestine. But, by day 4 or 5 of adulthood, live bacteria are found in the gut, and, as *C. elegans* age, the intestine becomes distended and filled with bacteria (Garigan et al., 2002; Gems and Riddle, 2000). Though the standard lab culture of *Escherichia coli* OP50 is considered non-pathogenic, worms fed by heat-killed, UV-killed, or antibiotic-arrested bacteria have longer lifespans than those fed live OP50 (Garigan et al., 2002; Gems and Riddle, 2000). Thus, OP50 can become an opportunistic pathogen in old worms, and may even be a cause of death. It will be interesting to figure out how and if pathogen resistance contributes to the extended lifespan of *daf-2* mutants grown on OP50 bacteria.

Chapter 2: RNAi Screen for "Progeria" Genes

Introduction

Although much is known about the genes and signaling pathways that influence aging in *C. elegans*, characterization of the worm's physiology during aging is still in a nascent state. Several recent studies investigated physiological changes that occur with age in *C. elegans* (Garigan et al., 2002; Gerstbrein et al., 2005; Herndon et al., 2002; Huang et al., 2004; Johnston et al., 2008). All found significant changes with age, confirming that old animals do indeed look different from young animals. This evidence is very helpful to the study of aging in *C. elegans*; the ability to differentiate old from young makes it possible to identify and discern mutations or manipulations that cause rapid aging from those that simply make an animal sick.

In an effort to better understand rapid aging, or progeria, part of my graduate career has been spent characterizing genetic manipulations that cause shortened lifespan in *C. elegans*. This work builds on a genome-wide RNAi screen undertaken by former members of the lab to identify genes that regulate aging. This screen identified 382 RNAi clones as causing a decrease in lifespan – defined as 80% or more animal death by Day 15 of adulthood, a time when wild-type mortality is only ~10%. I used Nomarski optics, an approach developed by Delia Garigan, to perform secondary screening on animals fed bacteria expressing dsRNA for these 382 RNAi clones. I found that exposure to many of the RNAi clones did simply make the animals sick (e.g. blistered cuticle, larval arrest, uncoordinated movement, *etc.*). However, animals treated with

RNAi for a subset of genes were scored as healthy at a young age and exhibited a reproducible progeric morphology and shortened lifespan.

Although I primarily used Nomarski microscopy to study the effects of these RNAi clones, I was also interested in using transcriptional GFP fusion proteins. By using GFP proteins specifically expressed in one tissue, one can characterize and compare aging in different tissue types in *C. elegans*. Herndon *et al.* employed this method; using GFP-tagged proteins in conjunction with electron microscopy, they presented a detailed histological description of aging muscles and neurons in *C. elegans.* Surprisingly, they found that, although muscle structure declines with age, neuronal morphology remains intact even in very old animals (Herndon et al., 2002). As I was already using high-powered microscopy to characterize progeria in *C. elegans*, I decided to also look for changes in neuronal structure. Perhaps the "progeric" RNAi clones found in our screen, might also affect neuronal integrity. Although my initial observations were interesting – I found that exposure to the respiratory inhibitor sodium azide caused an age-dependent formation of puncta in neuronal processes – this phenotype could not be replicated.

Results

Screen for genes that regulate rate of aging in C. elegans

As the worm ages several distinctive phenotypes become apparent: the pharynx becomes packed with bacteria and often starts to bend, the intestine develops a grainy, curdled appearance and also fills with bacteria, the gonad becomes disorganized, necrotic cavities appear, and autofluorescence increases (Garigan et al., 2002). Our lab previously identified the heat-shock transcription factor HSF-1 as a longevity factor. Knocking

down *hsf-1* either by RNAi or mutation causes shortened lifespan and accelerated tissue degradation (Garigan et al., 2002; Hsu et al., 2003). Thus, the wild-type function of *hsf-1* is to promote longevity.

To identify additional pro-longevity genes, I examined neuronal morphology and tissue integrity in animals exposed to a sublibrary of 382 RNAi clones. Because neurons are somewhat refractory to RNAi, I used the RNAi-sensitive mutant *rrf-3(pk1426)* and scored for progeric phenotypes during the second generation of growth on each RNAi clone. These clones were found in a genome-wide RNAi screen for genes that, when knocked down, shorten the lifespan of *C. elegans* (data unpublished). This screen was performed in the context of a larger screen for RNAi clones that both shorten and lengthen lifespan (Hansen et al., 2005). In this study animals were cultured on RNAi bacteria from the time of hatching and plates were then selected that contained few to no live individuals at a time when ~80-90% of age-matched controls were still living. Out of approximately 16,000 RNAi clones, 382 resulted in increased death at day 15 of adulthood compared to wild-type controls (Table 2.1).

Of these 382, it was possible that some clones simply resulted in sickness or developmental defects. To rule these conditions out, animals were scored on day one or two of adulthood and those that were not healthy or had obvious developmental defects were excluded from further analysis. Of 326 clones examined, 141 (43%) produced either sickness (such as blistered cuticle or molting defect) or developmental arrest (Table 2.1). However, 185 (57%) appeared normal and were subsequently examined by Nomarski and fluorescent optics at later time points for accelerated aging phenotypes and neuronal morphology. These animals were analyzed at days 2, 5 and 10 of adulthood.

For, even though a worm can appear healthy at day 1 or 2 of adulthood and still have a shortened lifespan, this does not necessarily indicate progeria, as some animals may become sick later in life. The use of Nomarski optics allowed us to exclude additional RNAi clones as simply causing sickness in adult animals. Thus, my initial screen identified 38 clones that produced one or more progeric phenotypes similar to those seen with knockdown of *hsf-1*, including a bent pharynx, bacterial packing, and a disorganized gonad (Table 2.1).

In addition to scoring progeric phenotypes, we also wished to assay the affect of the 382 RNAi clones on lifespan itself. To this end, we performed event-only lifespan analysis. This type of analysis allows one to quickly assess many RNAi clones at one time. To eliminate contamination from progeny, we used the sterile strain *fer-15(b26); fem-1(hc17)*. While animals on 133 clones were scored as sick, those on 185 were sufficiently healthy for lifespan analysis. Of these, 129 significantly shortened lifespan (Table 2.2 and 2.3). Although many of these clones also caused progeria, we did not see complete overlap with the results from our Nomarski analysis. This may be due to the variable effects of RNAi, small sample sizes, or strain differences.

Knockdown of five genes causes a reproducible progeric phenotype

From our morphology screen we found five genes whose knockdown produced a strong progeric phenotype (Table 2.4). Animals grown on dsRNA for these five genes did not display obvious phenotypes at the young adult stage, but exhibited early onset of aging-associated characteristics. In addition, knockdown of these genes significantly reduced the lifespan of treated animals, not only for whole-life RNAi treatment but for

adult-only treatment as well (Table 2.5). One gene identified from our screen, *cyp-42A.1*, decreased lifespan when knocked down by whole-life RNAi treatment. However, RNAi during adulthood-only did not significantly affect lifespan (Tables 2.4 and 2.5). This is interesting considering that *cyp-42A.1* encodes a cytochrome P450. In humans, these proteins metabolize both endogenous and exogenous compounds and make cholesterol, steroids and other important lipids (Nelson, 2009). The *C. elegans* genome contains at least 75 cytochrome P450 genes (Kulas et al., 2008), one of which, *cyp-22A.1* (*daf-9*), produces a dauer hormone that binds to the DAF-12 receptor to regulate lifespan (Gerisch et al., 2007; Jia et al., 2002). *cyp-42A.1* has previously been shown to have a role in lifespan; RNAi knockdown decreases mean lifespan in both *daf-2* and *daf-2; daf-16* mutants (Samuelson et al., 2007). RNAi of this gene also inhibits metabolism of eicosapentaenoic acid, the predominant polyunsaturated fatty acid (PUFA) in *C. elegans* (Kulas et al., 2008). PUFAs are important signaling molecules in various behavioral and developmental processes including sensory transduction and recruitment of sperm to the spermatheca (Kahn-Kirby et al., 2004; Kubagawa et al., 2006). The human homolog of *cyp-42A.1*, isoform 1 of cytochrome P450 4V2, is thought to have a role in fatty acid and steroid metabolism, and defects in it cause Bietti crystalline corneoretinal dystrophy – a disease which progresses to legal blindness by the patient's 50s or 60s (Li et al., 2004). It will be interesting to determine the exact role of *cyp-42A.1* in the synthesis and/or degradation of signaling molecules, and how this relates to lifespan regulation.

Other genes identified in our RNAi screen seem to affect lifespan in a broader manner. The first of these, *cel-1*, encodes an mRNA capping enzyme that modifies the 5' end of nascent transcripts to promote their processing and stability. This enzyme is

required for *C. elegans* development and viability (Srinivasan et al., 2003; Takagi et al., 2003). Since animals exposed to our *cel-1* RNAi clone were able to reach adulthood, we must conclude that our RNAi treatment produces less robust knockdown than that in previous studies. In our hands, *cel-1* RNAi treatment resulted in a pharynx packed with bacteria, a curdled intestine, and necrosis by day 9 of adulthood (data not shown). Consistent with our results, *cel-1* was independently described as a lifespan gene by Samuelson *et al.* These authors report that *cel-1* RNAi treatment increases the rate of aging and shortens the lifespan of both *daf-2* and *daf-2; daf-16* mutants. Because they observed a more dramatic shortening of *daf-2* lifespan, they hypothesize that *cel-1* is both parallel to and converging with the *daf-2*/IIS pathway (Samuelson et al., 2007). We find that *cel-1* RNAi treatment causes extreme shortening of *daf-2* lifespan, up to 72%, bringing *daf-2* lifespan to below wild-type levels (Table 2.6). Interestingly, the RNAi screen for longevity genes in Samuelson *et al.* identified other components of mRNA processing. This suggests a possible link between aging and mRNA fidelity.

Another gene identified in our screen is F43D2.1. This encodes a putative G1/S-specific cyclin C-like protein with homology to the human cyclin K. A previous study reports that F43D2.1 is one of 61 genes identified in a screen for genes that protect the *C. elegans* genome against mutations (Pothof et al., 2003). Another study from the same group finds that knockdown of F43D2.1 causes failure of cell-cycle arrest after DNA injury and reduced apoptosis after irradiation, thus indicating a role for this gene in the DNA damage response (van Haaften et al., 2006). Interestingly, the authors note that the human homolog of F43D2.1, cyclin K, is a direct target of the tumor suppressor p53

(Mori et al., 2002). Given these results, it would be interesting to ascertain the effect of F43D2.1 on the *C. elegans* tumor model, *gld-1(q485)*.

We find that F43D2.1 may have a role in the regulation of lifespan. By day five of adulthood, animals exposed to RNAi for this gene exhibit all the hallmarks of early aging (data not shown). While knockdown significantly shortens lifespan of wild-type animals (Table 2.4) RNAi inhibition of this gene can also shorten the lifespan of long-lived *daf-2(e1370)*, *eat-2(ad1116)*, *glp-1(e2141)*, and *clk-1(qm30)* mutants as well as further shorten the lifespan of the progeric mutants *hsf-1(sy441)* and *daf-16(mu86)* (Table 2.6). Thus, F43D2.1 may be acting generally to regulate lifespan. Further study will be needed to determine if this regulation is connected to genome stability and/or tumorigenesis. Our preliminary results suggest that F43D2.1 knockdown can affect lifespan independently of germline tumorigenesis as RNAi treatment significantly shortens lifespan of *gon-2(q388)* mutants which completely lack a gonad (Table 2.7).

vps-16 encodes a vacuolar assembly and sorting protein homologous to a component of a Golgi-to-vacuole trafficking pathway in yeast. We find that animals grown on bacteria expressing dsRNA for *vps-16* are progeric by day 9 of adulthood and have up to a 42% decrease in mean lifespan (data not shown and Table 2.4). When exposed to *vps-16* RNAi, *daf-2* mutants also have a shortened lifespan; knockdown produces more than a 55% decrease in mean lifespan (Table 2.6). Samuelson *et al.* find that *vps-16* functions specifically within the *daf-2*/IIS pathway to regulate lifespan as knockdown reduces *daf-2(e1370)* lifespan but not the lifespan of *daf-2(e1370); daf-16(mgDf47)* double mutants (Samuelson et al., 2007). It is unclear why, in our hands, *vps-16* RNAi shortens lifespan of wild-type animals, while it has no affect on *daf-*

2(e1370); daf-16(mgDf47) mutants which have similar lifespan to wildtype. Perhaps the varying effects of RNAi or small sample size can explain this discrepancy. It is interesting to note that while Samuelson *et al.* find an early age-associated increase in intestinal autofluorescence with *vps-16* knockdown, a previous study shows that *vps-16* is required for the formation of autofluorescent gut granules (Hermann et al., 2005; Samuelson et al., 2007).

Two other genes were identified in our screen for progeric RNAi clones. The first of these, *usp-48*, encodes an ubiquitin carboxyl-terminal hydrolase. Previously reported to cause a tumerous germline phenotype and sterility with RNAi knockdown (Colaiacovo et al., 2002), *usp-48* may also be required for inhibition of transgene expression in the germline and for germline maintenance (Cui et al., 2006). In our hands, animals grown on *usp-48* dsRNA exhibit necrosis by day 5 and a packed pharynx and curdled intestine by day 9 (data not shown). Furthermore, RNAi knockdown shortens lifespan of wild-type animals by ~20% (Table 2.4). Because of the tumerous germline phenotype found by Colaiacovo *et al.*, we wished to determine if the shortened lifespan seen with RNAi knockdown is due simply to over-proliferation of the germ cells (similar to *gld-1(q485)* mutations). To this end, we assayed the effect of *usp-48* inhibition on the lifespan of *gon-2(q388)* mutants which, if raised at the non-permissive temperature, completely lack a gonad. Surprisingly, *usp-48* knockdown significantly shortens the lifespan of *gon-2(q388)* mutants compared to control (Table 2.7). Thus, the lifespan shortening effects of *usp-48* RNAi are not due to a tumerous germline.

Inhibition of *usp-48* can also shorten lifespan of *daf-2(e1370)*, *eat-2(ad1116)*, *glp-1(e2141)*, and *clk-1(qm30)* mutants as well as that of the short-lived mutants *hsf-1(sy441)*

and *daf-16(mu86)* (Table 2.6). Interestingly, Samuelson *et al.* classify this gene as working in parallel and within the *daf-2*/IIS pathway. Our results support the classification of this gene as having an effect on multiple longevity pathways.

Lastly, we find that RNAi knockdown of ZC123.3 results in progeria in *C. elegans.* This gene encodes a protein containing three homeodomains and several Zn-finger domains (Wacker et al., 2003). RNAi for this gene produces a strong progeric phenotype with animals exhibiting early aging in all tissues by day 5 of adulthood and up to a 21.9% shortening of mean lifespan (data not shown and Table 2.4). RNAi of ZC123.3 also shortens lifespan of all longevity mutants tested (Table 2.6), suggesting it has a role as a general lifespan gene. ZC123.3 knockdown has been found to produce abnormal vulval development (Hurd, 2008). In our hands, inhibition of this gene causes sterility (data not shown) and, interestingly, a GFP transcriptional fusion shows strongest expression in the vulval muscles (Reece-Hoyes et al., 2007). As a transcription factor, the ZC123.3 protein is a prime candidate for transcriptional profiling. This type of analysis could reveal other genes involved in the aging process.

GFP expression analysis

To further explore the relationship between these newly identified "progeria" genes and known longevity pathways, we also examined whether knockdown affects expression levels and patterns of several DAF-16 and HSF-1 target genes. None of the RNAi clones significantly altered expression of *sod-3::gfp*, a known target of DAF-16 (data not shown). However, knockdown of one gene, *usp-48*, affects expression of *ins-7::gfp* (Fig. 2.1A). *ins-7* is a target of HSF-1 and DAF-16 and an agonist of the DAF-2

insulin/IGF-1-like receptor (Murphy et al., 2007). Perhaps *usp-48* helps control the INS-7-DAF-2 feedback loop, or, as a member of the ubiquitination pathway, it may work to clear GFP proteins from the cytosol.

We also tested expression levels of *dod-8::gfp* in a *glp-1(e2141ts)* mutant background. This putative steroid dehydrogenase (also called *stdh-1)* is upregulated in both *daf-2* and *glp-1* mutants in a *daf-16*-dependent fashion (Murphy et al., 2003). We found that *cel-1* knockdown reduced *dod-8::gfp* intensity by ~80% while ZC123.3 knockdown reduced it by ~25% (Fig. 2.1B). Other clones had no significant affect on *dod-8* expression (data not shown).

Analysis of touch neurons

To follow changes in the integrity of neurons with age, we used a strain in which a touch neuron-specific promoter, *mec-4*, drives the expression of green fluorescent protein. This strain was previously used in a study that did not observe any neuronal changes with age (Herndon et al., 2002). In order to visualize the neurons, we first mounted animals on glass slides with 10μl of the electron-transport chain inhibitor sodium azide to minimize movement. Treatment with this compound has been used to mimic hypoxic conditions and has been shown to lead to axonal beading in the neuronal processes (Scott et al., 2002). This phenotype, as well as other responses to hypoxia, is regulated by mutations in both *daf-16* and *daf-2* (Scott et al., 2002). We wondered if this regulation could be age-dependent. Thus, we followed axonal morphology with the *mec-4::gfp* marker at days 5, 10, and 15 of adulthood. We ascertained the effect of *daf-*

16(mu86) and *daf-2(e1370)* mutations as well as that of our "progeric" RNAi clones on neuronal morphology.

Our preliminary results were very promising. These show that sodium azide-induced formation of axonal beading is age-dependent. Animals at day 15 of adulthood appeared to exhibit more puncta in their touch neurons than in those of young, day 5 of adulthood, animals (Fig. 2.2A). We also observed differential puncta formation in the presences of the aging mutations *daf-16(mu86)*, *daf-2(e1370)*, and *hsf-1(RNAi)*. We found that the *daf-16(mu86)* mutation as well as *hsf-1* RNAi treatment, which both lead to accelerated aging, cause axonal beading in the touch neurons earlier than normal (Fig. 2.2B and 2.3A). Conversely, *daf-2(e1370)* mutations, which slow down aging, cause a delay in puncta formation (Fig. 2.3A). Many of the "progeric" clones identified in our screen also accelerated formation of puncta in the processes of touch neurons (data not shown). Because this phenotype seemed to occur mainly in the presence of sodium azide (Fig. 2.3B), we hypothesized that although the touch neurons may not have an inherent aging phenotype, they become more vulnerable to hypoxic insult with age. Unfortunately, further exploration of this phenomenon failed to reproduce our original results. Whether *C. elegans* neurons are more sensitive to hypoxia, which seems likely given the role of the *daf-2*/IIS pathway in the hypoxic response, will need further, careful study.

Discussion

Recently, Herndon *et al.* (2002) studied changes in cell and organ systems in aging *C. elegans.* Using GFP-tagged proteins in conjunction with electron microscopy,

they present a detailed histological description of aging muscles and neurons in *C. elegans* and find that, while there is gradual progressive deterioration of muscle tissue, the nervous system remains relatively intact (Herndon et al., 2002). This study and others have presented methods of identifying morphological changes between old and young *C. elegans* (Garigan et al., 2002; Gerstbrein et al., 2005; Herndon et al., 2002; Huang et al., 2004; Johnston et al., 2008). Their work makes it possible to confirm progeric mutations and provides valuable resources for describing how aging mutations affect the lifespan and healthspan of a worm.

Using the techniques of Garigan *et al.* (2002), we were able to identify five genes as positive regulators of lifespan in *C. elegans*. Inhibiting these genes with RNAi causes shortened lifespan and accelerates aging. Though none appear to be major regulatory nodes in the aging axis, each contributes to our understanding of the aging process and hints at how this process is controlled. Already, other evidence confirms the role of vesicular sorting and mRNA processing in lifespan regulation (Samuelson et al., 2007). For example, *vps-16* may be required for cycling of cellular components necessary for the extended lifespan of *daf-2* mutants. Likewise, *cel-1* may be important for the proper translation of other lifespan genes. Further analysis of these genes will surely provide insight into the aging process. Another gene, F43D2.1, seems particularly interesting given the connection between genome stability and progeria in humans. Several progeroid diseases are caused by defects in DNA repair (Ding and Shen, 2008; Schumacher et al., 2008). A detailed examination of how F43D2.1 protects against mutation and DNA damage may help clarify the link between genome stability and aging.

Confirming that these genes affect not only lifespan, but also healthspan is important too. Age-related diseases, as well as problems associated with normal aging, will become a growing challenge as our society ages. The findings from our screen may pave the way for studies that explore how mutations affecting life span influence aging and healthspan in humans. It is disappointing, then, that we could not identify a robust neuronal aging phenotype. One of the most devastating classes of aging diseases is neurodegeneration. Many age-related diseases in humans affect the nervous system, and neuronal and synaptic loss is thought to lead to the cognitive deficits associated with normal aging (Joseph et al., 2009). Given the conservation of aging pathways between nematodes and higher organisms, it is interesting that worms appear to lack a robust neuronal aging phenotype. Elucidating the mechanisms of neuronal protection in *C. elegans* could identify genes that protect neuronal tissue and could ultimately aid in the discovery of therapies that confer protective qualities on neurons, thus extending the healthspan of aged individuals. Perhaps future work from our lab will identify just such genes.

Experimental Procedures

Strains

All strains were maintained as described previously (Brenner, 1974). CF512 *fer-15(b26); fem-1(hc17)*, wild-type N2, CF1041 *daf-2(e1370)*, CF1903 *glp-1(e2141)*, CF2354 *clk-1(qm30)*, CF1908 *eat-2(ad1116)*, NL2099 *rrf-3(pk1426)*, CF1850 *eat-2(ad1116) rrf-3(pk1426)*, CF2485 *rrf-3(pk1426); clk-1(qm30)*, CF1814 *rrf-3(pk1426); daf-2(e1370)*, CF2481 *rrf-3(pk1426); glp-1(e2141)*, CF2495 *hsf-1(sy441)*, CF1037 *daf-16(mu86)*,

ZB154 *bzIs5* [*mec-4::gfp*] (generously given to us by M. Driscoll), CF2271 *daf-2(e1370); zdIs5*, CF2272 *daf-16(mu86); zdIs5*, CF2566 *rrf-3(pk1426); zdIs5*, CE284A *ins-7::gfp + rol-6*, CF2253 *gon-2(q388)*, CF2573 *glp-1(e2141); sIs10314* [*pdod-8::gfp + pCeh361*].

RNA interference

RNAi was performed by feeding as described (Kamath et al., 2001). Bacteria expressing each RNAi clone were grown overnight at 37°C in LB plus 10 μg/ml tetracycline and 100 μg/ml carbenicillin, then seeded onto NG plates containing carbenicillin. 80 μl of 0.1M IPTG was added exogenously after two days of bacterial growth at room temperature. The identities of the five RNAi clones subject to in depth analysis were verified by sequencing the inserts using the M13-forward primer. All clones were from Julie Ahringer's RNAi library (Kamath *et al.*, 2003).

Morphology analysis

CF2566 animals were grown from hatching, on HT-1115 RNAi bacteria. If animals were fertile, their progeny were transferred to new RNAi plates and assayed for accelerated aging phenotypes at days 2, 5,10, and sometimes 15 of adulthood. Experiments were done both with and without 2′fluoro-5′deoxyuridine (FUDR, Sigma, St Louis, MO, USA), a chemical that inhibits DNA synthesis and prevents progeny from developing. FUDR was exogenously added to either L4 stage or day one adults at 100 μM. FUDR was used to prevent progeny production. On days 1, 5, and 10 of adulthood, animals were mounted on agarose pads and anaesthetized with a 10μl drop of 0.5% Sodium Azide.

Images were captured using a Retiga EXi Fast1394 CCD digital camera (QImaging) on a Zeiss Axioplan 2 compound microscope (Zeiss Corporation, Jena, Germany). Openlab 4.0.2 software (Improvision, Coventry, U.K.) was used for image acquisition.

GFP fluorescence microscopy and quantification

On day 1 or 2 of adulthood, animals were anaesthetized on agarose pads containing with 0.5% Sodium Azide. Images were taken using a Retiga EXi Fast1394 CCD digital camera (QImaging) using the 10x objective on a Zeiss Axioplan 2 compound microscope (Zeiss Corporation, Jena, Germany). For *dod-8::gfp* analysis, Openlab 4.0.2 software (Improvision, Coventry, U.K.) was used to quantify total intensity of fluorescence per worm as measured by intensity of each pixel in selected area of a frame (i.e. the worm).

Lifespan analysis

In addition to morphological analysis, clones from our RNAi sublibrary were tested for affects on mean lifespan. For this analysis, *fer-15(b26); fem-1(hc17)* animals were fed bacteria expressing dsRNA from hatching and numbers of dead animals were scored every two days. These animals were allowed to develop at 25°C until stage L4 to induce sterility. For the five clones that represent our most promising candidates, lifespan analysis was performed in more detail; in this case, animals were fed bacteria expressing dsRNA from hatching and were scored every other day for death. Animals that crawled off the plate, bagged with progeny, or displayed extruded internal organs were censored. All experiments were carried out at 20°C. Statview 4.5 software (SAS) was used for statistical analysis. P-values were calculated using the Mantel-Cox log rank test.

Figure and Table Legends

Figure 2.1. GFP expression analysis of DAF-16 target genes.

A. Expression of a transcriptional *ins-7::gfp* transgene is upregulated upon *usp-48* RNAi knockdown. All pictures are of day 2 adults, taken at 10X. Top panels are brightfield whereas bottom left and middle were taken with a 900ms exposure and bottom right with a 500ms exposure.

B. GFP intensity of *dod-8::gfp* in a *glp-1(e2141)* background. Pictures were taken of whole animals at day 2 of adulthood. Numbers refer to number of animals observed.

Figure 2.2. Touch neurons may be susceptible to age-regulated, hypoxia-induced puncta formation.

A. ALM neurons in *mec-4::gfp* expressing animals after treatment with 0.5% sodium azide. Anterior is left. Pictures taken at 40X with a 300ms exposure.

B. Progeric mutants develop age-related puncta at earlier time-points than wildtype. *hsf-1* RNAi animal expressing *mec-4::gfp*. Pictures taken at day 10 of adulthood in either brightfield (a) or with a 900ms exposure to UV light (b).

Figure 2.3. Puncta formation in the touch neurons is dependent on age, genotype, and hypoxic treatment.

A. Aging mutations *daf-16(mu86)* and *daf-2(e1370)* affect puncta formation in the ALM touch neurons. Animals were incubated in 0.5% Sodium Azide. No numbers are

reported for *daf-16(mu86)* mutants on days 18 or 21 because all animals of this genotype were dead. N ranges from 1 to 6 animals/condition.

B. Puncta appear in ALM touch neurons in response to sodium azide. *mec-4::gfp* expressing animals were exposed to either M9, 0.5% sodium azide or 1mM levamisole before puncta were counted. N = 50/condition.

Table 2.1. Results of screen for progeric RNAi clones.

CF512 *fer-15(b26); fem-1(hc17)* or ZB154 *bzIs5* [*mec-4::gfp*] animals were grown on RNAi bacteria for two generations before being scored for progeric phenotypes. CF512 was subjected to a 25°C heat-pulse to induce sterility whereas FUDR was exogenously applied to ZB154 animals. Progeric phenotypes included bacterial packing in the gut and pharynx, a disorganized gonad, a bent pharynx and neuronal puncta. Animals were scored progeric if they displayed any of these characteristics at an earlier time-point than wildtype. Those scored as sick, were either developmentally arrested or had gross morphological defects unrelated to aging. Plate position refers to position in RNAi "SLC" sublibrary (made by MJ).

Table 2.2. Event-only lifespan analysis of putative progeric RNAi clones.

CF512 *fer-15(b26); fem-1(hc17)* animals were grown on RNAi bacteria from the time of hatching. A 25°C heat-pulse from the L1 to L4 stage was applied to induce sterility. Otherwise animals were kept at 20°C. Animals were scored for developmental arrest or gross morphological defects at day 1 of adulthood and those who were not wildtype, were excluded from further analysis and thus not shown in this graph. Dead animals were

counted every other day – censored animals were not scored. RNAi animals were fed bacteria expressing dsRNA for candidate genes while control animals were fed bacteria containing an empty vector. % lifespan change is relative difference in mean lifespan between RNAi animals and controls. *P* values obtained from Mantel-Cox Logrank test.

Table 2.3. RNAi knockdown of some candidate genes resulted in sickness or developmental arrest.

List of genes whose knockdown by RNAi caused either developmental arrest or gross morphological changes unrelated to aging. Lifespan analysis of these RNAi clones was not performed in our event-only lifespan experiment.

Table 2.4. Mean lifespan observed in wild-type and rrf-3(pk1426) animals grown on "progeric" RNAi clones for entire life.

RNAi lifespan of either N2 or *rrf-3(pk1426)* (denoted with asterisk) animals grown on specific RNAi bacteria from hatching (whole-life RNAi). Number of RNAi animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control lifespan is mean lifespan of animals grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. Experiments using N2 animals were carried out at 20 °C. *rrf-3(pk1426)* eggs were incubated at 25 °C until adulthood, and lifespan analysis of adult animals was performed at 20 °C

Table 2.5. Mean lifespan observed in wild-type animals grown on "progeric" RNAi clones during adulthood only.

Adult-only RNAi lifespan of N2 animals grown on specific RNAi bacteria during adulthood only (Adult-only RNAi lifespan). Animals were allowed to develop to stage L4 on control bacteria (vector only). Number of RNAi animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control lifespan is mean adult lifespan of animals grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. Experiments were carried out at 20 °C.

Table 2.6. Lifespan analysis of "progeric" RNAi treatment on longevity mutants.

RNAi lifespan of either longevity mutants grown on specific RNAi bacteria from hatching (RNAi lifespan). Number of RNAi animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control lifespan is mean lifespan of animals grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. All experiments were carried out at 20°C.

Table 2.7. Lifespan analysis of "progeric" RNAi treatment on sterile gon-2(q388) mutants.

RNAi lifespan of either *gon-2(q388)* mutant animals grown on specific RNAi bacteria from hatching (whole-life RNAi). Number of RNAi animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control lifespan is mean lifespan of animals grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. *gon-2(q388)* eggs were incubated at 15°C until stage L3/L4 when they were transferred to 25°C. These animals were then bleached, and resulting eggs were plated onto RNAi bacteria and allowed to develop at 25°C until stage L4. Lifespan analysis of adult animals was performed at 20 °C

Figure 2.1. GFP expression analysis of DAF-16 target genes.

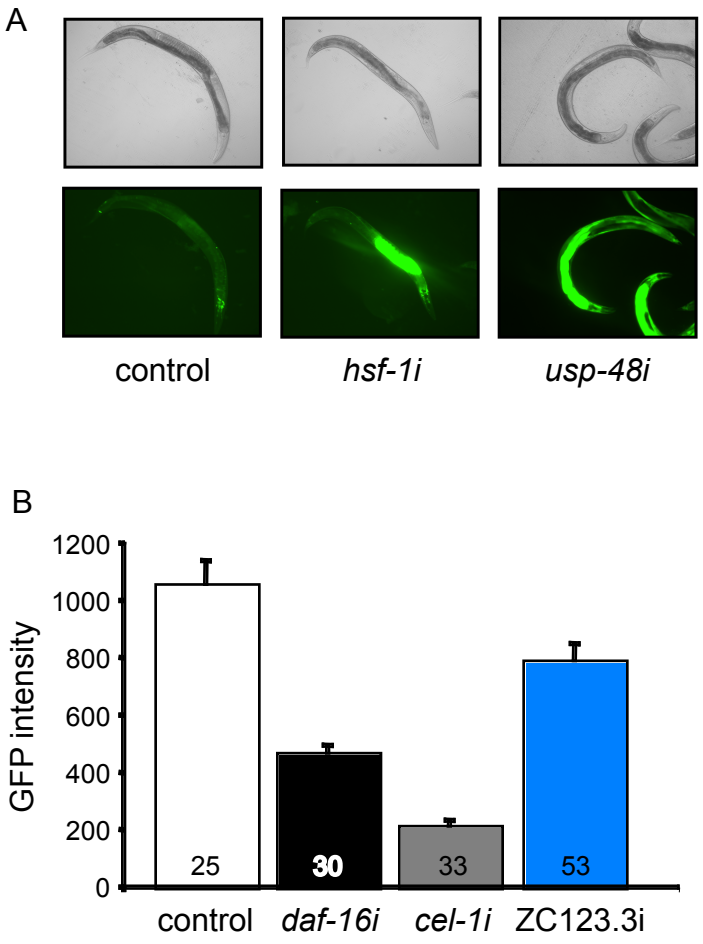

Figure 2.2. Touch neurons may be susceptible to age-regulated, hypoxia-induced puncta formation.

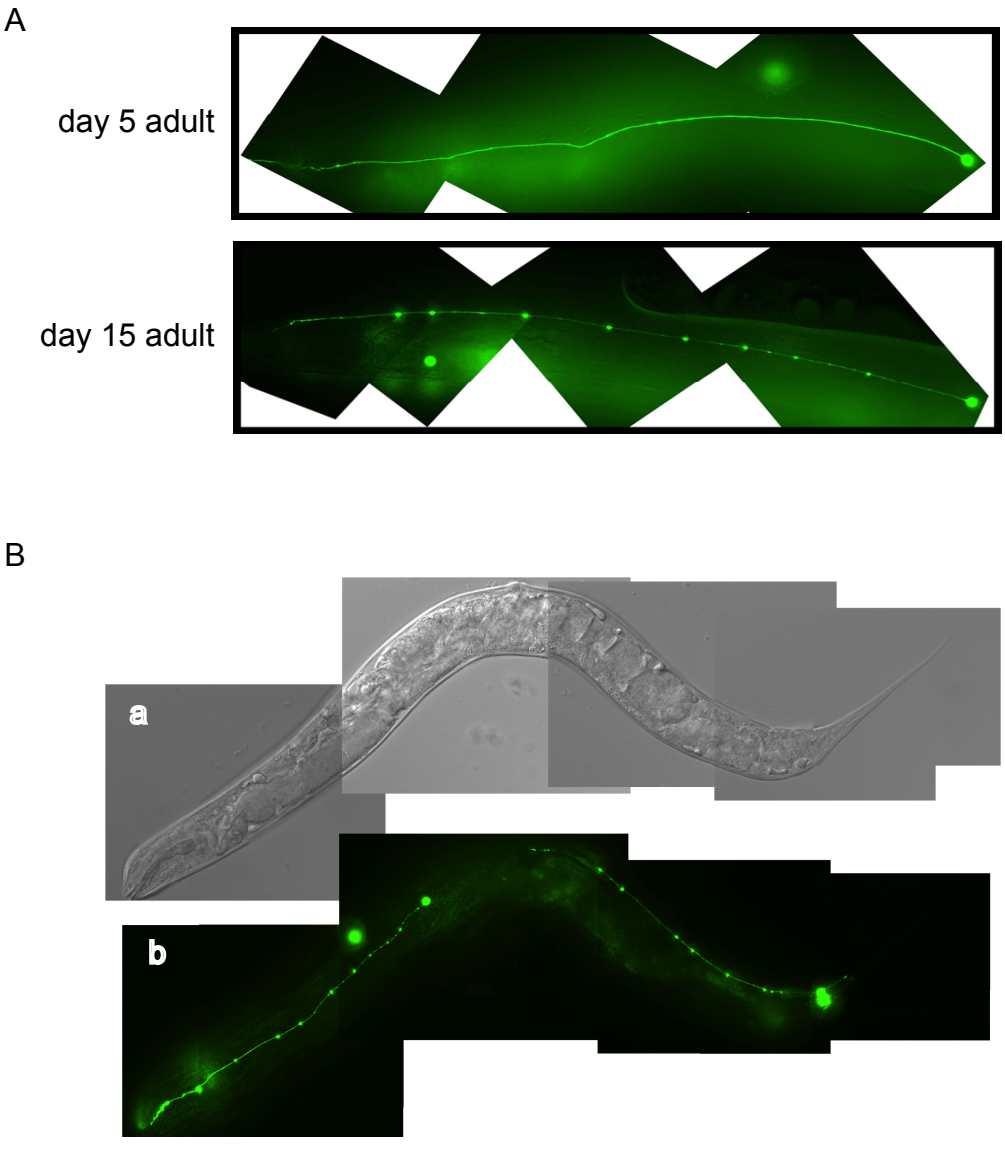

Figure 2.3. Puncta formation in the touch neurons is dependent on age, genotype, and hypoxic treatment.

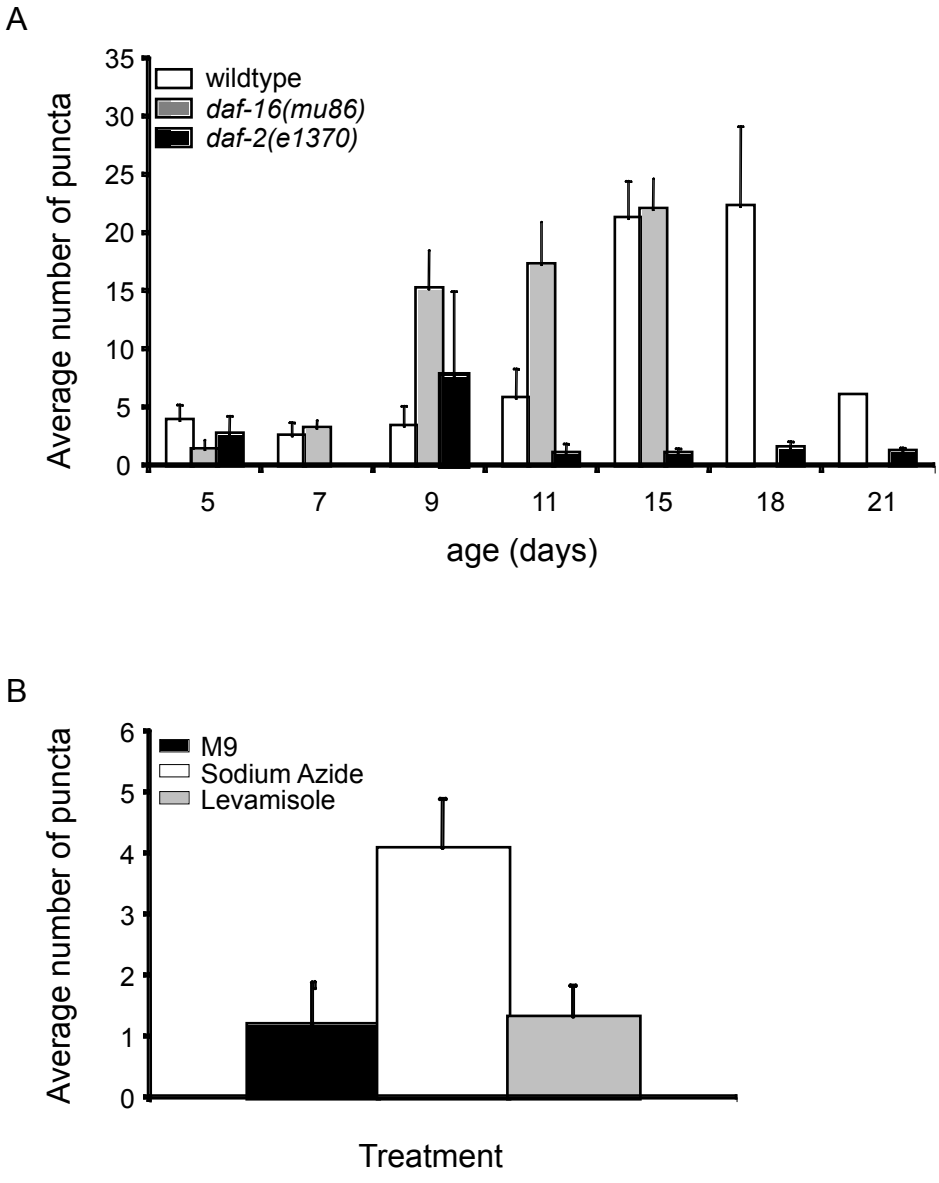

Table 2.1. Results of screen for progeric RNAi clones.

Gene	Cosmid	RNAi Phenotype	Plate position
rpl-13	C32E8.2	progeric	1A10
	ZC123.3	progeric	1A5
	ZC123.3	progeric	1A6
	C06A5.1	progeric	1C1
nrs-1	F22D6.3	progeric	1C8
cel-1	C03D6.3	progeric	1E10
	F30A10.10	progeric	1E8
	Y53C10A.1	progeric	1F12
hsf-1	Y53C10A.12	progeric	1G1
snr-7	Y71F9B.4	progeric	1H9
	C16C8.15	progeric	2A5
	F59E12.4	progeric	2B1
	C18E9.6	progeric	2B10
	F54C4.3	progeric	2D9
vps-16	C05D11.2	progeric	2F12
cgh-1	C07H6.5	progeric	2H1
ina-1	F54G8.3	progeric	3A3
	M03C11.7	progeric	3B1
	Y75B8A.11	progeric	3B10
arx-3	Y79H2A.6	progeric	3B9
	Y73B6A.G	progeric	3E11
	H04M03.4	progeric	3E6
	F54D1.6	progeric	3G2
	?	progeric	3G4
	?	progeric	3G6
	H02I12.7	progeric	3G8
let-60	ZK792.6	progeric	3H3
	Y39C12A.1	progeric	3H8
	F19B6.2A	progeric	3H9
	Y41D4A_2768.a	progeric	4A6
	CD4.4	progeric	4B8
	R07B5.8	progeric	4D2
	F43D2.1	progeric	4E7
	Y80D3A.6	progeric	4F10
cyp-42A.1	Y80D3A.5	progeric	4F9
	C52G5.1	progeric	4G12
	Y61A9LA.5	progeric	4G6
let-2	F01G12.5A	progeric	4H9
rpl-7	F53G12.10	wild-type	1A2
imb-3	C53D5.6	wild-type	1A3
	W05F2.5	wild-type	1A8
ccb-1	T28F2.5	wild-type	1A9

Gene	Cosmid	RNAi Phenotype	Plate position
	C41D11.1	wild-type	1B3
	Y110A7A.8	wild-type	1B5
	T05E8.1	wild-type	1B8
pnk-1	C10G11.5	wild-type	1C2
bli-4	K04F10.4A	wild-type	1C3
	T23B3.2	wild-type	1C6
unc-37	W02D3.9	wild-type	1C7
rpl-30	Y106G6H.3	wild-type	1E12
gei-17	W10D5.3A	wild-type	1E3
	F25H2.7	wild-type	1F1
	C35E7.5	wild-type	1F7
	F36D1.7	wild-type	1F8
srw-111	M01G12.4	wild-type	1G2
	F56H6.4	wild-type	1G3
	T06G6.8	wild-type	1G5
	F23F1.5	wild-type	1H12
	F32A7.5	wild-type	1H2
rpt-4	F23F1.8	wild-type	2A1
sptl-1	C23H3.4	wild-type	2A2
	D1069.3	wild-type	2A3
alg-2	T07D3.7	wild-type	2A4
nhr-75	C49D10.6	wild-type	2A6
ntl-2	B0286.4	wild-type	2A8
vrk-1	F28B12.3	wild-type	2B2
	C32D5.3	wild-type	2B3
lin-23	K10B2.1	wild-type	2B4
cdc-42	R07G3.1	wild-type	2B8
	T14D7.2	wild-type	2B9
pqn-47	F59B10.1	wild-type	2C1
	Y53F4B.6	wild-type	2C10
	F54A3.2	wild-type	2C11
trr-1	C47D12.1	wild-type	2C3
		wild-type	2C6
	Y48B6A.3	wild-type	2C7
	K02B7.2	wild-type	2C9
	Y46G5A.1	wild-type	2D1
mxl-2	F40G9.11	wild-type	2D10
	Y46G5A.5	wild-type	2D2
	Y46G5A.13	wild-type	2D3
sru-41	Y51H7BR.6	wild-type	2D4
	Y51H7C.13	wild-type	2D5
	Y51H7C.1	wild-type	2D6
	H10E21.5	wild-type	2D8

Gene	Cosmid	RNAi Phenotype	Plate position
	F42G9.5	wild-type	2E1
	H06I04.3A	wild-type	2E3
npp-9	F59A2.1	wild-type	2E4
	C34E10.8	wild-type	2F2
par-3	F54E7.3A	wild-type	2F3
	Y42G9A.4	wild-type	2F8
	F08F8.2	wild-type	2G10
	T20B12.6	wild-type	2G11
rps-21	F37C12.11	wild-type	2G6
	B0361.10	wild-type	2G9
rpn-3	C30C11.2	wild-type	2H10
	C30C11.4	wild-type	2H11
	ZK652.1	wild-type	2H5
phi-20	K12H4.4	wild-type	2H6
	C50C3.6	wild-type	2H7
	ZK1128.4	wild-type	3A11
	R01H10.1	wild-type	3A12
emb-30	F54C8.3	wild-type	3A4
	F54C8.5	wild-type	3A5
	Y75B8A.27	wild-type	3B11
cul-1	D2045.6	wild-type	3B2
	Y47D3A.29	wild-type	3B6
	Y47D3B.7	wild-type	3B8
	Y49E10.2	wild-type	3C1
	Y37D8A.16	wild-type	3C6
	F55F10.1	wild-type	3E1
	F38A5.8	wild-type	3E10
his-61	F55G1.10	wild-type	3E12
	F55F10.2	wild-type	3E2
	ZK180.3	wild-type	3E3
	C02B10.5	wild-type	3E4
lam-1	W03F8.5	wild-type	3E5
	C46G7.1	wild-type	3E7
	C49A9.6	wild-type	3E8
srv-29	T13A10.6	wild-type	3E9
	C33H5.7	wild-type	3F1
	C46C2.1	wild-type	3F10
rack-1	K04D7.1	wild-type	3F12
	C33H5.18A	wild-type	3F2
ntl-4	C49H3.5	wild-type	3F3
arf-3	F57H12.1	wild-type	3F4
	T01B11.3	wild-type	3F7
lin-49	F42A9.2	wild-type	3F8

Gene	Cosmid	RNAi Phenotype	Plate position
	F54D1.3	wild-type	3G1
kin-4	C10C6.1	wild-type	3G10
	M04B2.3	wild-type	3G11
	B0035.7	wild-type	3G3
	?	wild-type	3G5
	H02I12.6	wild-type	3G7
	F22B3.2	wild-type	3G9
	F28D1.9	wild-type	3H10
	Y62E10A.1	wild-type	3H11
	Y37A1B.7	wild-type	3H12
	F11A10.2	wild-type	3H7
rps-18	Y57G11C.16	wild-type	4A2
	Y38F2AL.3	wild-type	4A5
	Y50D4B.7	wild-type	4B2
	F59D6.3	wild-type	4B5
	F54D11.2	wild-type	4B6
	C18G1.4A	wild-type	4B7
	C50F4.5	wild-type	4C11
his-37	C50F4.7	wild-type	4C12
	C13F10.4	wild-type	4C2
let-413	F26D11.11	wild-type	4C8
	F41E6.3	wild-type	4C9
	F55C5.8	wild-type	4D10
	H09F14.1	wild-type	4D4
	R10D12.10	wild-type	4E1
	T10C6.5	wild-type	4E10
his-4	T10C6.11	wild-type	4E11
	W06H3.3	wild-type	4E12
	T08G5.5	wild-type	4E2
	D1086.4	wild-type	4E3
par-1	H39E23.1A	wild-type	4E4
	C15H11.2	wild-type	4E5
sdc-3	C25D7.3	wild-type	4E8
rpl-38	C06B8.8	wild-type	4E9
	F09C6.9	wild-type	4F1
	Y38H6C.1	wild-type	4F12
	Y102A5D.1	wild-type	4F2
fat-5	W06D12.3	wild-type	4F3
		wild-type	4F4
	Y59A8B.6	wild-type	4F5
	ZK262.8	wild-type	4F7
wrs-1	Y80D3A.1	wild-type	4F8
	T05A10.5	wild-type	4G10

Gene	Cosmid	RNAi Phenotype	Plate position
ldb-1	F58A3.1A	wild-type	4G11
dnj-25	W07A8.3	wild-type	4G2
pha-4	F38A6.1	wild-type	4G3
unc-68	K11C4.5	wild-type	4G5
jam-1	C25A11.4A	wild-type	4G7
	ZK899.2	wild-type	4G8
	F49E2.1A	wild-type	4G9
	C52G5.2	wild-type	4H1
	C36E6.2	wild-type	4H10
	C31E10.7	wild-type	4H2
nkat-1	F28H6.3	wild-type	4H3
epn-1	T04C10.2A	wild-type	4H4
nhx-1	B0395.1	wild-type	4H7
ifa-3	F52E10.5	wild-type	4H8
bli-3	F56C11.1	sick	1A1
	C50F2.3	sick	1A11
pbs-4	T20F5.2	sick	1A12
pqn-59	R119.4	sick	1A4
	F23C8.6	sick	1A7
ars-2	F28H1.3	sick	1B1
npp-7	T19B4.2	sick	1B10
rpt-5	F56H1.4	sick	1B11
noah-1	C34G6.6	sick	1B12
rpn-8	R12E2.3	sick	1B2
pat-10	F54C1.7	sick	1B4
	C01G8.9	sick	1B6
apm-1	F55A12.7	sick	1B7
vha-10	F46F11.5	sick	1B9
	F37E3.1	sick	1C4
	ZC581.1	sick	1C5
spd-2	F32H2.3	sick	1E1
	F32H2.5	sick	1E2
rab-5	F26H9.6	sick	1E4
lin-41	C12C8.3A	sick	1E5
skr-1	F46A9.5	sick	1E6
	F30A10.6	sick	1E7
smn-1	C41G7.1A	sick	1E9
	ZK1151.2A	sick	1F10
		sick	1F11
pas-5	F25H2.9	sick	1F2
	B0511.8	sick	1F3
	F55A3.3	sick	1F5
		sick	1F6

Gene	Cosmid	RNAi Phenotype	Plate position
vab-10	ZK1151.1	sick	1F9
	Y6B3A.1	sick	1G10
	W04A4.5	sick	1G11
	Y105E8A.M	sick	1G12
	E03H4.8	sick	1G4
gsk-3	Y18D10A.5	sick	1G6
pbs-2	C47B2.4	sick	1G7
snr-2	W08E3.1	sick	1G8
vrs-2	Y87G2A.5	sick	1G9
pbs-5	K05C4.1	sick	1H1
	Y71G12B.11A	sick	1H10
	Y71G12B.11A	sick	1H11
imb-5	Y48G1A.5	sick	1H4
imb-5	Y48G1A.5	sick	1H5
	Y65B4A.3	sick	1H6
	Y65B4BL.2	sick	1H7
nxt-1	Y71F9AM.5	sick	1H8
pbs-3	Y38A8.2	sick	2A10
	C34F11.3A	sick	2A12
rpn-11	K07D4.3	sick	2A7
phi-48	ZK430.8	sick	2A9
pqn-95	ZK1067.7	sick	2B11
vha-9	ZK970.4	sick	2B12
tag-309	C56C10.3	sick	2B6
dpy-2	T14B4.6	sick	2B7
rsp-7	D2089.1	sick	2C2
trs-1	C47D12.6	sick	2C4
imb-2	R06A4.4A	sick	2C8
gei-4	W07B3.2A	sick	2D11
unc-45	F30H5.1	sick	2D12
cogc-4	Y51H7C.6A	sick	2D7
emb-5	T04A8.14	sick	2E10
rps-0	B0393.1	sick	2E11
	T24C4.5	sick	2E2
(pat-3) rpn-1	ZK1058.2	sick	2E5
	H38K22.2A	sick	2E6
	B0285.1	sick	2E7
	R144.2	sick	2F1
	C23G10.8	sick	2F10
	C23G10.8	sick	2F11
	R12B2.5	sick	2F4
ubq-1	F25B5.4	sick	2F5
	ZK328.5B	sick	2F6

Gene	Cosmid	RNAi Phenotype	Plate position
rpn-2	C23G10.4A	sick	2F9
rpt-3	F23F12.6	sick	2G1
hmg-4	T20B12.8	sick	2G12
	F57B9.10	sick	2G4
rps-14	F37C12.9	sick	2G5
	T20H4.3	sick	2G8
phi-28	K02D10.5	sick	2H12
rpl-9	R13A5.8	sick	2H2
	R13A5.12	sick	2H3
	R13A5.12	sick	2H4
pbs-6	C02F5.9	sick	2H8
	ZK1236.3	sick	2H9
unc-32	ZK637.8A	sick	3A1
vha-1	R10E11.8	sick	3A10
	R08D7.3	sick	3A2
	B0464.1	sick	3A6
pri-1	F58A4.4	sick	3A7
gei-13	F58A4.11	sick	3A8
cbp-1	R10E11.1A	sick	3A9
rpt-6	Y49E10.1	sick	3B12
	F43D9.3	sick	3B3
	Y47D3A.29	sick	3B5
teg-1	Y47D3A.27	sick	3B7
act-5	T25C8.2	sick	3C12
	Y49E10.15	sick	3C2
	Y41D4B.19A	sick	3C3
	Y111B2A.14	sick	3C4
	Y37D8A.1	sick	3C5
ubq-2	ZK1010.1	sick	3C7
	F56A8.6	sick	3C8
gon-1	F25H8.3	sick	3F11
vha-5	F35H10.4	sick	3F5
	T26A8.4	sick	3F6
icl-1	C01F6.8	sick	3F9
	F12F6.6	sick	3G12
rps-11	F40F11.1	sick	3H1
	F40F11.2	sick	3H2
ftt-1	M117.2	sick	3H4
fat-6	VZK822L.1	sick	3H5
spt-5	K08E4.1	sick	3H6
	F52B11.3	sick	4A1
	Y55F3AR.3	sick	4A10
	Y55F3AR.3	sick	4A11

Gene	Cosmid	RNAi Phenotype	Plate position
		sick	4A12
	Y116A8C.35	sick	4A3
hsp-1	F26D10.3	sick	4A4
	Y41D4B.19B	sick	4A7
	Y41D4B.19B	sick	4A8
	Y41D4B.19A	sick	4A9
	Y67D8C.5	sick	4B1
	M03F8.3	sick	4B10
	F29G9.3	sick	4B11
pas-6	CD4.6	sick	4B9
	F09G2.4	sick	4C1
	C37C3.2	sick	4C6
ppn-1	C37C3.6A	sick	4C7
arx-2	K07C5.1	sick	4D3
	F17C11.8	sick	4D5
	T19B10.4A	sick	4D6
	F55A11.2	sick	4D7
snr-4	C52E4.3	sick	4D8
cyl-1	C52E4.6A	sick	4D9
hda-1	C53A5.3	sick	4E6
rpl-2	B0250.1	sick	4F11
phi-33	E01B7.1	sick	4F6
mlt-11	W01F3.3	sick	4G1
mdt-6	Y57E12AL.5	sick	4G4
gob-1	H13N06.3A	sick	4H5
	C02C6.2	sick	4H6
nhr-23	C01H6.5	not done	1C10
unc-15	F07A5.7	not done	1C11
gld-1	T23G11.3	not done	1C12
	M05B5.2	not done	1C9
adr-1	H15N14.1	not done	1D1
cdk-8	F39H11.3	not done	1D10
pbs-7	F39H11.5	not done	1D11
pas-4	C36B1.4	not done	1D12
adr-1	H15N14.1	not done	1D2
	F20G4.1	not done	1D3
	F18C12.2A	not done	1D4
kin-10	T01G9.6A	not done	1D5
	D1081.8	not done	1D6
	K02B12.3	not done	1D7
ran-4	R05D11.3	not done	1D8
tlf-1	F39H11.2	not done	1D9
blmp-1	F25D7.3	not done	1E11

Gene	Cosmid	RNAi Phenotype	Plate position
	B0205.10	not done	1F4
	Y34D9A.10	not done	1H3
rpl-22	C27A2.2A	not done	2A11
tag-184	F18C5.3	not done	2B5
	Y110A2AL.2	not done	2C12
		not done	2C5
	C27F2.5	not done	2E12
pqn-45	F56F3.1	not done	2E8
cct-5	C07G2.3	not done	2E9
	T17E9.2A	not done	2F7
	F57B9.2	not done	2G2
inf-1	F57B9.6	not done	2G3
rpl-6	R151.3	not done	2G7
	K01G5.4	not done	3B4
cua-1	Y76A2A.2	not done	3C10
	T27E9.2	not done	3C11
cua-1	Y76A2A.2	not done	3C9
	T12D8.6	not done	3D1
	F42A6.5	not done	3D10
srz-23	F58E2.9	not done	3D11
	M57.2	not done	3D12
	Y79H2A.3	not done	3D2
	Y79H2A.3	not done	3D3
	Y82E9BR.13	not done	3D4
unc-45	F30H5.1	not done	3D5
	Y55B1BM.1	not done	3D6
	Y71H2AM.17	not done	3D7
	Y77E11A.7	not done	3D8
	Y77E11A.6	not done	3D9
unc-68	K11C4.5	not done	4B12
	Y46H3A.5	not done	4B3
	C29G2.3	not done	4B4
	F41E6.13	not done	4C10
	C54F6.12	not done	4C3
	C50E3.2	not done	4C4
	F07C4.11	not done	4C5
	C50C10.5	not done	4D1
	T01D3.1	not done	4D11
	T22G5.4	not done	4D12

Table 2.2. Event-only lifespan analysis of putative progeric RNAi clones.

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
C02C6.2		5.8	65	20.0	89	-71.1	<0.0001
C13F10.4		8.1	80	20.0	89	-59.6	<0.0001
Y61A9LA.5		8.2	74	20.0	89	-58.8	<0.0001
H13N06.3A	*gob-1*	8.7	33	20.0	89	-56.3	<0.0001
F37E3.1		7.0	11	15.5	55	-55.0	<0.0001
CD4.4		9.1	23	20.0	89	-54.2	<0.0001
Y67D8C.5		9.1	26	20.0	89	-54.2	<0.0001
C33H5.18A		9.0	34	19.4	177	-53.7	<0.0001
C03D6.3	*cel-1*	7.3	7	15.5	55	-53.1	<0.0001
F28D1.9		9.3	6	19.4	177	-51.8	<0.0001
C06A5.1		9.3	63	19.4	177	-51.8	<0.0001
T26A8.4		9.4	23	19.4	177	-51.3	<0.0001
F40F11.2		9.5	30	19.4	177	-51.0	<0.0001
C52G5.1		9.8	27	20.0	89	-50.9	<0.0001
T17E9.2A		7.7	6	15.5	55	-50.7	<0.0001
F30A10.10		9.6	46	19.4	177	-50.6	<0.0001
1F6		7.7	7	15.5	55	-50.4	<0.0001
F08F8.2		7.9	7	15.5	55	-49.5	<0.0001
C50F4.5		10.2	28	20.0	89	-49.0	<0.0001
W06D12.3	*fat-5*	10.2	51	20.0	89	-48.9	<0.0001
F17C11.8		10.3	46	20.0	89	-48.6	<0.0001
Y53C10A.12	*hsf-1*	8.1	9	15.5	55	-47.8	<0.0001
Y18D10A.5	*gsk-3*	8.1	7	15.5	55	-47.6	<0.0001
F58A3.1A		10.6	50	20.0	89	-47.0	<0.0001

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
F30H5.1	*unc-45*	10.3	28	19.4	177	-46.7	<0.0001
Y55F3AR.3		10.7	36	20.0	89	-46.6	<0.0001
E01B7.1		10.8	36	20.0	89	-46.2	<0.0001
F43D2.1		10.8	19	20.0	89	-45.8	<0.0001
C49H3.5	*ntl-4*	10.5	37	19.4	177	-45.7	<0.0001
F54D1.6		10.5	15	19.4	177	-45.6	<0.0001
ZK637.8A	*unc-32*	10.5	11	19.4	177	-45.6	<0.0001
W04A4.5		8.5	8	15.5	55	-45.3	<0.0001
Y49E10.19		10.6	19	19.4	177	-45.1	<0.0001
F55A11.2		11.0	10	20.0	89	-45.0	<0.0001
F42A9.2	*lin-49*	10.7	21	19.4	177	-44.9	<0.0001
K07C5.1	*arx-2*	11.1	36	20.0	89	-44.7	<0.0001
C46C2.1		10.8	15	19.4	177	-44.3	<0.0001
Y71G12B.11A		8.7	6	15.5	55	-44.2	<0.0001
Y76A2A.2	*cua-1*	10.8	16	19.4	177	-44.2	<0.0001
W10D5.3A		8.7	7	15.5	55	-43.9	<0.0001
B0361.10		8.8	12	15.5	55	-43.7	<0.0001
T20F5.2	*pbs-4*	8.8	6	15.5	55	-43.2	<0.0001
R12B2.5		8.8	13	15.5	55	-43.1	<0.0001
W06H3.3		11.4	41	20.0	89	-42.9	<0.0001
K11C4.5	*unc-68*	11.4	83	20.0	89	-42.8	<0.0001
F09G2.4		11.5	56	20.0	89	-42.5	<0.0001
Y79H2A.6	*arx-3*	11.2	24	19.4	177	-42.1	<0.0001
Y65B4A.3		9.0	9	15.5	55	-42.1	<0.0001
Y37D8A.1		11.3	31	19.4	177	-41.6	<0.0001
Y77E11A.7		11.4	29	19.4	177	-40.9	<0.0001

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
R07B5.8		11.8	36	20.0	89	-40.8	<0.0001
C33H5.7		11.5	38	19.4	177	-40.6	<0.0001
M01G12.4	*srw-111*	9.2	21	15.5	55	-40.6	<0.0001
C05D11.2	*vps-16*	11.6	24	19.4	177	-40.2	<0.0001
D2045.6	*cul-1*	11.6	15	19.4	177	-40.1	<0.0001
T04C10.2A		12.0	66	20.0	89	-40.1	<0.0001
T12D8.6		11.7	19	19.4	177	-39.4	<0.0001
W07B3.2A	*gei-4*	9.5	15	15.5	55	-39.1	<0.0001
C23G10.8		9.5	11	15.5	55	-38.6	<0.0001
Y55B1BM.1		11.9	19	19.4	177	-38.3	<0.0001
T01G9.6A		9.7	12	15.5	55	-37.8	<0.0001
F54G8.3	*ina-1*	12.1	18	19.4	177	-37.8	<0.0001
W03F8.5	*lam-1*	12.1	28	19.4	177	-37.5	<0.0001
4F4		12.5	46	20.0	89	-37.5	<0.0001
C25D7.3	*sdc-3*	12.6	27	20.0	89	-37.0	<0.0001
C01F6.8		12.3	37	19.4	177	-36.7	<0.0001
F39H11.2	*tlf-1*	9.9	15	15.5	55	-36.5	<0.0001
Y80D3A.6		12.7	80	20.0	89	-36.3	<0.0001
F41E6.3		12.9	39	20.0	89	-35.3	<0.0001
Y59A8B.6		12.9	160	20.0	89	-35.3	<0.0001
Y42G9A.4		10.1	17	15.5	55	-35.3	<0.0001
Y62E10A.1		12.6	29	19.4	177	-35.2	<0.0001
Y80D3A.5	*cyp-42A.1*	13.0	49	20.0	89	-35.2	<0.0001
Y75B8A.11		12.6	32	19.4	177	-34.8	<0.0001
F54C8.3	*emb-30*	12.7	27	19.4	177	-34.4	<0.0001
Y47D3A.29		12.8	24	19.4	177	-33.8	<0.0001

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
F49E2.1A		13.4	34	20.0	89	-33.1	<0.0001
C50F4.7	*his-37*	13.4	31	20.0	89	-32.8	<0.0001
Y49E10.2		13.1	17	19.4	177	-32.6	<0.0001
F38A6.1	*fkh-1*	13.5	29	20.0	89	-32.5	<0.0001
K04D7.1		13.6	28	19.4	177	-29.9	<0.0001
C49A9.6		13.6	33	19.4	177	-29.9	<0.0001
Y77E11A.6		13.6	35	19.4	177	-29.8	<0.0001
T08G5.5		14.3	52	20.0	89	-28.6	<0.0001
F54C8.5		13.9	21	19.4	177	-28.2	<0.0001
T19B10.4A		14.5	31	20.0	89	-27.4	<0.0001
T05A10.5		14.6	31	20.0	89	-27.1	<0.0001
C31E10.7		14.7	44	20.0	89	-26.6	<0.0001
T20B12.6		11.6	12	15.5	55	-25.5	<0.0001
F58A4.4	*pri-1*	14.5	19	19.4	177	-25.3	<0.0001
C25A11.4A	*jam-1*	15.2	41	20.0	89	-24.1	<0.0001
C15H11.2		15.3	50	20.0	89	-23.6	<0.0001
ZK262.8		15.4	74	20.0	89	-23.2	<0.0001
F52E10.5	*ifa-3*	15.8	52	20.0	89	-21.1	<0.0001
ZK180.3		15.3	29	19.4	177	-20.8	<0.0001
C02B10.5		15.8	28	19.4	177	-18.5	<0.0001
C10C6.1	*kin-4*	16.1	39	19.4	177	-17.1	<0.0001
R01H10.1		16.6	37	19.4	177	-14.3	<0.0001
T07D3.7	*alg-2*	9.0	5	15.5	55	-42.1	0.0002
Y41D4B.19A		16.4	25	20.0	89	-18.2	0.0002
F18C12.2A		11.0	22	15.5	55	-29.5	0.0003
H38K22.2A		10.0	12	15.5	55	-35.7	0.0004

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
Y79H2A.3		14.4	8	19.4	177	-25.8	0.0004
B0395.1		16.5	41	20.0	89	-17.4	0.0004
ZC123.3		10.1	13	15.5	55	-35.2	0.0005
Y65B4BL.2		7.3	3	15.5	55	-52.8	0.0006
Y46G5A.13		11.0	21	15.5	55	-28.9	0.0006
F39H11.3	*cdk-8*	10.4	12	15.5	55	-33.0	0.0007
Y46G5A.1		10.6	14	15.5	55	-32.0	0.0007
R119.4		8.8	5	15.5	55	-43.4	0.0011
B0285.1		11.1	16	15.5	55	-28.8	0.0011
C06B8.8	*rpl-38*	18.0	45	20.0	89	-10.2	0.0011
Y51H7BR.6		11.3	14	15.5	55	-27.4	0.0031
K02B12.3		10.0	6	15.5	55	-35.7	0.0032
C05D11.2	*vps-16*	11.8	21	15.5	55	-24.3	0.0034
W08E3.1	*snr-2*	9.0	2	15.5	55	-42.1	0.0042
F25D7.3	*blmp-1*	10.8	12	15.5	55	-30.8	0.0055
B0511.8		10.2	6	15.5	55	-34.6	0.0072
R08D7.3		16.4	12	19.4	177	-15.3	0.0077
Y71F9AM.5	*nxt-1*	9.5	4	15.5	55	-38.9	0.0078
R10D12.10		17.4	31	20.0	89	-12.8	0.0081
F54E7.3A	*par-3*	11.5	14	15.5	55	-26.0	0.009
Y51H7C.13		11.6	15	15.5	55	-25.4	0.0095
Y79H2A.3		16.1	18	19.4	177	-16.8	0.0119
K12H4.4		10.4	5	15.5	55	-33.1	0.0131
H09F14.1		17.2	23	20.0	89	-14.2	0.0195
C47D12.1	*trr-1*	10.7	10	15.5	55	-31.0	0.0267
F36D1.7		10.3	4	15.5	55	-34.1	0.0327

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
R07G3.1	*cdc-42*	12.0	9	15.5	55	-22.8	0.0434
Y41D4A_2768.a		17.5	19	20.0	89	-12.7	0.0589
W02D3.9	*unc-37*	21.0	5	15.5	55	35.1	0.1216
C18G1.4A		18.0	25	20.0	89	-10.2	0.1272
F56H6.4		11.0	4	15.5	55	-29.2	0.1425
T13A10.6	*srv-29*	18.1	35	19.4	177	-6.6	0.2007
Y102A5D.1		18.3	67	20.0	89	-8.3	0.2111
F46A9.5	*skr-1*	12.6	12	15.5	55	-19.1	0.2162
Y46G5A.5		12.1	9	15.5	55	-22.1	0.218
C23H3.4	*sptl-1*	19.4	17	15.5	55	24.9	0.2402
F28B12.3	*vrk-1*	18.1	37	15.5	55	16.1	0.2681
F23F1.5		11.0	2	15.5	55	-29.2	0.2905
R06A4.4A	*imb-2*	13.4	20	15.5	55	-14.1	0.3033
B0393.1	*rps-0*	18.2	17	15.5	55	16.9	0.3272
T23G11.3	*gld-1*	12.8	15	15.5	55	-17.7	0.3441
Y51H7C.6A		13.3	11	15.5	55	-14.6	0.3574
H10E21.5		18.3	9	15.5	55	17.9	0.3582
C32D5.3		18.1	21	15.5	55	16.4	0.3799
F40G9.11		12.9	19	15.5	55	-16.7	0.388
F26D11.11	*let-413*	20.6	36	20.0	89	3.3	0.4197
C23G10.8		13.2	20	15.5	55	-15.1	0.451
W05F2.5		18.0	9	15.5	55	15.8	0.4546
T23B3.2		17.9	7	15.5	55	14.9	0.4746
T28F2.5	*ccb-1*	14.2	21	15.5	55	-8.7	0.4977
F22D6.3		12.3	4	15.5	55	-21.2	0.5032
F54C4.3		15.1	9	15.5	55	-2.8	0.6521

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
C53D5.6		16.0	17	15.5	55	2.9	0.6524
C18E9.6		16.6	21	15.5	55	6.6	0.6858
D1086.4		19.5	27	20.0	89	-2.5	0.7394
F23C8.6		15.1	12	15.5	55	-3.0	0.7426
C16C8.15		16.6	28	15.5	55	6.8	0.7434
Y53C10A.1		16.0	6	15.5	55	2.9	0.7571
C32E8.2	*rpl-13*	14.9	19	15.5	55	-3.8	0.7999
T05E8.1		14.4	7	15.5	55	-7.2	0.8285
F18C5.3	*tag-184*	14.7	24	15.5	55	-5.6	0.8289
ZK1128.4		20.2	22	19.4	177	4.2	0.8358
C49D10.6	*nhr-75*	16.8	52	15.5	55	7.8	0.8365
K02B7.2		16.6	14	15.5	55	7.1	0.8378
C41D11.1		16.3	20	15.5	55	4.5	0.8388
F55F10.1		19.8	9	19.4	177	2.1	0.8392
F30A10.6		13.5	2	15.5	55	-13.2	0.8453
C34E10.8		14.3	11	15.5	55	-8.2	0.8493
D1069.3		16.6	36	15.5	55	6.7	0.8704
C41G7.1A	*smn-1*	14.2	5	15.5	55	-8.7	0.8858
F28H6.3		19.8	24	20.0	89	-1.0	0.9
F32H2.3	*spd-2*	14.0	2	15.5	55	-9.9	0.9009
C27F2.5		14.2	10	15.5	55	-8.7	0.9053
Y37A1B.7		19.3	22	19.4	177	-0.5	0.9226
F59E12.4		16.6	17	15.5	55	6.7	0.9235
T06G6.8		15.1	7	15.5	55	-2.6	0.9336
R05D11.3	*ran-4*	14.7	20	15.5	55	-5.8	0.9517
H06I04.3A		14.8	12	15.5	55	-4.6	0.9611

Cosmid	Gene	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p value*
F23F1.8	*rpt-4*	14.1	7	15.5	55	-9.0	0.9735
F42A6.5		19.9	32	19.4	177	2.8	0.9796
T14D7.2		15.6	10	15.5	55	0.4	0.9853
2C6		14.8	8	15.5	55	-5.1	0.9918
C34G6.6		14.6	7	15.5	55	-6.3	0.9974
control	*daf-16*	13.3	43	15.5	55	-14.1	0.0243
control	*daf-16*	14.5	64	19.4	177	-24.9	<0.0001
control	*hsf-1*	7.6	24	15.5	55	-50.9	<0.0001
control	*hsf-1*	8.9	201	19.4	177	-53.9	<0.0001
control	*hsf-1*	8.386	237	20.0	89	-58.0	<0.0001
control	*daf-16*	12.242	113	20.0	89	-38.8	<0.0001

Table 2.3. RNAi knockdown of some candidate genes resulted in sickness or developmental arrest.

Cosmid	Gene
F56C11.1	
ZC123.3	
F28H1.3	
T19B4.2	
F54C1.7	
C01H6.5	*nhr-23*
ZC581.1	
H15N14.1	*adr-1*
F39H11.5	*pbs-7*
C36B1.4	*pas-4*
H15N14.1	*adr-1*
F20G4.1	
D1081.8	
Y106G6H.3	*rpl-30*
F32H2.5	
F26H9.6	*rab-5*
C12C8.3A	
ZK1151.2A	
F25H2.9	*pas-5*
F55A3.3	
Y6B3A.1	
Y105E8A.M	
E03H4.8	
C47B2.4	*pbs-2*
K05C4.1	*pbs-5*
Y71G12B.11A	
Y71F9B.4	*snr-7*
Y38A8.2	*pbs-3*
C27A2.2A	*rpl-22*
K07D4.3	*rpn-11*
ZK430.8	
ZK1067.7	*pqn-95*
ZK970.4	*vha-9*
C56C10.3	*tag-309*
T14B4.6	*dpy-2*
F59B10.1	*pqn-47*
Y110A2AL.2	
D2089.1	*rsp-7*
C47D12.6	*trs-1*

Cosmid	Gene
Y48B6A.3	
T04A8.14	*emb-5*
F59A2.1	
ZK1058.2	*(pat-3) rpn-1*
C07G2.3	*cct-5*
R144.2	
F25B5.4	*ubq-1*
ZK328.5B	
C23G10.4A	*rpn-2*
F23F12.6	*rpt-3*
T20B12.8	*hmg-4*
F57B9.2	
F57B9.6	*inf-1*
F57B9.10	
F37C12.9	*rps-14*
F37C12.11	*rps-21*
R151.3	*rpl-6*
T20H4.3	
C30C11.2	*rpn-3*
K02D10.5	
R13A5.8	*rpl-9*
R13A5.12	
R13A5.12	
ZK652.1	
C50C3.6	
C02F5.9	*pbs-6*
R10E11.8	*vha-1*
B0464.1	
F58A4.11	*gei-13*
R10E11.1A	*cbp-1*
M03C11.7	
Y49E10.1	*rpt-6*
F43D9.3	
K01G5.4	
Y47D3A.29	
Y47D3B.7	
T25C8.2	*act-5*
Y49E10.15	
Y111B2A.14	
Y37D8A.16	
ZK1010.1	*ubq-2*
F58E2.9	*srz-23*

Cosmid	Gene
Y71H2AM.17	
F55G1.10	*his-61*
H04M03.4	
C46G7.1	
F25H8.3	*gon-1*
F57H12.1	*arf-3*
F35H10.4	*vha-5*
F12F6.6	
B0035.7	
?	
?	
H02I12.6	
H02I12.7	
F22B3.2	
F40F11.1	*rps-11*
M117.2	*ftt-1*
VZK822L.1	*fat-6*
K08E4.1	*spt-5*
F11A10.2	
Y39C12A.1	
F52B11.3	
Y57G11C.16	*rps-18*
F26D10.3	*hsp-1*
Y38F2AL.3	
Y41D4B.19B	
Y55F3AR.3	
?	
Y46H3A.5	
C29G2.3	
CD4.6	*pas-6*
M03F8.3	
F29G9.3	
K11C4.5	*unc-68*
C54F6.12	
C50E3.2	
F07C4.11	
C37C3.2	
C37C3.6A	*ppn-1*
F41E6.13	
C50C10.5	
C52E4.3	
C52E4.6A	*cyl-1*

Cosmid	Gene
F55C5.8	
T01D3.1	
T22G5.4	
C53A5.3	*hda-1*
T10C6.11	*his-4*
Y80D3A.1	
B0250.1	*rpl-2*
W01F3.3	
Y57E12AL.5	*mdt-6*

Table 2.4. Mean lifespan observed in wild-type and rrf-3(pk1426) animals grown on "progeric" RNAi clones for entire life.

Gene	Cosmid	Chr	Function/Domain	Exp.	Whole-life RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value
	ZC123.3	I	*Homeobox protein*	2	10.4	38/53	21.9	47/70	-52.5	<0.0001
				4*	14.7	133/173	18.5	159/165	-20.1	<0.0001
				5	13.2	70/120	21.8	74/124	-39.3	<0.0001
				6*	15.5	125/160	21.0	149/160	-26.2	<0.0001
usp-48	F30A10.10	I	*Ubiquitin carboxyl-terminal hydrolase*	1	9.9	7/90	21.4	30/88	-53.6	<0.0001
				3	11.0	17/99	22.5	49/107	-50.9	<0.0001
				4*	13.7	169/173	18.5	159/165	-25.9	<0.0001
				5	13.4	103/128	21.8	74/124	-38.5	<0.0001
				6*	14.1	145/160	21.0	149/160	-32.8	<0.0001
cel-1	C03D6.3	I	*mRNA capping enzyme*	1	9.4	53/90	21.4	30/88	-56.2	<0.0001
				3	10.2	85/140	22.5	49/107	-54.7	<0.0001
vps-16	C05D11.2	III	*vacuolar sorting protein*	1	13.4	39/90	21.4	30/88	-37.3	<0.0001
				3	13.0	56/119	22.5	49/107	-42.0	<0.0001
	F43D2.1	V	*Cyclin*	1	16.0	36/75	21.4	30/88	-25.5	<0.0001
				3	15.7	57/105	22.5	49/107	-30.2	<0.0001
				4*	15.1	174/177	18.5	159/165	-18.1	<0.0001
				5	15.5	91/120	21.8	74/124	-29.0	<0.0001
				6*	17.6	152/160	21.0	149/160	-16.0	<0.0001
cyp-42A.1	Y80D3A.5	V	*Cytochrome P450*	2	19.4	40/51	21.9	47/70	-11.3	0.0053
hsf-1	Y53C10A.12	I	*Heat shock factor*	1	9.1	47/90	21.4	30/88	-57.5	<0.0001
				2	10.9	54/58	21.9	47/70	-50.2	<0.0001
				3	10.0	71/105	22.5	49/107	-55.6	<0.0001
				4*	11.4	159/164	18.5	159/165	-38.4	<0.0001
daf-16	pAD43	I	*FOXO transcription factor*	1	14.8	64/90	21.4	30/88	-30.8	<0.0001
				2	17.1	37/54	21.9	47/70	-21.9	<0.0001
				3	17.7	50/105	22.5	49/107	-21.3	<0.0001
				4*	16.5	166/170	18.5	159/165	-10.4	<0.0001

*Experiment done in an *rrf-3(pk1426)* RNAi-sensitized background

Table 2.5. Mean lifespan observed in wild-type animals grown on "progeric" RNAi clones during adulthood only.

Gene	Cosmid	Chr	Function/Domain	Exp.	Adult-only RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*p* value
	ZC123.3	I	*Homeobox protein*	2	19.8	41/70	21.9	47/70	-9.8	0.03
usp-48	F30A10.10	I	*Ubiquitin carboxyl-terminal hydrolase*	1	12.9	24/92	22.7	43/90	-43.0	<0.0001
				3	13.3	22/51	22.5	49/107	-40.9	<0.0001
cel-1	C03D6.3	I	*mRNA capping enzyme*	1	10.1	18/90	22.7	43/90	-55.3	<0.0001
vps-16	C05D11.2	III	*vacuolar sorting protein*	1	18.5	33/90	22.7	43/90	-18.3	0.0024
				3	19.4	28/46	22.5	49/107	-13.9	0.0061
	F43D2.1	V	*Cyclin*	1	19.2	24/75	22.7	43/90	-15.1	0.04
				3	19.7	34/45	22.5	49/107	-12.4	0.004
cyp-42A.1	Y80D3A.5	V	*Cytochrome P450*	2	21.1	40/70	21.9	47/70	-3.7	0.515
hsf-1	Y53C10A.12	I	*Heat shock factor*	1	12.4	24/90	22.7	43/90	-45.3	<0.0001
				2	16.0	51/70	21.9	47/70	-26.8	<0.0001
daf-16	pAD43	I	*FOXO transcription factor*	1	16.7	48/90	22.7	43/90	-26.4	<0.0001
				2	17.6	55/70	21.9	47/70	-19.6	<0.0001

Table 2.6. Lifespan analysis of "progeric" RNAi treatment on longevity mutants.

daf-2(e1370)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	20.4	194/201	29.0	147/160	-29.8	<0.0001
	14.2	41/90	49.5	51/120	-71.3	<0.0001
usp-48	20.8	146/162	29.0	147/160	-28.5	<0.0001
	16.0	41/130	49.5	51/120	-67.7	<0.0001
cel-1	13.4	105/119	49.5	51/120	-72.9	<0.0001
vps-16	22.0	57/119	49.5	51/120	-55.6	<0.0001
F43D2.1	26.0	163/167	29.0	147/160	-10.3	<0.0001
hsf-1	19.4	146/159	29.0	147/160	-33.1	<0.0001
	15.8	79/120	49.5	51/120	-68.0	<0.0001
daf-16	16.6	121/145	29.0	147/160	-42.9	<0.0001
	21.6	80/120	49.5	51/120	-56.4	<0.0001

hsf-1(sy441)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	12.6	151/162	12.782	129/162	-1.3	NS
usp-48	11.8	143/161	12.782	129/162	-7.6	<0.0001
F43D2.1	11.1	136/160	12.782	129/162	-13.3	<0.0001
daf-16	12.217	151/158	12.782	129/162	-4.4	0.0529

daf-16(mu86)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	11.8	128/161	13.547	145/160	-12.8	<0.0001
usp-48	11.7	144/161	13.547	145/160	-13.6	<0.0001
F43D2.1	11.1	149/160	13.547	145/160	-18.4	<0.0001
hsf-1	11.698	152/160	13.547	145/160	-13.6	<0.0001

glp-1(e2141)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	16.8	144/168	20.2	97/106	-16.9	<0.0001
usp-48	13.4	155/167	20.2	97/106	-33.6	<0.0001
F43D2.1	14.4	95/147	20.2	97/106	-28.6	<0.0001
hsf-1	10.7	156/160	20.2	97/106	-47.1	<0.0001
daf-16	15.4	145/160	20.2	97/106	-23.8	<0.0001

eat-2(ad1116)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	16.6	166/175	19.0	147/160	-13.0	<0.0001
usp-48	13.4	157/168	19.0	147/160	-29.9	<0.0001
F43D2.1	12.8	160/164	19.0	147/160	-33.0	<0.0001
hsf-1	9.5	138/165	19.0	147/160	-50.0	<0.0001
daf-16	17.8	149/163	19.0	147/160	-6.5	0.02

clk-1(qm30)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
ZC123.3	24.2	138/160	31.103	102/130	-22.2	<0.0001
usp-48	12.4	101/160	31.103	102/130	-60.0	<0.0001
F43D2.1	25.8	109/160	31.103	102/130	-17.2	<0.0001

Table 2.7. Lifespan analysis of "progeric" RNAi treatment on sterile gon-2(q388) mutants.

gon-2(q388)

RNAi clone	RNAi lifespan (days)	Number of RNAi animals	Control lifespan (days)	Number of control animals	% lifespan change	*P* value vs. control
usp-48	11.4	86/105	18.4	85/105	-38.3	<0.0001
	11.3	81/95	19.4	96/106	-41.9	<0.0001
F43D2.1	14.1	97/1105	19.4	96/106	-27.3	<0.0001
daf-16	14.6	102/105	19.4	96/106	-24.9	<0.0001
hsf-1	9.7	84/105	18.4	85/105	-47.6	<0.0001

Chapter 3: RNAi Screen for Regulators of *dod-11::RFP*

Introduction

Food quality and quantity directly impact how organisms age. One of the most robust methods of lifespan extension is caloric or dietary restriction (DR). Reducing food intake while maintaining proper nutrition can extend lifespan in many organisms, from single-celled yeast to primates (Colman et al., 2009; Jiang et al., 2000). And, in addition to lifespan increases, animals undergoing DR regimes show reductions in the incidence of age-related diseases (Mair and Dillin, 2008). DR may have evolved as an adaptation to reduce growth and prolong fecundity during times of famine. Yet, despite this knowledge of DR-mediated lifespan extension, its mechanisms are not yet well understood. Studies in model organisms may help elucidate the puzzle.

In *C. elegans*, dietary restriction can be studied using the eating defective mutant *eat-2(ad1116)* (Lakowski and Hekimi, 1998). *eat-2* encodes a nicotinic acetylcholine receptor subunit that functions in the pharyngeal muscle. Mutations in this gene reduce the animal's ability to pump bacterial food through its pharynx, thus causing these food-limited animals to live ~30% longer than fully fed controls (Hansen et al., 2007; Lakowski and Hekimi, 1998). The *eat-2(ad1116)* regime of dietary restriction is independent of the FOXO transcription factor DAF-16 (Houthoofd et al., 2003; Lakowski and Hekimi, 1998) but may involve the deacetylase SIR2 (Wang and Tissenbaum, 2006) the nutrient sensor CeTOR (Hansen et al., 2007), and autophagy (Hansen et al., 2008). A few additional members of a putative dietary restriction response pathway in worms are also known (Hansen et al., 2005).

In a cDNA microarray study Mitic and Kenyon (personal communication) detected a significant transcriptional upregulation of the putative alcohol dehydrogenase DOD-11 in *eat-2(ad1116)* animals. A *dod-11::rfp* transcriptional reporter confirmed increased expression in food-limited animals. Although *dod-11* expression is a reliable indicator of nutritional status, it does not influence longevity, as neither knockdown nor overexpression affect lifespan. Thus, we decided to use the *dod-11::rfp* transcriptional reporter to identify additional genes functioning within the dietary restriction pathway. We conducted an RNAi-based screen and identified approximately 100 genes whose reduced function influenced expression of *dod-11::rfp* without causing additional gross morphologic or developmental changes. Analysis of these candidates may enhance our understanding of the molecules involved in promoting longevity in response to dietary restriction.

Results

A screen for genes involved in dietary restriction-mediated longevity

Since relatively little is known about the genes controlling the longevity of dietary restricted (DR) animals, we performed an RNAi screen to identify new components of the DR pathway. We looked for changes in expression of the transcriptional *Pdod-11::RFP* fusion construct. This gene is upregulated in starved as well as in *eat-2(ad1116)* mutant animals, but does not affect lifespan itself (data not shown and Figure 3.1A). Thus it can be used as a nutritional read-out. To find genes whose knockdown affected the expression of the *dod-11* gene, we exposed *eat-2(ad1116) rrf-3(pk1426); Pdod-11::rfp* animals to RNAi clones from chromosome I of the Julie Ahringer RNAi

library, as well as clones from a kinase/phosphatase RNAi sub-library. Initially, we expected *dod-11::rfp* expression to go either up or down. Our reasoning was that if an RNAi treatment lowers *dod-11::rfp* expression, that clone would correspond to a gene whose wild-type function would promote DR-mediated longevity. Likewise, a gene whose knockdown causes elevated *dod-11::rfp* expression, would most likely act to inhibit DR-mediated longevity. Contrary to our expectations, most RNAi treatments affected not levels of *dod-11::rfp* but the pattern of expression, especially tissue-specific patterns (Fig. 3.1B). Each RNAi clone was independently screened in duplicate. As confirmation that our screen could identify components of DR-mediated lifespan extension pathway, we found that knockdown of the *skn-1* gene increased hypodermal expression of *dod-11:rfp* in the tail and head regions of the animal (Fig. 3.1B). SKN-1 is an Nrf2 transcription factor known to be necessary for DR-mediated lifespan extension in *C. elegans* (Bishop and Guarente, 2007). That inhibition of a known DR gene can change the pattern of *dod-11::rfp* expression is a good indication that other genes identified in our screen may also regulate DR-mediated longevity.

Out of 4,083 RNAi clones screened, we found that 148 change *dod-11::rfp* expression without grossly affecting development or morphology (Table 3.1 and data not shown). To verify that these genes are part of the DR pathway, we next decided to establish whether knockdown affects the longevity of *eat-2(ad1116)* mutants. If a gene is necessary for the extended lifespan of *eat-2(ad1116)* mutants, then RNAi knockdown should shorten *eat-2(ad1116)* lifespan to wild-type levels. While most RNAi clones had no effect, 15 enhanced the lifespan of RNAi-sensitized *eat-2(ad1116) rrf-3(pk1426)* animals while ~80 suppressed it (Table 3.1). Since the enhancers could correspond to

genes that promote aging and that are not necessarily in the DR lifespan pathway, we decided to focus our efforts on the genes whose knockdown decreases lifespan of *eat-2(ad1116) rrf-3(pk1426)* animals. With further analysis, we confirmed that six clones reproducibly shorten lifespan of *eat-2(ad1116) rrf-3(pk1426)* mutants compared to that of wild-type (Table 3.2). We also established that these clones have no effect on intestinal morphology as seen with a translational GFP reporter for *dlg-1*, a protein normally localized to intestinal adherens junctions (Firestein and Rongo, 2001) (Fig. 3.3).

Six genes that change dod-11::rfp expression pattern affect lifespan

Of the six genes further analyzed, only five consistently shortened *eat-2(ad1116)* lifespan; upon further examination, *srh-49* RNAi did not affect lifespan of any strains tested (Table 3.2). One of six, K04G2.9, had considerable affects on lifespan and will be further discussed in Chapter four of this thesis. Another, *cel-1*, was also identified in our screen for progeria genes and is thus discussed in Chapter two.

Of the three remaining genes, T23G11.6 seemed the most appealing. This gene encodes a protein containing a leucine rich repeat. These domains are generally thought to mediate protein-protein interactions. Although not well characterized, T23G11.6 has homology to a mammalian insulin-like growth factor binding protein acid-labile subunit (IGFALS), which forms a complex with IGF-1 and IGF-1 binding proteins to regulate protein transport and blood sugar levels. Interestingly, this gene affects timing of puberty; defects in IGFALS delay and slow puberty, while elevated serum levels of ALS are found in patients with precocious puberty (Cisternino et al., 2002; Domene et al.,

2004). Because of the connection between insulin/IGF-1 signaling and longevity, we decided to further investigate the role of T23G11.6 on lifespan.

We found that knockdown of this gene alters hypodermal expression of *dod-11::rfp* and shortens lifespan of *eat-2(ad1116) rrf-3(pk1426)* mutants (Fig. 3.1B and Table 3.2). This effect is specific to the *eat-2* pathway; knockdown does not significantly affect lifespan of RNAi-sensitized *clk-1(qm30)* or *daf-2(e1370)* mutants and actually lengthens the lifespan of *rrf-3(pk1426); glp-1(e2141)* animals (Table 3.2). Interestingly, T23G11.6 knockdown has no effect on *eat-2(ad1116)* lifespan if the RNAi-sensitizing mutation *rrf-3(pk1426)* is not present (Table 3.2). This suggests that either T23G11.6 levels must be very low to suppress *eat-2*-mediated longevity, or that T23G11.6 is acting in neurons, which are refractory to RNAi, to promote longevity.

To see if T23G11.6 might act in the neurons, we constructed transgenic animals expressing the T23G11.6 cDNA fused to mCherry and driven by the endogenous promoter. Although we saw expression in the intestine and muscle tissues, we did not observe any neuronal expression (Fig. 3.3A). One caveat is that intestinal and muscle expression is very dim in these animals; it is possible that our microscopy techniques are not sufficiently powerful to pick up expression in the neurons – a tissue which makes up a significantly smaller portion of the animal than either intestine or muscle. However, T23G11.6 seems to be expressed at higher levels in larval stages and we did not observe any neuronal expression in these animals either (Fig. 3.3B). Thus, it seems more likely that the requirement of *rrf-3(pk1426)* for decreased lifespan, may be due to increased RNAi or perhaps some interaction between this mutation and T23G11.6 activity.

Because inhibition of T23G11.6 suppresses *eat-2*-mediated longevity, we wished to determine if overexpression could extend lifespan. Unfortunately, our transgene did not affect lifespan of either wild-type or *eat-2(ad1116)* animals (Figure 3.3C). This may be due to the low level of overexpression in our transgenic animals. Another possibility is that the *rrf-3(pk1426)* mutation must be present for T23G11.6 to extend lifespan. Although, this mutation is usually used simply to sensitize an animal to the effects of RNAi, it is possible that *rrf-3(pk1426)* can affect lifespan itself, and that this effect might interact with or be mediated by changes in T23G11.6. Further analysis will be necessary to establish which of these possibilities is more likely; RT-PCR analysis of transcript levels could determine how low T23G11.6 levels must be in order to influence lifespan.

Although we did not investigate the last two genes in much detail, preliminary experiments were done. *phi-15* encodes a gene with homology to a human prolactin regulatory element-binding protein. Although uncharacterized in *C. elegans*, this protein may be involved in intracellular trafficking and vesicular transport, as its yeast homolog is required for initiation of transport vesicle budding from the endoplasmic reticulum. RNAi inhibition of *phi-15* globally lowers *dod-11::rfp* levels and decreases lifespan in all strains tested (Tables 3.1 and 3.2). The other gene identified in our screen, *agef-1* encodes a putative ARF guanine nucleotide exchange factor. *agef-1* is required for transport of caveolin from the Golgi to CAV-1 bodies during oogenesis and is more generally involved in embryonic osmotic integrity, neurotransmission, locomotion, and growth. RNAi knockdown of this gene decreases *dod-11::rfp* expression in the intestine, but elevates it in the hypodermis (Table 3.1). Furthermore, inhibition of *agef-1* shortens

lifespan of RNAi-sensitized *eat-2(ad1116)* and *glp-1(e2141)* animals, but has no affect on the lifespan of *rrf-3(pk1426); clk-1(qm30)* mutants (Table 3.2).

Discussion

We undertook this RNAi screen in an attempt to find components of the dietary restriction pathway in *C. elegans*. As an indicator of nutritional status, *dod-11* expression is a good candidate for finding genes necessary for a lifespan extension that relies on reduced food intake. However, although we found many modulators of *dod-11::rfp*, we identified only one, T23G11.6, which seems to specifically affect the lifespan of dietary-restricted *eat-2(ad1116)* mutants (Tables 3.1 and 3.2). And, given that this effect requires the *rrf-3(pk1426)* mutation, it is still unclear whether this gene is really part of the DR pathway. Several experiments could clarify the role of T23G11.6 in lifespan regulation. First, it is important to determine how much the *rrf-3(pk1426)* mutation affects RNAi-induced knockdown of T23G11.6. If expression is significantly lower in an *rrf-3(pk1426)* background, it suggests that the lack of effect of T23G11.6 RNAi on *eat-2(ad1116)* single mutants is a result of protein levels not being lowered enough. It will also be interesting to determine the effect of T23G11.6 overexpression in an *eat-2(ad1116) rrf-3(pk1426)* background. Although an overexpression construct did not extend lifespan of the *eat-2(ad1116)* mutant alone, the effect of T23G11.6 inhibition on lifespan indicates that this gene merits further testing. Another important analysis will be to establish the effect of T23G11.6 knockdown on lifespan extension induced by other DR regimes. There are now multiple treatments from bacterial dilution to growth on axenic media that extend lifespan in *C. elegans* (Greer and Brunet, 2009). These

different regimes act through both independent and overlapping genetic pathways to regulate lifespan (Greer and Brunet, 2009). Thus, it will be interesting to compare how inhibition of T23G11.6 affects the lifespan extension produced by each of these regimes.

Despite our failure to recover DR pathway genes, our screen did identify several interesting candidates for lifespan regulation. The mRNA processing gene, *cel-1*, appears to be very important for aging rate, as it was also identified in our screen for progeria genes (discussed in Chapter 2) and severely shortens lifespan when inhibited by RNAi (Table 3.2). Likewise, our analysis of K04G2.9 reveals an important regulator of both aging and immune function (Chapter 4). As for *agef-1* and *phi-15*, further work could uncover novel lifespan regulators. Although neither has yet been shown to affect aging, it is interesting that both are predicted to be involved in vesicle trafficking. Recent evidence demonstrates a role for vacuolar sorting proteins in aging (Samuelson et al., 2007). Furthermore, as we will see in Chapter four, K04G2.9 is a Golgi-localized protein that may affect lifespan through altered vesicle trafficking.

Experimental Procedures

Strains

All strains used in this study were maintained as previously described (Brenner, 1974). Strains analyzed in this study were: wild-type N2, CF512 *fer-15(b26); fem-1(hc17),* CF1908 *eat-2(ad1116)*, NL2099 *rrf-3(pk1426)*, CF1850 *eat-2(ad1116) rrf-3(pk1426)*, CF2485 *rrf-3(pk1426); clk-1(qm30)*, CF1814 *rrf-3(pk1426); daf-2(e1370)*, CF2481 *rrf-3(pk1426); glp-1(e2141)*, CF2190 *eat-2(ad1116) rrf-3(pk1426); muIs139* [*Pdod-11::dod-*

11::rfp], CF3205 *muEx490* [T23G11.6::T23G11.6::mCherry], CF3217 *eat-2(ad1116); muEx490*, FT0048 *xnIs16;him-8(e1489)* [*dlg-1::dlg-1::gfp + rol-6Dn*].

Lifespan Analysis

Lifespan analysis was performed at 20°C as described previously (Kenyon et al., 1993). Statview 4.5 software (SAS) was used for statistical analysis. *P*-values were calculated using the Mantel-Cox log rank test.

RNA mediated interference (RNAi)

RNAi was performed through feeding as previously described (Kamath et al., 2001). RNAi clones were grown overnight at 37°C in LB plus 10 μg/ml tetracycline and 100 μg/ml carbenicillin. Two days after seeding on NG plates containing carbenicillin, dsRNA production was induced by adding 80 μl 0.1M IPTG. Unless otherwise noted, worms were exposed to RNAi bacteria from hatching.

dod-11:rfp RNAi screen

RNAi plates were seed in duplicate and allowed to grow at room temperature for 1-2 days. At this point, gravid CF2190 adults were bleached, and isolated eggs were plated on RNAi plates in an M9/0.1M IPTG/carbenicillin mix. Plates were placed at 25°C to induce sterility. When animals reached the L4 stage (approximately 2 days later), plates were taken out of 25°C and placed at 20°C. *dod-11::rfp* expression was analyzed on day 3 of adulthood using a Leica MZ16F (Wetzlar, Germany) dissecting microscope with fluorescence attachment.

Figure and Table Legends

Figure 3.1. dod-11::rfp expression changes with nutritional status.

A. *dod-11::rfp* expression is increased in dietary restricted *eat-2(ad1116) rrf-3(pk1426)* animals (c and d) compared to *rrf-3(pk1426)* controls (a and b).

B. Examples of changes in expression pattern of *dod-11:rfp* in *eat-2(ad1116) rrf-3(pk1426)* animals upon RNAi knockdown. a) vector control-treated animals display *dod-11* expression in the intestine. b) *skn-1i*-treated animals have upregulated *dod-11* expression in the anterior and posterior portions of the body. c) *cel-1i* treatment globally downregulates *dod-11* expression while d) K04G2.9i and e) T23G11.6i upregulated hypodermal expression. All pictures are of day 3 adults taken at 10X magnification.

Figure 3.2. Modifiers of dod-11::rfp expression do not affect intestinal morphology.

Shown are *dlg-1::dlg-1::gfp* animals exposed to A) vector control, B) *cel-1i*, C) *srh-49i*, D) T23G11.6i, E) *agef-1i*, F) *phi-15i*, and G) K04G2.9i. DLG-1 localizes to adherens junctions in gut epithelia. Expression of this translational GFP fusion is not affected by any RNAi treatment. All pictures are of day 2-3 adults taken at 10X magnification.

Figure 3.3. T23G11.6 is expressed in the intestine and muscle and does not increase lifespan when overexpressed.

A pT23G11.6::T23G11.6::*rfp* expression construct is localized to intestine (arrows) and muscle (arrowheads) in A) adult animals and B) larval stage animals.

C) This overexpression construct does not affect lifespan in either a wild-type or *eat-2(ad1116)* mutant background.

Table 3.1. Genes whose knockdown affects dod-11::rfp expression pattern in an eat-2(ad1116) rrf-3(pk1426) background.

Analysis of *eat-2(ad1116) rrf-3(pk1426); dod-11::rfp* animals grown on bacteria expressing dsRNA of Chromosome I genes. RNAi clones that caused sickness or developmental arrest, or, that produced no changes in *dod-11:rfp* expression, are not shown. Animals were grown on RNAi bacteria from the time of hatching. Development was allowed to take place at 25°C to induce sterility and sensitize the animals to the RNAi treatment. *dod-11::rfp* expression pattern was observed at day 3 of adulthood. RNAi lifespan refers to event-only lifespan analysis of *eat-2(ad1116) rrf-3(pk1426)* exposed to specific RNAi bacteria. Censored animals were not counted. % Lifespan change refers to difference in mean lifespan between RNAi treated animals and animals that were grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. Lifespan analysis of adult animals was performed at 25°C. Sublibrary position refers to position of RNAi clone in the "*dod-11::rfp"* sublibrary (made by MJ).

Table 3.2. Lifespan analysis of progeric RNAi clones.

Lifespan of animals fed bacteria expressing dsRNA for specific genes. Number of animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals

censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control refers to animals grown on control bacteria (vector only). *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test. A 25°C pulse was given to *rrf-3(pk1426)*-containing animals from the L1 to L4 stage in order to induce sterility and sensitize them to RNAi. Lifespan analysis of adult animals was performed at 20°C.

Figure 3.1. *dod-11::rfp* expression changes with nutritional status.

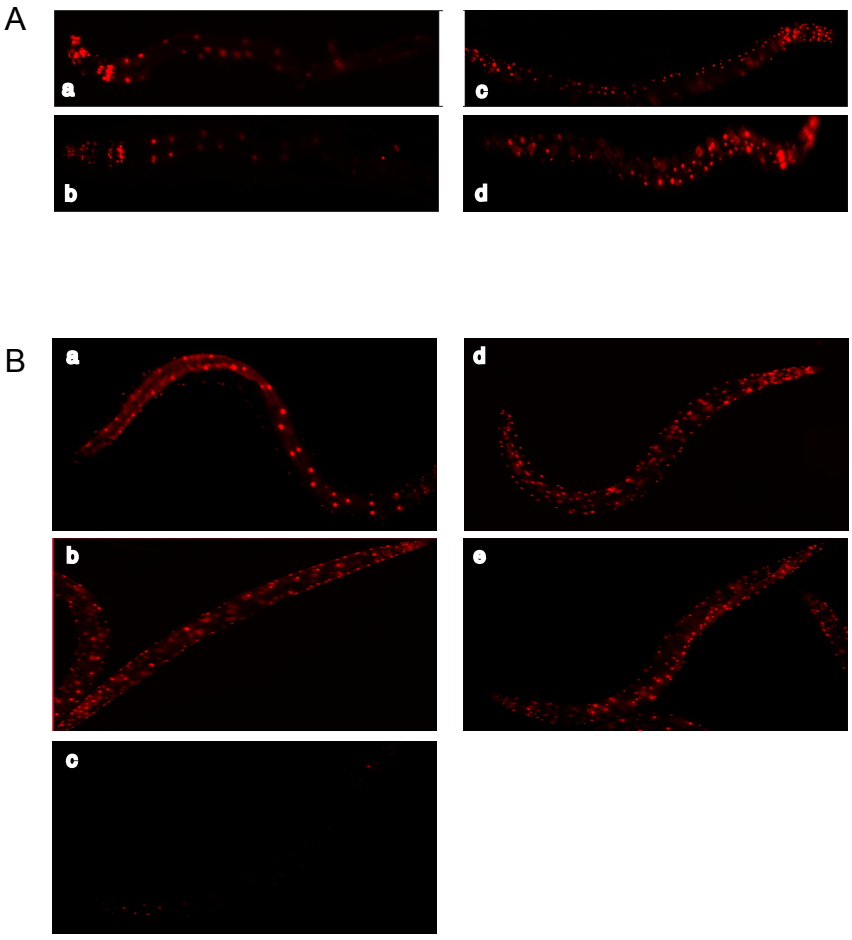

Figure 3.2. Modifiers of *dod-11::rfp* expression do not affect intestinal morphology.

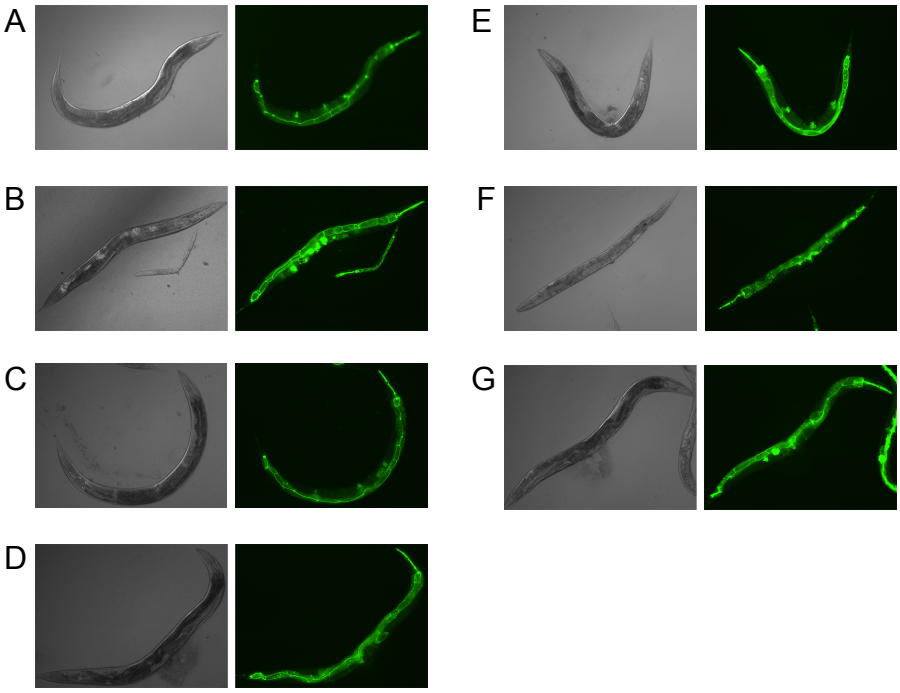

Figure 3.3. T23G11.6 is expressed in the intestine and muscle and does not increase lifespan when overexpressed.

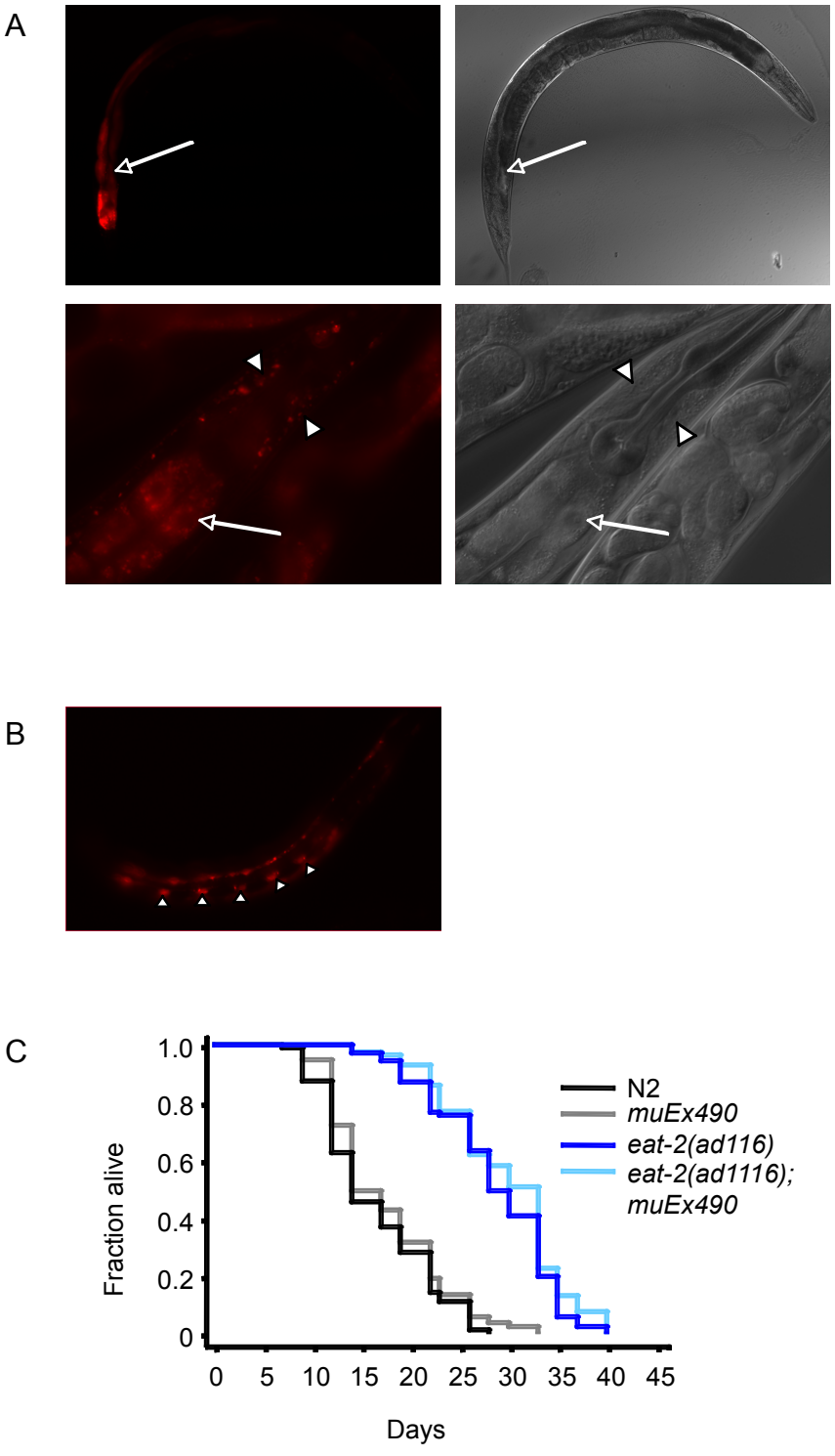

Table 3.1. Genes whose knockdown affects dod-11::rfp expression pattern in an eat-2(ad1116) rrf-3(pk1426) background.

Gene	Cosmid	*dod-11::rfp* pattern	Gene function/domain	RNAi lifespan (days)	% lifespan change	*p value*	Sublibrary position
	B0280.1	hypo up, intestine down	unknown	9.5	-50.0	<0.0001	2A12
	B0285.1	global up	Cdc2-related protein kinase	10.5	-44.2	<0.0001	2D5
cel-1	C03D6.3	global down	mRNA capping enzyme, guanylyltransferase (alpha) subunit	7.7	-59.3	<0.0001	1H9
cel-1	C03D6.3	hypo up/ intestine down	mRNA capping enzyme, guanylyltransferase (alpha) subunit	8.4	-55.3	<0.0001	1D9
pnk-1	C10G11.5	hypo up	Pantothenate kinase PanK and related proteins	8.1	-57.3	<0.0001	1H7
pnk-1	C10G11.5	NO intestinal RFP	Pantothenate kinase PanK and related proteins	8.4	-55.5	<0.0001	1B3
pab-3	C17E4.5	hypo up/ intestine down	Splicing factor RNPS1, SR protein superfamily	10.9	-42.4	<0.0001	1D4
	C25A1.5	hypo up/ intestine down	Cytochrome b5; Sphingolipid fatty acid hydroxylase	13.3	-29.6	<0.0001	1E6
	C34B2.8	hypo up/ intestine down	NADH:ubiquinone oxidoreductase, B16.6 subunit/cell death-regulatory protein	24.7	30.9	<0.0001	1F1
smn-1	C41G7.1	intestine down/global up	mRNA splicing protein SMN (survival motor neuron)	11.9	-36.8	<0.0001	1D7
pqn-25	D1044.3	some hypo up	Proteins containing Ca2+-binding EGF-like domains	12.8	-32.4	<0.0001	2A10
hpk-1	F20B6.8	variable down	Dual-specificity tyrosine-phosphorylation regulated kinase	12.3	-35.2	<0.0001	2C11
	F21C3.5	intestine down	Prefoldin subunit 6, KE2 family	13.9	-26.6	<0.0001	1C1
	F26A3.2	global down	Nuclear cap-binding protein complex, subunit CBP20	9.2	-51.4	<0.0001	1C3
taf-5	F30F8.8	intestine down	TAF (TBP-associated transcription factor) family	14.9	-20.9	<0.0001	1C5
ceh-20	F31E3.1	global down	Transcription factor PBX and related HOX domain proteins	11.9	-37.1	<0.0001	2A11
itr-1	F33D4.2a	hypo up/ global down	putative inositol (1,4,5) trisphosphate receptor	10.3	-45.6	<0.0001	2C1
tag-214	F36F2.3	hypo up/ intestine down	Predicted E3 ubiquitin ligase	12.2	-35.5	<0.0001	1D2
ppk-1	F55A12.3	intestine down	Phosphatidylinositol-4-phosphate 5-kinase	11.4	-39.5	<0.0001	1A7
	F56H6.12	hypo up/ intestine down	Integral membrane O-acyltransferase	10.9	-42.5	<0.0001	1H4
cdk-9	H25P06.2a	hypo up/ intestine down	ortholog of the metazoan transcription elongation factor kinase CDK-9	13.1	-30.8	<0.0001	1F6
cdk-9	H25P06.2a	global down	Cyclin T-dependent kinase CDK9	13.1	-30.8	<0.0001	1H11
gfi-1	K02A11.1a	hypo up/ intestine down	Myosin phosphatase, regulatory subunit	10.0	-47.3	<0.0001	1D11
gfi-2	K02A11.1a	??	Myosin phosphatase, regulatory subunit	12.3	-34.9	<0.0001	1H10
phi-15	K02B12.3	global down	Prolactin regulatory element-binding protein/Protein transport protein SEC12p	6.2	-67.1	<0.0001	1C11
	K04G2.9	hypo up	MARVEL-domain containing protein	6.8	-64.1	<0.0001	1C7
nhr-49	K10C3.6a	hypo up/ hypo down	Hepatocyte nuclear factor 4 and similar steroid hormone receptors	12.6	-33.5	<0.0001	1E3
kin-10	T01G9.6A	intestine down/intestine up	Casein kinase II, beta subunit	14.2	-24.8	<0.0001	1C9
pps-1	T14G10.1	intestine down	Bifunctional ATP sulfurylase/adenosine 5'-phosphosulfate kinase	12.6	-33.2	<0.0001	2C3
skn-1	T19E7.2	hypo up, intestine down	transcription factor NRF1	9.3	-50.6	<0.0001	2B8
pri-2	W02D9.1	hypo up/ intestine down	Eukaryotic-type DNA primase, large subunit	10.5	-44.3	<0.0001	1G1
rpl-30	Y106G6H.3	hypo up/ intestine down	large ribosomal subunit L30 protein	12.6	-33.5	<0.0001	1E10

Gene	Cosmid	*dod-11::rfp* pattern	Gene function/domain	RNAi lifespan (days)	% lifespan change	*p value*	Sublibrary position
lpd-3	Y47G6A.23	hypo up/ intestine down	required for normal lipid metabolism	7.7	-59.4	<0.0001	1G10
imb-5	Y48G1A.5	hypo up	Nuclear export receptor CSE1/CAS (importin beta superfamily)	6.1	-67.5	<0.0001	1G11
cdt-1	Y54E10A.15	intestine nuc up	CDT (S. pombe licensing factor) homolog	13.0	-31.0	<0.0001	1H1
	Y54E10BR.6	global down/hypo up, int down	DNA-directed RNA polymerase subunit E'	7.4	-60.7	<0.0001	1H2
agef-1	Y6B3A.1	hypo up/ intestine down	Guanine nucleotide exchange factor	6.4	-65.9	<0.0001	1G8
agef-1	Y6B3A.1	hypo up/ intestine down	Guanine nucleotide exchange factor	9.9	-47.7	<0.0001	1G7
zyg-8	Y79H2A.11	hypo up, intestine down	Ca2+/calmodulin-dependent protein kinase,	10.2	-46.2	<0.0001	2B6
	control?	hypo up, intestine down		14.8	-21.7	0.0001	2C7
frs-1	T08B2.9	up	predicted phenylalanyl-t-RNA synthetase.	14.3	-24.2	0.0001	1B2
lin-53	K07A1.12	down/hypo up	Nucleosome remodeling factor, subunit CAF1/NURF55/MSI1	14.7	-22.2	0.0002	1D8
prp-17	F49D11.1	intestine down	mRNA splicing factor	14.2	-24.8	0.0003	1F2
pmk-1	B0218.3	intestine down	mitogen-activated protein kinase	11.3	-40.0	0.0005	2C2
	B0511.6	global up	ATP-dependent RNA helicase pitchoune	14.6	-22.7	0.0006	1E12
inx-17	R12E2.4	global down	Innexin-type channels	14.5	-23.0	0.0006	1A4
	F43G9.12	hypo up/ intestine down	Transcriptional regulators binding to the GC-rich sequences	14.3	-24.2	0.0007	1C12
his-67	T23D8.5	hypo up	H4 histone required for embryonic viability and growth	14.8	-21.7	0.0008	1E5
sem-4	F15C11.1	hypo up	Transcriptional repressor SALM	13.5	-28.4	0.0009	1B10
lys-9	C54C8.6	global up	N-acetylmuraminidase/lysozyme	14.0	-26.1	0.001	1H5
	K10C3.5	hypo up/ var	Translation elongation factor 2/ribosome biogenesis protein RIA1	14.1	-25.2	0.0013	1H3
	VZC374L.1	global up	Mitogen-activated protein kinase (MAPK) kinase MKK4	14.1	-25.5	0.0013	2D1
	ZK39.3	global up	C-type lectin	14.8	-21.5	0.0014	1F4
ccb-1	T28F2.5	striped, head up, tail down	calcium channel, beta subunit	15.4	-18.5	0.0016	1A2
unc-22	ZK617.1a	hypo up, intestine down	Projectin/twitchin and related proteins	14.3	-24.4	0.0017	2C5
skr-1	F46A9.5	intestine down	SCF ubiquitin ligase, Skp1 component	13.6	-27.9	0.0025	1D5
	Y48G1A.4a	hypo down?/ hypo, int up	Nucleolar protein involved in 40S ribosome biogenesis	8.7	-54.0	0.0028	1G12
	Y48G1A.4a	hypo up/ intestine down	Nucleolar protein involved in 40S ribosome biogenesis	8.7	-54.0	0.0028	X
	ZK563.6	global up	Lysosomal & prostatic acid phosphatases	15.1	-20.3	0.0032	2C10
hil-8	T05E8.2	down	Serine/threonine kinase (haspin family)	14.4	-23.9	0.0036	1A8
	Y71H2AM.20/ Y71H2AM.5	global down?/ var	Phosphotyrosyl phosphatase activator	23.0	21.9	0.0037	2B7
	D2030.9	intestine down/ hypo up	WD40 repeat-containing protein	14.3	-24.3	0.0045	1C2
	W04C9.3	intestine down	Cuticulin precursor	14.9	-21.2	0.0049	1A1
	F37E3.1	intestine up	Nuclear cap-binding complex, subunit NCBP1/CBP80	16.2	-14.1	0.005	1B7
	F33H2.5	global down/ intestine nuc up	DNA polymerase epsilon, catalytic subunit A	15.9	-16.0	0.0054	1G9
	K04C1.5	hypo up, intestine down	Casein kinase (serine/threonine/tyrosine protein kinase)	16.2	-14.4	0.0065	2D3

Gene	Cosmid	*dod-11::rfp* pattern	Gene function/domain	RNAi lifespan (days)	% lifespan change	*p value*	Sublibrary position
	F32E10.2/ F32E10.5	hypo up/ global down	Heterochromatin-associated protein HP1 and related CHROMO domain proteins	15.1	-20.2	0.0069	2B12
rack-1	K04D7.1	intestine down	G protein beta subunit-like protein	16.2	-14.1	0.0069	2C4
	Y106G6A.2	global up	Predicted coiled-coil protein	15.6	-17.3	0.009	1E4
	F46F11.9	hypo down	Protein with predicted involvement in meiosis (GSG1)	15.5	-18.1	0.0097	1A10
	F32B4.2	hypo up/ intestine down	Translocase of outer mitochondrial membrane complex, subunit TOM20	15.7	-16.8	0.0105	1F7
ckb-1	B0285.8	hypo up	Choline kinase	15.9	-16.0	0.0112	2D6
ngp-1	T19A6.2a	global up	Nucleolar GTPase	16.4	-13.1	0.0123	1C10
	control?	global up		15.4	-18.3	0.0128	1H6
	control?	hypo up		15.1	-20.1	0.0129	2A8
	F59C6.5	hypo up/ intestine down	NADH-ubiquinone oxidoreductase, subunit NDUFB10/PDSW	22.9	21.4	0.0129	1E11
ima-2	F26B1.3	intestina up proximally	Karyopherin (importin) alpha	16.3	-13.6	0.0141	1B5
par-1	H39E23.1a	hypo up	serine-threonine kinase;	16.1	-15.0	0.0149	2C9
	control?	hypo up		15.7	-17.1	0.0184	2C8
pef-1	F23H11.8	up	Protein serine/threonine phosphatase RDGC/PPEF	15.6	-17.5	0.0186	2A4
lag-1	K08B4.1	hypo up	Recombination signal binding protein-J kappa	16.3	-13.8	0.0212	2B9
gei-11	F32H2.1a	global down/global up	Transcription factor, Myb superfamily	16.7	-11.5	0.0224	1D1
	F58E1.7	intestine, hypo down	unknown	16.4	-13.4	0.0320	1H12
	ZC477.10	hypo up, intestine down	Serine/threonine specific protein phosphatase PP1, catalytic subunit	15.9	-16.0	0.0438	2B11
nhr-191	F55D12.3	hypo up	Nuclear hormone receptor	15.7	-16.9	0.0441	1C6
	R12E2.11	down	Uridine 5'- monophosphate synthase/orotate phosphoribosyltransferase	16.4	-13.5	0.450	1A5
daf-7	B0412.2	up	Transforming growth factor beta, bone morphogenetic protein and related proteins	16.6	-12.1	NS	2A3
	B0464.9	intestine down?/global up?	Predicted acetyltransferases and hydrolases with the alpha/beta hydrolase fold	17.5	-7.5	NS	2B3
	C03D6.1	hypo up/ intestine down	Translation initiation factor 2C (eIF-2C)	17.2	-8.7	NS	1D10
tlk-1	C07A9.3	hypo up	Tousled-like protein kinase	18.7	-0.9	NS	2B4
	C11D9.1	down	Invasion-inducing protein TIAM1/CDC24 and related RhoGEF GTPases	18.2	-3.9	NS	1A6
plk-1	C14B9.4	intestine down?	Polo-like serine/threonine protein kinase	18.1	-4.0	NS	2B2
	C17H12.3	hypo up, intestine down	Protein tyrosine phosphatase	16.5	-12.4	NS	2B10
	C25A1.6	hypo up/ intestine down	H/ACA snoRNP complex, subunit NOP10	16.2	-14.3	NS	1E7
	C29E4.8	hypo up, intestine down?	Adenylate kinase	17.9	-5.3	NS	2B1
tag-255	C45G3.1	intestine nuc up	Microtubule-associated protein Asp	17.4	-7.9	NS	1D3
tba-2	C47B2.3a	global down/global up	Alpha tubulin	16.2	-14.3	NS	1G3

Gene	Cosmid	*dod-11::rfp* pattern	Gene function/domain	RNAi lifespan (days)	% lifespan change	*p value*	Sublibrary position
eif-6	C47B2.5	hypo up/ intestine down	Translation initiation factor 6 (eIF-6)	17.5	-7.5	NS	1G4
tag-172	E02H4.3	global up	LAMMER dual specificity kinases	18.0	-4.7	NS	2D4
gcy-12	F08B1.2	up	Natriuretic peptide receptor, guanylate cyclase	17.0	-9.8	NS	2A1
nrs-1	F22D6.3	global down	Asparaginyl-tRNA synthetase	19.1	1.0	NS	1B11
	F22D6.4	intestine down/global up?	NADH:ubiquinone oxidoreductase, NDUFS6/13 kDa subunit	20.9	10.9	NS	1B12
cco-1	F26E4.9	hypo up/ intestine down	Cytochrome c oxidase, subunit Vb/COX4	20.5	8.6	NS	1E1
	F46A8.10	hypo up	Phospholipid scramblase	18.6	-1.4	NS	1F5
	F46F6.2	global up	Serine/threonine protein kinase	17.3	-8.6	NS	2D2
	F49D11.4	hypo up	Predicted peptidase	17.1	-9.3	NS	1F3
dad-1	F57B10.10	intestine down	inhibits cell death	17.6	-6.8	NS	1B9
par-2	F58B6.3b	up	specialized type of zinc-finger found in E3 ubiquitin ligase subunits;	17.8	-5.8	NS	2A6
	H28O16.2	hypo up	Daxx-interacting protein MSP58/p78, contains FHA domain	16.1	-14.6	NS	1G2
	K12D12.1		DNA topoisomerase type II	19.7	4.1	NS	2A2
	R06B10.2	hypo up	unknown	18.3	-3.0	NS	2A5
cdk-1	T05G5.3	intestine nuc up	cyclin-dependent kinase	17.5	-7.6	NS	2B5
	T08B2.8	global up, esp. intestine	Predicted mitochondrial ribosomal protein L23	18.3	-3.0	NS	1B1
nuo-2	T10E9.7	hypo down, intestine up	NADH-ubiquinone oxidoreductase	22.1	17.2	NS	1B8
rab-10	T23H2.5	hypo up	GTP-binding protein SEC4	18.2	-3.9	NS	1B6
clec-12	T27F6.2	variable down	C-type lectin	17.7	-6.6	NS	1F12
col-50	T28F2.6	intestine up	collagens (type IV and type XIII)	17.6	-6.6	NS	1A3
	Y106G6E.6	hypo up/ intestine down	Casein kinase (serine/threonine/tyrosine protein kinase)	18.7	-1.2	NS	1E8
pab-1	Y106G6H.2	hypo up/ intestine down	Polyadenylate-binding protein (RRM superfamily)	17.8	-5.9	NS	1E9
nhr-62	Y67A6A.2	intestina up proximally	Nuclear Hormone Receptor family	19.3	1.9	NS	1E2
gpi-1	Y87G2A.8	hypo up	Glucose-6-phosphate isomerase	17.7	-6.1	NS	1G6
sre-23	ZK265.5	global up	Sre G protein-coupled chemoreceptor	18.6	-1.6	NS	1C8
	C04F12.1	hypo up/ intestine down	Translation initiation factor 2C (eIF-2C)	not done			
srh-49	C10G11.4	intestine up	Predicted olfactory G-protein coupled receptor [L	not done			1B4
	C48E7.2	intestine up	RNA polymerase III (C) subunit	not done			1A12
	C54C8.4	intestina up proximally	Glycosyltranferase	not done			1F11
	control?	proximal intestine down		not done			2A7
	F10F2.1	intestine down/global up	Kinase A-anchor protein Neurobeachin and related BEACH and WD40 repeat proteins	not done			2A9
	F15D3.7	hypo up/ intestine down	Mitochondrial import inner membrane translocase, subunit TIM23	not done			
	F19C6.3	global up	von Willebrand factor and related coagulation proteins	not done			2C12
tba-1	F26E4.8	hypo up/ intestine down	Alpha tubulin	not done			1D12

Gene	Cosmid	*dod-11::rfp* pattern	Gene function/domain	RNAi lifespan (days)	% lifespan change	*p value*	Sublibrary position
	F30A10.1	intestine nuc up	Ca2+-binding kinase interacting protein (KIP) (EF-Hand protein superfamily)	not done			1D6
tag-214	F36F2.3	intestine down	Predicted E3 ubiquitin ligase	not done			1H8
klp-15	M01E11.6	variable down	Kinesin (KAR3 subfamily)	not done			1A9
spe-12	T02E1.1	hypo up/ intestine down	defective SPErmatogenesis	not done			
	T09E11.4	hypo up/ intestine down	Integral membrane O-acyltransferase	not done			
unc-40	T19B4.7	down	receptor mediating netrin-dependent axon guidance	not done			1A11
	T23G11.6	hypo up/ hypo down	Leucine rich repeat	not done			1C4
rab-11.2	W04G5.2	hypo up/ intestine down	RAB family	not done			1F8
ztf-6	W06H12.1	global up	Zinc finger Transcription Factor family	not done			
rpl-18	Y45F10D.12	hypo up?	large ribosomal subunit L18 protein.	not done			2C6
smg-2	Y48G8AL.6	hypo up/ intestine down	RNA helicase nonsense mRNA reducing factor (pNORF1)	not done			
	Y52B11A.3	hypo up	Flavohemoprotein b5+b5R; NADH-cytochrome b-5 reductase	not done			
hsf-1	Y53C10A.12	hypo up/ intestine down	Heat shock transcription factor	not done			1F9
rom-5	Y54E10A.14	hypo up/ intestine down	RhOMboid (Drosophila) related	not done			
	Y54E10BL.5	hypo up/ intestine down	NADH:ubiquinone oxidoreductase, NDUFS5/15kDa	not done			
vrs-2	Y87G2A.5	hypo down	predicted cytoplasmic valyl-tRNA synthetase	not done			1G5
lrs-2	ZK524.3	hypo down	Leucyl-tRNA synthetase	not done			

Table 3.2. Lifespan analysis of progeric RNAi clones.

RNAi treatment	Genetic Background	Mean lifespan (days)	Number of animals	% lifespan change	*p value*
cel-1	*rrf-3(pk1426)*	13.3	81/90	-33	<0.0001
	eat-2(ad1116); rrf-3(pk1426)	12.4	86/90	-51	<0.0001
	glp-1(e2141); rrf-3(pk1426)	11.4	68/90	-51	<0.0001
	rrf-3(pk1426); clk-1(qm30)	13.8	87/90	-35	<0.0001
srh-49	*rrf-3(pk1426)*	20.0	81/90	0	NS
	eat-2(ad1116); rrf-3(pk1426)	25.6	67/90	1	NS
	glp-1(e2141); rrf-3(pk1426)	21.3	67/90	-8	NS
	rrf-3(pk1426); clk-1(qm30)	24.0	83/90	13	NS
T23G11.6	*rrf-3(pk1426)*	19.4	83/90	-3	NS
	eat-2(ad1116); rrf-3(pk1426)	15.9	14/90	-37	<0.0001
	glp-1(e2141); rrf-3(pk1426)	26.5	71/90	14	0.0027
	rrf-3(pk1426); clk-1(qm30)	24.9	67/95	17	NS
	rrf-3(pk1426); daf-2(e1370)	51.8	101/150	-1	NS
	N2	19.7	106/150	-4	NS
	eat-2(ad1116)	24.4	94/150	2	NS
agef-1	*rrf-3(pk1426)*	14.9	89/90	-25	<0.0001
	eat-2(ad1116); rrf-3(pk1426)	13.0	76/90	-49	<0.0001
	glp-1(e2141); rrf-3(pk1426)	14.4	89/90	-38	<0.0001
	rrf-3(pk1426); clk-1(qm30)	20.9	70/90	-2	NS
phi-15	*rrf-3(pk1426)*	10.5	88/90	-48	<0.0001
	eat-2(ad1116); rrf-3(pk1426)	7.9	85/90	-69	<0.0001
	glp-1(e2141); rrf-3(pk1426)	9.4	88/90	-59	<0.0001
	rrf-3(pk1426); clk-1(qm30)	11.4	85/90	-47	<0.0001
K04G2.9	*rrf-3(pk1426)*	11.8	88/90	-41	<0.0001
	eat-2(ad1116); rrf-3(pk1426)	7.3	77/90	-71	<0.0001
	glp-1(e2141); rrf-3(pk1426)	9.9	79/90	-57	<0.0001
	rrf-3(pk1426); clk-1(qm30)	13.4	77/90	-37	<0.0001
	rrf-3(pk1426); daf-2(e1370)	38.4	122/150	-27	<0.0001
control	*rrf-3(pk1426)*	20.0	83/90	-	-
	eat-2(ad1116); rrf-3(pk1426)	25.4	77/90	-	-
	glp-1(e2141); rrf-3(pk1426)	23.1	71/90	-	-
	rrf-3(pk1426); clk-1(qm30)	21.3	48/75	-	-
	rrf-3(pk1426); daf-2(e1370)	52.5	24/36	-	-
	N2	20.5	98/150	-	-
	eat-2(ad1116)	23.8	94/150	-	-

Chapter 4: Characterization of an Interesting Lifespan Gene

Abstract

In this study, we identify and analyze a gene that regulates lifespan and controls the rate of aging in *Caenorhabditis elegans*. Knockdown of *mrvl-1* by RNAi shortens lifespan and causes progeria while overexpression promotes longevity. This membrane protein is expressed in many tissues and partially localizes to the Golgi apparatus, implicating a role in vesicle trafficking. Here, we use expression profiling to identify genes involved in *mrvl-1*-regulation of aging. Microarray analysis reveals overlap with both the lifespan-regulating *daf-2*/insulin/IGF-1 signaling pathway and the innate immune response in *C. elegans*. Consistent with these findings, *mrvl-1* RNAi treatment not only shortens lifespan but also causes increased sensitivity to the bacterial pathogen *Pseudomonas aeruginosa*. Bacterial food sources may pose a potential hazard to *C. elegans* and contribute to their eventual death. *mrvl-1* likely helps the worm cope with the detrimental effects of proliferating bacteria. Indeed, *mrvl-1* overexpression no longer extends the lifespan of animals fed non-proliferating, UV-killed bacteria. Our study describes a novel lifespan gene that potentially acts within the Golgi to protect the worm from pathogenic or proliferating bacteria, thus promoting survival and extending lifespan.

Introduction

Aging is a genetically regulated process and research over the past 20 to 30 years has improved our knowledge of the genes and pathways that control aging. One of the best characterized of these is the insulin/IGF-1 signaling pathway. Mutations in the

insulin/IGF-1 receptor homolog, *daf-2*, can double the lifespan of *C. elegans* (Kenyon et al., 1993; Kimura et al., 1997; Morris et al., 1996). This lifespan extension is mediated by the FOXO-family transcription factor DAF-16 (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997), which regulates expression of a number of genes, including antioxidant and antimicrobial genes which work in concert to promote longevity (McElwee et al., 2003; Murphy et al., 2003). Loss-of-function mutations in *daf-16* suppress the extended lifespan of *daf-2* mutants as well as shorten the lifespan of wild-type animals (Lin et al., 2001). Thus, *daf-16* influences the longevity of wild-type animals as well as long-lived *daf-2* mutants.

Not only do mutations in *daf-2* extend lifespan, but they also influence rate of aging (Garigan et al., 2002). As *C. elegans* become older, they exhibit characteristic age-related changes in morphology including a decline in tissue integrity and bacterial packing in the intestine and pharynx (Garigan et al., 2002; Herndon et al., 2002). This accumulation of bacteria may hold more significance than simply existing as a consequence of aging. Evidence suggests that live bacteria in the body of old animals may contribute to the cause of death in *C. elegans*; if animals are grown on a lawn of non-proliferating or dead bacteria, mean lifespan can be increased up to 40% (Garigan et al., 2002). Although the standard laboratory diet of *Escherichia coli* OP50 is assumed to be non-toxic to *C. elegans*, it is possible that this bacterium becomes an opportunistic pathogen in aged animals.

Significantly, the lifespan promoting *daf-16* transcription factor positively regulates expression of stress resistance genes including antimicrobials (McElwee et al., 2003; Murphy et al., 2003). Perhaps the extended lifespan of *daf-2* mutants is due in part to

better coping mechanisms against bacterial pathogens. Not surprisingly, long-lived *daf-2* mutants are resistant to pathogen infection (Garsin et al., 2003). Recent work shows a link between the *daf-2*/IIS pathway and immune function in *C. elegans*. In addition to the TGF-β pathway, p38 MAPK pathway and the GATA transcription factor *elt-2*, insulin/IGF-1 signaling also regulates innate immune function in *C. elegans* (Evans et al., 2008a; Mallo et al., 2002; Shapira et al., 2006; Troemel et al., 2006). Intestinal DAF-16 expression is required for resistance to the pathogenic bacterium *Pseudomonas aeruginosa* (Evans et al., 2008b) and the pathogen resistance of *daf-2* mutants requires the IIS pathway members AKT-1 and AKT-2 (Evans et al., 2008a). Furthermore, transcriptional profiling shows overlap between genes upregulated by pathogen infection and those regulated by the *daf-2*/IIS pathway (McElwee et al., 2003; Murphy et al., 2003; Shapira et al., 2006; Troemel et al., 2006).

However, this overlap is not complete; for example, the immune effectors *lys-1* and *nlp-29,* which are upregulated upon pathogen infection, are not regulated by DAF-16 (Mallo et al., 2002; Murphy et al., 2003) and furthermore, some genes positively regulated by the p38 MAPK pathway are in fact negatively regulated by DAF-16 (Troemel et al., 2006). Since susceptibility to pathogens increases with age in *C. elegans* (Kurz et al., 2003; Laws et al., 2004), it seems surprising that regulation of aging and immunity is distinct within the *daf-2*/IIS pathway. Further studies may elucidate how this pathway contributes to the immune response of both long-lived mutants and wild-type animals.

Here we describe the effect of a previously uncharacterized gene, which we call *mrvl-1*, on the regulation of aging and innate immunity in *C. elegans*. We show that

knockdown of *mrvl-1* leads to shortened lifespan and increased sensitivity to the bacterial pathogen *P. aeruginosa*. Furthermore, overexpression of this gene produces lifespan extension and greater resistance to *P. aeruginosa*. *mrvl-1*-regulation of aging and innate immunity, which appears to overlap with the insulin/IGF-1 signaling pathway, may be important for *C. elegans* response to environmental bacterial pathogens encountered in its natural environment. Furthermore, this gene may be critical for maintaining basal levels of pathogen response genes that are essential for resistance to pathogenic bacteria such as *P. aeruginosa* as well as for defenses against the age-induced pathogenic effects of the usually non-toxic *E. coli* OP50.

Results

mrvl-1 knockdown results in shortened lifespan and progeria in C. elegans

We performed an RNAi screen in *C. elegans* to identify genes regulating lifespan (discussed in Chapter 3). In this preliminary screen, knockdown of K04G2.9 appeared to shorten lifespan. K04G2.9, which we have named *mrvl-1*, encodes a heretofore-uncharacterized protein containing a four-transmembrane MARVEL domain (for MAL and related proteins for vesicle trafficking and membrane link). Although these domains are proposed to be involved in membrane apposition events (Sanchez-Pulido et al., 2002), *mrvl-1* function has not yet been determined. We decided to investigate the role of this gene in lifespan regulation. To assess the lifespan phenotype more rigorously, we cultured animals on bacteria expressing dsRNA from the time of hatching and scored every two days for survival. We found that RNAi knockdown of this gene results in a dramatic shortening of lifespan in *C. elegans*, producing up to a 35% decrease in the

mean lifespan of wild-type animals and up to a 53% reduction in the RNAi-sensitized strain *rrf-3(pk1426)* (Fig. 4.1A, Table 4.1).

Because the severe shortening of lifespan upon *mrvl-1* knockdown could be due to sickness and not premature aging, we chose to examine RNAi-treated animals for known phenotypic hallmarks of aging. These characteristics, which allow one to distinguish between progeria and early death unrelated to premature aging, include bacterial packing in the intestine and pharynx, a disorganized gonad, and the presence of necrotic cavities (Garigan et al., 2002). By scoring animals for the appearance of these age-related phenotypes, we found that knockdown of *mrvl-1* by RNAi not only shortens lifespan, but also causes progeric phenotypes (Fig. 4.1B and C, Table 4.2). These animals appear normal at young ages, but develop age-specific phenotypes at earlier time-points than wild-type animals.

mrvl-1 overexpression extends lifespan

If knockdown of *mrvl-1* causes early aging and a shortened lifespan, overexpression of the protein may lead to increased survival. In order to ascertain the effect of overexpression of this gene, we generated transgenic animals expressing the *mrvl-1* cDNA fused to mCherry and driven by the endogenous promoter. Overexpression in wild-type animals consistently increased lifespan, though to a varying extent (Fig. 4.2A, Table 4.3). This variability may be due to the fact that the effect on longevity is relatively small, ranging from a 5-30% increase in mean lifespan. Additional variability may result from uneven transmission of the extrachromosomal array containing the construct.

As noted previously, *mrvl-1* encodes a protein with a MARVEL domain. These four-transmembrane domains are proposed to have a role in membrane apposition events such as biogenesis of vesicular transport carriers and formation and stabilization of tight junctions (Sanchez-Pulido et al., 2002). Another MARVEL domain-containing protein, MAL, is involved in transport between the *trans*-Golgi network and the plasma membrane (Puertollano and Alonso, 1999). MRVL-1 may be involved in one such role. Using the full-length fusion protein, we found that *mrvl-1* is expressed in multiple tissues and that the protein forms a punctate, subcellular localization in the cells in which it is expressed. To determine in which subcellular compartments MRVL-1 is expressed, we looked for overlap with known, subcellularly localized proteins. We found no overlap with a mitochondrial-expressed protein (data not shown), but partial co-localization of MRVL-1 and a Golgi-localized protein, MIG-23 (Fig. 4.2B). This overlap is intriguing given the Golgi function of the MARVEL domain protein MAL. Perhaps, like MAL, MRVL-1 acts to transport proteins between subcellular compartments.

Microarray analysis reveals transcriptional changes upon mrvl-1 inhibition

While our lifespan analysis indicated a clear role for *mrvl-1* in the regulation of aging, the mechanism was unknown. We wished to determine if *mrvl-1* knockdown produced gene expression changes similar to those previously associated with aging regulatory pathways. Toward this aim, we examined the transcriptional profile of animals exposed to *mrvl-1* RNAi. Using whole-genome Agilent *C. elegans* oligo arrays, we identified 100 upregulated and 720 downregulated genes (Table 4.4). An initial analysis of the downregulated genes revealed a striking number of pathogen response

genes. These include a number encoding members of specific protein families, such as lysozymes (7 genes), antimicrobial effectors which degrade bacterial peptidoglycan (Nicholas and Hodgkin, 2004), as well as proteins with CUB-like (19 genes) and C-type lectin (17 genes) domains. CUB-like domains, first found in human complement proteins C1r/C1s, are found on extracellular and membrane proteins and are involved in a diverse range of functions including tissue repair and inflammation (Shivers et al., 2008). C-type lectins are involved in many immune functions and can recognize and bind to sugar moieties on the surface of pathogens (Shivers et al., 2008).

The abundance of genes involved in innate immunity led us to compare our array data with studies examining the transcriptional response to pathogen infection in *C. elegans.* Recently, the worm has emerged as a model for the study of innate immunity (Nicholas and Hodgkin, 2004; Tan and Ausubel, 2000). *C. elegans*, a free-living, soil-dwelling microbivore, is normally fed a diet of *Escherichia coli* OP50 in the laboratory. However, exposure to microbial pathogens including *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Serratia marcescens* (Labrousse et al., 2000; Mallo et al., 2002; Tan et al., 1999) leads to infection and early death. Given its natural life history and probable exposure to microbial pathogens in the soil, it is not surprising that the worm is able to mount a robust defense against invading microbes. In an effort to identify the genetic machinery of these host defense-response pathways, several groups have conducted microarray-based transcriptional profiling studies in *C. elegans* (Mallo et al., 2002; Shapira et al., 2006; Troemel et al., 2006; Wong et al., 2007). We compared *mrvl-1*-regulated genes to these data and found significant overlap ($p<10^{-15}$, hypergeometric distribution) between genes downregulated upon *mrvl-1* inhibition and those upregulated

in *C. elegans* by infection with the bacterial pathogen *P. aeruginosa* (Shapira et al., 2006; Troemel et al., 2006). Thus, molecules induced in defense against *P. aeruginosa* infection are downregulated upon *mrvl-1* inhibition. The downregulation of these genes may lead to increased susceptibility to pathogens and thus to a shorter lifespan.

mrvl-1 affects pathogen sensitivity and resistance in C. elegans

The strong overlap with innate immunity genes compelled us to examine the effect of *mrvl-1* knockdown on the response of *C. elegans* to pathogenic bacteria. Because we found a highly significant transcriptional overlap between *mrvl-1*-regulated genes and those upregulated by the human Gram-negative bacterial pathogen *P. aeruginosa*, we chose to expose animals to PA14, a *P. aeruginosa* strain which, when cultured in low salt medium, is capable of killing *C. elegans* over a period of several days (Tan et al., 1999). To ascertain the effect of *mrvl-1* inhibition on pathogen resistance, we grew *C. elegans* first on bacteria expressing dsRNA for *mrvl-1*, then moved second generation animals to PA14 at day 2 of adulthood. Consistent with our microarray results, we found that exposure to *mrvl-1* RNAi leads to increased sensitivity to PA14 (Fig. 4.3, Table 4.5), suggesting that *mrvl-1* regulates the expression of host defense-response pathways and that, without wild-type activity of the protein, *C. elegans* is more susceptible to pathogens.

If the wild-type function of MRVL-1 is to maintain basal expression levels of pathogen and stress response genes, it is possible that overexpression of the protein may protect animals from pathogen infection and thus lead to increased survival. We decided to assay the effect of MRVL-1 overexpression on pathogen sensitivity. Overexpression

significantly increased wild-type survival on the *P. aeruginosa* strain PA14 (Fig. 4.3, Table 4.6). This increase was somewhat higher than the increase in lifespan observed when *C. elegans* is grown on its standard *E. coli* food source. Perhaps the augmentation of pathogen resistance genes has a greater effect when the bacterial food source is more toxic. Conversely, *mrvl-1* activity may not be as important when bacterial food sources are not toxic. If bacteria are incapable of proliferation, *C. elegans* exhibit decreased amounts of bacterial packing in the intestine and pharynx and live 30-40% longer (Garigan et al., 2002). Indeed, non-proliferating, UV-killed *E. coli* extended the lifespan of wild-type *C. elegans*. However, overexpression of *mrvl-1* did not further prolong the lifespan of these animals (Fig. 4.3, Table 4.7). This suggests that when food sources are not toxic, *mrvl-1* activity may be less important.

mrvl-1 influences lifespan and pathogen resistance in multiple longevity mutants

Since knockdown of *mrvl-1* shortened lifespan in wild-type animals, we wished to test whether this gene functioned within a known longevity pathway. Toward this aim, we examined the effect of RNAi treatment and *mrvl-1* overexpression on a number of long-lived *C. elegans* mutant lines. There are several known genetic pathways regulating aging in *C. elegans*, each with representative long-lived mutants. We examined three such mutants, including *clk-1(qm30)*, which is long-lived due to lowered mitochondrial respiration (Cristina et al., 2009; Lakowski and Hekimi, 1996), *eat-2(ad1116)*, the genetic model for dietary restriction in *C. elegans* (Lakowski and Hekimi, 1998) and the long-lived *daf-2*/IIS-receptor mutant *daf-2(e1370)* (Kenyon et al., 1993). If any of these mutants requires *mrvl-1* activity for lifespan extension or pathogen resistance, we might

expect RNAi knockdown to have a more pronounced effect in that background. Similarly, one would expect *mrvl-1* overexpression to further increase lifespan of long-lived mutants if it acts in parallel to the longevity mutation.

To our surprise, RNAi exposure shortened the lifespan of all *C. elegans* strains tested, including the *clk-1(qm30)* mutant. As in wild-type, RNAi knockdown caused a significant decrease in mean lifespan while overexpression of *mrvl-1* increased lifespan in *clk-1(qm30)* mutants (Fig. 4.4, Tables 4.1 and 4.3). These data suggest that the lifespan extending mechanisms of *mrvl-1* act in a different pathway from mitochondrial respiration-mediated lifespan extension. Similarly, *clk-1(qm30)* animals responded to the pathogenic bacterium *P. aeruginosa* in the same way as wild-type. Knockdown of *mrvl-1* increased susceptibility of *clk-1(qm30)* mutants to PA14 while overexpression conferred resistance (Fig. 4.5, Tables 4.5 and 4.6).

In an *eat-2(ad1116)* mutant background, we also observed extreme shortening of lifespan. In this strain, knockdown of *mrvl-1* caused up to a 57% reduction in mean lifespan (Fig. 4.4, Table 4.1). Because of the dramatic effect *mrvl-1* knockdown has on *eat-2(ad1116)* mutants, we chose to score aging characteristics in these animals as well. Consistent with the greater decrease in lifespan, progeric symptoms occur at an earlier time-point in these animals than in wildtype (Fig. 4.6, Table 4.2). Overexpression of *mrvl-1* also increased lifespan in these mutants, resulting in up to a 35% increase in mean lifespan (Fig. 4.4, Table 4.3). This increase was more robust than in wild-type, suggesting that *eat-2(ad1116)* animals are more sensitive to changes in *mrvl-1* expression than wild-type.

mrvl-1 knockdown also affected susceptibility to *P. aeruginosa* in *eat-2(ad1116)* mutant animals. Previous studies have established that long-lived *eat-2* mutants are not resistant to *P. aeruginosa* infection (Evans et al., 2008a; Tan et al., 1999). Similar to these results, we observed that the dietary restriction model *eat-2(ad1116)*, although long-lived on standard *E. coli* bacteria, is not more resistant to *P. aeruginosa* than wild-type (Fig. 4.5, Table 4.5). Furthermore, we find that, as in wild-type, inhibition of *mrvl-1* further sensitizes *eat-2(ad1116)* mutants to the toxic effects of *P. aeruginosa* (Fig. 4.5, Table 4.5). Concordantly, overexpression of *mrvl-1* leads to increased resistance to *P. aeruginosa* in *eat-2(ad1116)* mutant animals (Fig. 4.5, Table 4.6). While the *eat-2* gene does not play a role in regulating pathogen recognition and host defense-response pathways, overexpression of *mrvl-1* may upregulate the pathogen response, thus conferring pathogen resistance to the *eat-2(ad1116)* strain. These results suggest that *mrvl-1* is important for pathogen recognition and/or host defense-response pathways in *C. elegans*. Interestingly, both *mrvl-1* overexpression and growth on non-proliferating, UV-killed *E. coli* can extend the lifespan of long-lived *eat-2(ad1116)* animals. However, *mrvl-1* overexpression cannot further extend the lifespan of *eat-2(ad1116)* mutants grown on UV-killed bacteria (Fig. 4.7, Table 4.7). As in wildtype, *mrvl-1* activity may be crucial only when animals are exposed to potentially pathogenic environments.

We next examined the effect of *mrvl-1* inhibition on *daf-2(e1370)* mutants and found that, as in all other strains tested, knockdown shortened lifespan of these long-lived animals (Fig. 4.4, Table 4.1). The sweeping effect of *mrvl-1* inhibition on all aging mutants tested, suggests that the gene may act more generally to regulate lifespan rather than within one of these aging pathways. However, while lifespan was shortened below

wild-type levels in the *clk-1(qm30)* and *eat-2(ad1116)* backgrounds, *daf-2(e1370)* mutants fed bacteria expressing dsRNA for *mrvl-1* lived longer than wild-type animals fed control bacteria (Fig. 4.4, Table 4.1). This finding indicates a potential genetic interaction between *mrvl-1* and the insulin signaling pathway. Adding further evidence to this possible interaction, we found that *mrvl-1* overexpression had little to no effect on the lifespan of *daf-2(e1370)* mutants. If *mrvl-1* regulates lifespan through expression of genes that are also regulated by the *daf-2*/IIS pathway, then we might expect only small or no increases in the lifespan of *daf-2* mutants with *mrvl-1* overexpression. In fact, in one experiment, *mrvl-1* overexpression gave only a slight (5.6%) increase in mean lifespan and in two more, did not extend lifespan at all (Fig. 4.4, Table 4.3).

One possible explanation for these data is that the mechanism of extension by *mrvl-1* overexpression is mirrored by a *daf-2* mutation. Supporting this hypothesis, microarray analysis revealed significant overlap between genes downregulated upon *mrvl-1* knockdown and genes both upregulated ($p<10^{-8}$, hypergeometric distribution) and downregulated ($p<10^{-13}$, hypergeometric distribution) in *daf-2*$^{-/-}$ long-lived mutant animals (Murphy et al., 2003). This finding is not surprising in light of the fact that the complement of stress resistance genes induced by pathogenic bacteria also overlaps with those regulated by the *daf-2*/IIS aging pathway (McElwee et al., 2004; Murphy et al., 2003; Shapira et al., 2006). Indeed, *daf-2*$^{-/-}$ mutants are resistant to pathogenic and other stressors such as UV and hypoxia (Garsin et al., 2003; Murakami and Johnson, 1996; Scott et al., 2002). However, the mechanisms of lifespan extension and pathogen resistance by the *daf-2*/IIS pathway are genetically distinct with downstream components playing differing roles (Evans et al., 2008a).

Given the partial overlap between lifespan and pathogen resistance regulation by the *daf-2*/IIS pathway, we wished to determine if *mrvl-1* contributes to the increased pathogen resistance of *daf-2* mutants. In one experiment, we found that *mrvl-1* RNAi significantly decreased survival of *daf-2(e1370)* mutants exposed to the *P. aeruginosa* strain PA14. However, survival was not reduced to wild-type levels and, furthermore, a second experiment revealed no differences in PA14 resistance between *mrvl-1* RNAi-treated animals and controls (Fig. 4.5, Table 4.5). These results are similar to those from the *E. coli* lifespan experiments. Perhaps *mrvl-1* inhibition has small effects on *daf-2* mutant lifespan and survival because of converging gene regulation. For example, genes knocked down by *mrvl-1* inhibition may already be upregulated in *daf-2* mutants. Concordantly, *mrvl-1* overexpression may regulate genes similarly regulated by the *daf-2* mutation, and thus have only small effects on lifespan and *P. aeruginosa* survival in *daf-2(e1370)* mutants. However, we found that *mrvl-1* overexpression significantly increased *daf-2(e1370)* resistance to PA14 (Fig. 4.5, Table 4.6). This suggests that the mechanism of *mrvl-1*-regulated resistance to *P. aeruginosa* is distinct from that of *daf-2*/IIS-mediated resistance. Perhaps *daf-2* mutants' resistance to bacterial pathogens is conferred by transcriptional gene changes that work in parallel with those regulated by *mrvl-1*. It is of note that two genes downregulated by *mrvl-1* inhibition, *lys-1* and *nlp-29*, were upregulated by pathogen exposure but remained unchanged in *daf-2*$^{-/-}$ mutants (Table 4.4) (Mallo et al., 2002; Murphy et al., 2003). Because the expression of these genes is pathogen-responsive, and is not regulated by *daf-2*, the mechanism of pathogen resistance in *mrvl-1* overexpressing animals may be due, in part, to a pathway distinct from the *daf-2*/IIS pathway.

Exploring the relationship between mrvl-1 and reproductive signaling

It is unclear how *mrvl-1* may act in a *glp-1* mutant background. *glp-1(e2141)* animals are long-lived due to germ cell loss (Arantes-Oliveira et al., 2002), and this longevity is due in part to downstream activity of the DAF-16 transcription factor. Furthermore, like *daf-2*/IIS pathway mutants, *glp-1(-)* animals are resistant to pathogen infection and this resistance may be partially dependent on DAF-16 activity (Alper et al., 2009; Evans et al., 2008a; Kim et al., 2002; Miyata et al., 2008). It is possible, then, that as in *daf-2(e1370)* mutants, *mrvl-1* may act in parallel to the *glp-1* pathway to regulate gene expression changes and further extend survival on *P. aeruginosa*.

To investigate the relationship between *mrvl-1* and the reproductive system's control of lifespan, we performed lifespan analysis of sterile, long-lived *glp-1(e2141)* mutants with either *mrvl-1* inhibition or overexpression. Similar to all other longevity mutants, knockdown of *mrvl-1* with RNAi caused significant shortening of lifespan in *glp-1(e2141)* animals (Table 4.1). However, contrary to the wild-type, *clk-1(qm30)*, and *eat-2(ad1116)* conditions, we found variable results with *mrvl-1* overexpression. While one lifespan analysis showed a significant increase in mean lifespan, another showed only a trend toward this result; in this experiment, overexpression of *mrvl-1* would have resulted in a significant increase in lifespan except for a single, very long-lived *glp-1(e2141)* worm (Table 4.3). Because of this inconsistency, it is difficult to interpret these results. It seems likely that *mrvl-1* overexpression can extend lifespan of *glp-1(e2141)* mutants, however, more lifespan analysis will be necessary to elucidate this relationship.

We also examined the effect of *mrvl-1* activity on survival of *glp-1(e2141)* mutants exposed to *P. aeruginosa*. As mentioned previously, sterile mutants are resistant to pathogenic bacteria. It was surprising, therefore, that we saw increased *glp-1(e2141)* survival in only one out of four experiments (Tables 4.5 and 4.6). Though *mrvl-1* RNAi treatment did severely shorten survival of *glp-1(e2141)* animals exposed to *P. aeruginosa* (Table 4.5), it is unclear whether *mrvl-1* inhibition has any effect on *glp-1(e2141)* resistance, since, in our hands, these animals do not consistently exhibit increased survival.

Inconsistency also obscured interpretation of the effect of *mrvl-1* overexpression on *glp-1(e2141)* pathogen resistance. While overexpression increased survival of *glp-1e2141)* mutants by almost 40% in one experiment, in another it had no significant influence (Table 4.6). Again, further lifespan analysis will be needed to clarify this situation. However, since *glp-1(e2141)* mutants are not long-lived in our hands, any results will be difficult to interpret.

It is possible that these conflicting data are due to our bacterial culturing conditions. As shown in Alper *et al.*, *P. aeruginosa* bacteria have different virulence factors depending on the temperature at which they are grown (Alper et al., 2009). It will be interesting to see if the effects of *mrvl-1* overexpression or knockdown on pathogen resistance change, if PA14 is cultured in conditions similar to those that others have used (Alper et al., 2009). Interestingly, these differing culture conditions may also explain why, in our hands, PA14 is not as effective at killing *C. elegans* as in others'. In most published results, PA14 kills *C. elegans* in several days. However, in our experiments, mean survival ranges from five to eight days (Alper et al., 2009; Nicholas and Hodgkin,

2004). This discrepancy may be resolved if we culture PA14 on peptone-rich media with an extra day of growth at 37C.

Tissue specific requirements for mrvl-1

Because *mrvl-1* is expressed in multiple tissues, we wished to determine if its activity in any single tissue could extend lifespan. Towards this aim, we expressed a *mrvl-1*::mCherry fusion in a tissue-specific fashion. We used the *myo-3*, *grl-21*, and *vit-2* and F08A8.4 promoters for muscle, hypodermal, and intestinal-specific expression respectively. While muscle and hypodermal expression was sufficient to extend lifespan in *eat-2(ad1116)* mutants, neither reproducibly extended lifespan in a wild-type background (Table 4.8). And, while an F08A8.4 promoter coupled to *mrvl-1* cDNA modestly extended lifespan of wild-type animals, the promoter for another intestinal-specific gene, *vit-2*, did not (Table 4.8). Interestingly, *vit-2* expression does not occur until the L4 stage. Thus, perhaps whole-life intestinal expression of *mrvl-1* is needed to extend lifespan. Intestinal-specific expression of *mrvl-1* was not examined in an *eat-2(ad1116)* background.

These data suggest several explanations. *mrvl-1* may be needed in multiple tissues for lifespan extension in wild-type animals. Perhaps tissue-specific expression does not produce high enough levels of MRVL-1 protein to enhance longevity, or, perhaps *mrvl-1* acts cell-autonomously to regulate lifespan and thus, must be expressed everywhere, or in certain tissues to modify aging rate. After all, the intestine is a relatively large tissue and is also a major site of aging regulation in *C. elegans*. Furthermore, given the possibility that OP50 may become pathogenic to *C. elegans* late

in life, it seems likely that a pathogen-fighting gene such as *mrvl-1* would be most important in the intestine. However, a major role for the intestine is not supported by our *eat-2(ad1116)* data. In *eat-2* mutants, *mrvl-1* expression in the muscle and hypodermis can extend lifespan. It is possible that *eat-2(ad1116)* animals are more sensitive to *mrvl-1* levels. This would explain why RNAi knockdown of *mrvl-1* has such a considerable effect in the *eat-2* mutant background, and also why the effects of *mrvl-1* overexpression are more consistent in these mutants compared to wildtype.

Epistasis with known longevity genes

In an effort to better understand *mrvl-1*-regulation of lifespan, we performed epistatic analysis with known longevity genes. We decided to investigate *daf-16* and *hsf-1*, two genes encoding transcription factors that are major regulatory nodes in the insulin/IGF-1 signaling pathway, as well as genes for the FOXO transcription factor *pha-4*, the Nrf2 transcription factor *skn-1*, and the autophagy gene *bec-1*. *pha-4* and *skn-1* are both necessary for *eat-2*-mediated extension of lifespan (Bishop and Guarente, 2007; Panowski et al., 2007), and autophagy has been shown to be required for *daf-2* mutations as well as dietary restriction to extend lifespan in *C. elegans* (Hansen et al., 2008; Melendez et al., 2003). To our surprise, RNAi inhibition of *pha-4*, *skn-1*, and *bec-1* suppressed *mrvl-1*-mediated lifespan extension. This would suggest that all three genes are necessary for *mrvl-1* overexpression to increase lifespan. Epistatic analysis of *daf-16* and *hsf-1* was more difficult to interpret. Lifespan analysis was performed twice for knockdown of each gene, and each repeat gave opposing results from those of the first

experiments performed. Thus, it is unclear whether either of these two transcription factors is necessary for *mrvl-1*-mediated lifespan extension.

Epistatic analysis was performed in an *eat-2(ad1116)* background as well. Similar to experiments done in wild-type animals, *skn-1* and *bec-1* were both necessary for *mrvl-1* overexpression to increase the lifespan of *eat-2(ad1116)* mutants. However, in only one out of two experiments was expression of the transcription factor gene, *pha-4*, necessary. Furthermore, *mrvl-1* overexpression was not able to increase lifespan in animals exposed to *hsf-1* RNAi treatment. Ambiguous results were also obtained with epistatic analysis of *daf-16*. The lifespan of *daf-16* RNAi-treated animals was extended by *mrvl-1* overexpression in the first experiment performed, but not in the second. However, a third experiment revealed once again, that *mrvl-1*-mediated lifespan extension is not dependent upon the transcription factor DAF-16.

Discussion

In the wild, *C. elegans* live freely in the soil, an environment potentially ripe with microorganisms. Some of these are food for *C. elegans*, but not all; numerous pathogens exist that cause infection and death (Darby, 2005). To combat these infections, *C. elegans* have developed a set of pathogen recognition and host defense-response pathways. Molecules such as lectins, lysozymes, and CUB-like proteins are expressed to protect the worm from invading microbes (Nicholas and Hodgkin, 2004). Some of the pathways that shape the immune response also regulate aging (Alper et al., 2009; Evans et al., 2008a; Evans et al., 2008b; Garsin et al., 2003). However, the exact overlap between these physiological processes remains unclear. Here, we have described a gene

whose knockdown results in both shortened lifespan and increased sensitivity to the bacterial pathogen *P. aeruginosa* (Fig. 4.1 and 4.3). Transcriptional profiling reveals that this knockdown is associated with a decrease in expression of many of the genes responsible for protecting *C. elegans* against pathogens (Table 4.4).

How does K04G2.9 regulate both lifespan and pathogen resistance? One possibility is that it employs the same mechanism to influence both responses. Bacterial proliferation may be a cause of death in *C. elegans*; as worms age, they progressively accumulate live, proliferating *E. coli* in their gastrointestinal tract (Garigan et al., 2002; Gems and Riddle, 2000). Whether this is due to defects in pharyngeal grinding, decreased clearance through the anal sphincter, or a less functional immune response is unclear, but it is possible that older animals are less able to produce the antibacterial substances needed to cope with this bacterial invasion. Consistent with OP50 being an opportunistic pathogen in old worms, animals fed non-proliferating or dead *E. coli* live up to 30% longer than those fed live bacteria, and furthermore, bacterial packing is delayed in these animals as well as in long-lived *daf-2* mutants (Garigan et al., 2002). A gene such as K04G2.9, that regulates antibacterial molecules, could thus influence both pathogen resistance and lifespan.

Microarray analysis of K04G2.9 and the long-lived *daf-2(e1370)* mutant suggests shared or overlapping mechanisms; many of the genes regulated by the *daf-2*/IIS pathway are downregulated by K04G2.9 inhibition (Table 4.4) (Murphy et al., 2003). Furthermore, analysis of these expression-profiling experiments reveals that this overlap is likely the result of common regulation of pathogen-response genes (Shapira et al., 2006; Troemel et al., 2006). Interestingly, *daf-2* mutants exhibit less bacterial

colonization and better clearance of pathogenic *P. aeruginosa* than do wild-type controls (Evans et al., 2008a) and evidence suggests that this may be accomplished by differential expression of immunity genes. However, it is unclear just how the *daf-2*/IIS pathway shapes immune response, as the overlap between genes regulated by DAF-16 and those induced by pathogen infection are often in the wrong direction (Shivers et al., 2008). For example, although DAF-16 positively regulates genes that are induced by *P. aeruginosa* infection (and downregulated by K04G2.9 RNAi), it also negatively regulates other genes including a C-type lectin, a glutaredoxin-related protein, and a putative protease inhibitor, which are induced by pathogen infection and are potentially part of the inducible antimicrobial defense system in *C. elegans*. Furthermore, although DAF-16 positively regulates the expression of *lys-7* and *dod-6*, genes known to have a role in immunity, these genes are not actually induced by *P. aeruginosa* infection (Mallo et al., 2002; O'Rourke et al., 2006; Shapira et al., 2006; Troemel et al., 2006). Perhaps DAF-16 regulates a more general pathogen resistance that may not be required for the particular conditions used in laboratory models of pathogen infection. Given the extreme longevity of *daf-2* mutants, this antimicrobial activity could be more important for lifespan regulation and resistance to OP50 in older ages, rather than *P. aeruginosa* survival responses. Interestingly, recent work demonstrates that insulin/IGF-1 signaling regulates pathogen resistance and lifespan by convergent signals acting on DAF-16; while the downstream kinases *pdk-1* and *sgk-1* seem to be necessary for lifespan extension in *daf-2* mutants, two other components of the pathway, *akt-1* and *akt-2,* modulate pathogen resistance (Evans et al., 2008a). Thus, DAF-16 could regulate lifespan and innate immunity in a distinct manner.

K04G2.9, itself, may act through distinct yet convergent mechanisms to regulate both lifespan and immunity. One interesting feature of our study is that K04G2.9 overexpression increases survival of *daf-2(e1370)* mutants exposed to *P. aeruginosa*, but does not extend the longevity of *daf-2(e1370)* animals raised on *E. coli*. This suggests that while K04G2.9 activity augments the pathogen defense responses of the insulin/IGF-1 signaling pathway, its effects on lifespan completely coincide with that of *daf-2(e1370)*. To elucidate this relationship, one could determine whether the lifespan and pathogen resistance phenotypes of K04G2.9-overexpressing animals are at all dependent upon the DAF-16 transcription factor. Preliminary work is ambiguous. While in one experiment, *daf-16* activity was needed for K04G2.9 to extend lifespan, in another it was not (Table 4.9). Likewise, similar results were obtained with an *hsf-1* epistasis test (Table 4.9). These experiments are difficult to interpret since the effect of K04G2.9 on wild-type lifespan is relatively small and variable. It will be particularly elucidating to test the requirement of *daf-16* for the exceptional pathogen resistance of K04G2.9-overexpressing *daf-2(e1370)* animals.

It is also possible that K04G2.9-mediated regulation of pathogen resistance acts through other immune response pathways. These run in parallel to the *daf-2*/IIS pathway and can independently regulate pathogen recognition molecules and antimicrobial effectors. Indeed, K04G2.9 RNAi knockdown downregulates the immune effectors *lys-1* and *nlp-29,* which are upregulated upon pathogen infection, but are not regulated by DAF-16 (Table 4.4) (Mallo et al., 2002; Murphy et al., 2003). This suggests the presence of other pathway components besides *daf-2/daf-16* in K04G2.9-mediated pathogen resistance. Epistasis experiments with members of the TGF-β and p38 MAPK pathways

as well as with the GATA transcription factor *elt-2*, may reveal how K04G2.9 regulates both lifespan and pathogen resistance. Perhaps K04G2.9 overexpression works within one of these pathways to boost survival of the already pathogen resistant *daf-2(e1370)*. Furthermore, as many of the gene components of these pathways do not modulate lifespan (Kurz and Tan, 2004), any pathogen-resistance dependence on them could reveal how K04G2.9 independently regulates both aging and immunity.

Because none of the components of these pathways were identified in our microarray experiments, K04G2.9 most likely acts in a downstream fashion. Just how knockdown of a MARVEL-domain containing protein regulates expression of immune effector genes is unclear. MARVEL domains are found in many proteins including the myelin and lymphocyte protein (MAL), which shuttles between the *trans*-Golgi network, the plasma membrane, and the endosomes (Puertollano and Alonso, 1999; Sanchez-Pulido et al., 2002) to regulate apical sorting and transport (Puertollano et al., 1999). As K04G2.9 possesses homology to a chemokine-like factor-like MARVEL domain-containing protein, which, itself, has high homology to MAL, it is especially interesting that we find partial co-localization of the K04G2.9 protein with the Golgi-expressed MIG-23 (Fig. 4.2). Further co-expression experiments will tell us whether K04G2.9 also localizes to endosomes, but, at any rate, it is possible that K04G2.9 may be involved in trafficking of the molecules necessary for antimicrobial responses in *C. elegans*.

In our microarray analysis of K04G2.9-regulated genes, we were struck by a large downregulation of the claudin homolog F44G3.10 (almost 7 fold, Table 4.4). Claudins are a group of cell adhesion molecules that work at vertebrate tight junctions to seal the intercellular space between cells (Asano et al., 2003; Sanchez-Pulido et al., 2002). *C.*

elegans lack typical tight junctions, but do express at least four claudin-like genes which are important for epithelial barrier function. In one set of experiments, RNAi of the claudin-like genes *clc-1* and *clc-2* allowed TRITC-dextran to permeate the internal cavity of the body across the epithelial layers of the pharynx and hypodermis respectively (Asano et al., 2003). With a possible role in normal cohesion of apical junctions in the epithelia, F44G3.10 may be an important defense against pathogen infection in *C. elegans*. Indeed, in addition to being downregulated by K04G2.9 inhibition, F44G3.10 is induced by infection with *P. aeruginosa* (Shapira et al., 2006). Likewise, *clc-1* is regulated in a similar manner with upregulation induced by multiple pathogens (Shapira et al., 2006; Troemel et al., 2006; Wong et al., 2007). One explanation is that without adequate barriers, invading bacteria could cross into the body cavity to spread infection. Indeed, bacterial infections in the intestine have been shown to penetrate the rest of the body only during the terminal stages of infection (Nicholas and Hodgkin, 2004). Upregulation of the molecules necessary for epithelial barrier formation may stave off this attack, thus reducing the virulence of intestinal pathogens.

As organisms age, their immune system declines and deteriorates. Elderly patients are more susceptible to infections and less responsive to vaccinations. Given the conservation of biological processes between *C. elegans* and other systems, the study of immunosenescence in this model organism could reveal cellular and molecular mechanisms relevant to humans. Determining the exact role of K04G2.9 in aging and immune function may elucidate how these two processes shape each other and provide insights into the mechanism of immunosenescence.

Experimental Procedures

Strains

All strains used in this study were maintained as previously described (Brenner, 1974). Strains analyzed in this study were: wild-type N2, CF1041 *daf-2(e1370)*, CF1903 *glp-1(e2141)*, CF2354 *clk-1(qm30)*, CF1908 *eat-2(ad1116)*, NL2099 *rrf-3(pk1426)*, CF1850 *eat-2(ad1116) rrf-3(pk1426)*, CF2485 *rrf-3(pk1426); clk-1(qm30)*, CF1814 *rrf-3(pk1426); daf-2(e1370)*, CF2481 *rrf-3(pk1426); glp-1(e2141)*, CF3012 *muEx430* [pK04G2.9::K04G2.9::mCherry], CF3469 *daf-2(e1370); muEx430*, CF3483 *glp-1(e2141); muEx430*, CF3487 *clk-1(qm30); muEx430*, CF3073 *eat-2(ad1116); muEx430*, CF3358 *muEx525* [*pmyo-3::*K04G2.9::mCherry], CF3361 *muEx528* [*pgrl-21::*K04G2.9::mCherry], CF3362 *muEx529* [*pgrl-21::*K04G2.9::mCherry], CF3378 *eat-2(ad1116); muEx525*, CF3377 *eat-2(ad1116); muEx528*, CF3382 *eat-2(ad1116); muEx529*, CF3573 *muEx574* [*pvit-2::*K04G2.9::mCherry], CF3574 *muEx575* [*pvit-2::*K04G2.9::mCherry], CF3575 *muEx576* [pF08A8.4*::*K04G2.9::mCherry], CF3576 *muEx577* [pF08A8.4*::*K04G2.9::mCherry].

Generation of overexpression constructs and transgenic strains

Plasmids for K04G2.9 overexpression were constructed using Gateway Technology (www.ivitrogen.com). The K04G2.9 ORF was obtained from the *C. elegans* ORFeome (www.openbiosystems.org). Promoters for K04G2.9, *myo-3*, *grl-21*, *vit-2*, and F08A8.4 were obtained from the *C. elegans* Promoterome (www.openbiosystems.org) or were generously donated by the Ashrafi Lab. All constructs were injected at 5 ng/μl into the

gonad of wild-type day 1 adults. These animals were then crossed to longevity mutants to obtain transgenic *eat-2(ad1116)*, *glp-1(e2141)*, *clk-1(qm30)*, and *daf-2(e1370)* animals.

Lifespan Analysis

Lifespan analysis was performed at 20°C as described previously (Kenyon et al., 1993). Statview 4.5 software (SAS) was used for statistical analysis. P-values were calculated using the Mantel-Cox log rank test.

RNA mediated interference (RNAi)

RNAi was performed through feeding as previously described (Kamath et al., 2001). RNAi clones were grown overnight at 37°C in LB plus 10 μg/ml tetracycline and 100 μg/ml carbenicillin. Two days after seeding on NG plates containing carbenicillin, dsRNA production was induced by adding 80 μl 0.1M IPTG. Unless otherwise noted, worms were exposed to RNAi bacteria from hatching.

Microarray

Standard RNA purification, labeling, and hybridization on Agilent 4 × 44K *C. elegans* arrays were performed by the University Health Network Microarray Centre (UHN, Canada). 43,803 *C. elegans* probes were represented on this platform. RNA from six biological replicates of day 1 adult, age-matched N2 animals exposed to either K04G2.9 RNAi or vector control were hybridized competitively. Analysis was performed using SAM (Significance Analysis for Microarrays, Stanford University, USA) to identify genes significantly upregulated or downregulated by K04G2.9 RNAi

treatment. Using a cut-off q-value of 0.1, 100 upregulated and 720 downregulated genes were chosen for further analysis.

Pseudomonas aeruginosa infection

To study bacterial infection in *C. elegans*, we used the PA14 strain of *Pseudomonas aeruginosa*. Assays were done as previously described (Tan et al., 1999) with some modifications; PA14 was grown in LB broth overnight at 37°C, then seeded onto NG plates and left to grow at room temperature for at least one day. *C. elegans* were allowed to develop on OP50 bacteria and then picked to PA14 plates at day one of adulthood. For RNAi experiments, animals were grown on plates seeded with RNAi bacteria for at least two generations. Second generation progeny were transferred to PA14 plates at day one or two of adulthood. Experiments were done both with and without 2′fluoro-5′deoxyuridine (FUDR, Sigma, St Louis, MO, USA), a chemical that inhibits DNA synthesis and prevents progeny from developing. FUDR was exogenously added to either L4 stage or day one adults at 100 μM. All experiments were carried out at 20°C. In all cases, death was scored once per day. Statview 4.5 software (SAS) was used for statistical analysis with P-values calculated using the Mantel-Cox log rank test.

Figure and Table Legends

Figure 4.1. RNAi inhibition of mrvl-1 shortens lifespan and accelerates tissue aging.

A. Survival curves of wild-type animals fed either control, vector-only bacteria or bacteria expressing *mrvl-1* dsRNA (Mantel-Cox Logrank $p<0.0001$). For these and other survival curves, see tables for details and experiment repetitions.

B. Percentages of animals of different genotypes displaying aging phenotypes. Scores indicate increasing deterioration with 1 representing most youthful and 4 corresponding to a very aged appearance. The Mann-Whitney test was used to carry out pair-wise comparisons between the individual groups at each time-point (* = $p<0.01$; N=10-17 animals for each condition).

C. Representative animals cultured either on control bacteria or bacteria expressing dsRNA for *mrvl-1*.

Figure 4.2. mrvl-1 overexpression extends lifespan

Survival curves of wild-type (N2) animals and animals expressing a *mrvl-1* translational fusion protein (*muEx430*) (Mantel-Cox Logrank $p<0.0001$).

Figure 4.3. mrvl-1 is partially localized to the Golgi apparatus.

Representative pictures of muscle expressed MRVL-1 (red) and the Golgi marker MIG-23 (green) in day 1 adult animals. Each row shows a separate animal.

Figure 4.4. mrvl-1 activity is important when bacteria are pathogenic.

A. RNAi inhibition of *mrvl-1* results in decreased survival upon exposure to *P. aeruginosa*. Survival curves of wild-type animals fed either control bacteria or bacteria expressing *mrvl-1* dsRNA and then switched at day 1 of adulthood to plates seeded with PA14 pathogenic bacteria (Mantel-Cox Logrank $p<0.0001$).

B. *mrvl-1* overexpression confers resistance to *P. aeruginosa*. Survival curves of wild-type (N2) and *mrvl-1* overexpressing (*muEx430*) animals fed OP50 bacteria then

switched at day 1 of adulthood to plates seeded with PA14 pathogenic bacteria (Mantel-Cox Logrank p<0.0001).

C. Overexpression increases lifespan when animals are grown on live OP50. Survival curves of wild-type (N2) and *mrvl-1* overexpressing (*muEx430*) animals fed live OP50 bacteria (Mantel-Cox Logrank p=0.0017).

D. Overexpression cannot increase lifespan when animals are grown on UV-killed OP50. Survival curves of wild-type (N2) and *mrvl-1* overexpressing (*muEx430*) animals fed UV-killed OP50 bacteria (Mantel-Cox Logrank p=0.0797).

Figure 4.5. Epistatic analysis of mrvl-1 and known longevity genes.

RNAi knockdown of *mrvl-1* shortens lifespan of A) *clk-1(qm30); rrf-3(pk1426)* mutants C) *eat-2(ad1116) rrf-3(pk1426)* mutants and E) *rrf-3(pk1426); daf-2(e1370)* mutants (wild-type refers to *rrf-3(pk1426)* single mutants; see Table 4.1 for details and p values). Overexpression of *mrvl-1* (*muEx430*) extends lifespan of B) *clk-1(qm30)* mutants and D) *eat-2(ad1116)* mutants, but not F) *daf-2(e1370)* mutants (See Table 4.3 for details and p values).

Figure 4.6. Effect of mrvl-1 knockdown and overexpression on pathogen resistance of known longevity mutants.

RNAi knockdown of *mrvl-1* increases susceptibility of A) *clk-1(qm30)* mutants, C) *eat-2(ad1116)* mutants, and E) *daf-2(e1370)* mutants to *P. aeruginosa* infection (see Table 4.5 for details and p values). Overexpression of *mrvl-1* (*muEx430*) increases resistance

of B) *clk-1(qm30)* mutants, D) *eat-2(ad1116)* mutants, and F) *daf-2(e1370)* mutants to PA14 infection (See Table 4.6 for details and p values).

Figure 4.7. mrvl-1 RNAi causes progeria in an eat-2(ad1116) background.

A. Percentages of animals of different genotypes displaying progeric phenotypes. Scores indicate increasing deterioration with 1 representing most youthful and 4 corresponding to a very aged appearance. The Mann-Whitney test was used to carry out pair-wise comparisons between the individual groups at each time-point (* = $p<0.01$; ** = $p<0.001$; N=10-17 animals for each condition).

B. Representative *eat-2(ad1116)* animals cultured either on control bacteria or bacteria expressing dsRNA for *mrvl-1*.

Figure 4.8. mrvl-1 activity is more important when bacteria are potentially pathogenic.

Survival curves of *eat-2(ad1116)* and *mrvl-1*-overexpressing *eat-2(ad1116)* animals fed either A) live OP50 bacteria (Mantel-Cox Logrank $p<0.0001$) or B) UV-killed OP50 bacteria (Mantel-Cox Logrank $p=0.04$).

Table 4.1. Knockdown of mrvl-1 by RNAi decreases mean lifespan.

Lifespan of animals fed bacteria expressing dsRNA for *mrvl-1*. Each strain was exposed to RNAi bacteria from the time of hatching. Number of animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control animals were

fed bacteria containing an empty vector. A 25°C pulse was given to *rrf-3(pk1426)*-containing animals from the L1 to L4 stage in order to induce sterility and sensitize them to RNAi. Lifespan analysis of adult animals was performed at 20°C. % lifespan change is relative difference in mean lifespan between *mrvl-1* RNAi animals and controls. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.2. mrvl-1 RNAi knockdown causes progeria.

Individual animals were evaluated and given a "progeria score" ranging from 1, for the most youthful, to 4, for the most decrepit. Included is the number of animals of each age and genotype, as well as the range, mean, and median scores for each set.

Table 4.3. Lifespan analysis of mrvl-1 overexpression.

Lifespan of animals expressing a *mrvl-1::mrvl-1::mCherry* construct (overexpression lifespan). Number of animals refers to number of observed deaths/total number of animals in experiment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. "wt lifespan" refers to mean lifespan of corresponding non-transgenic strains. % lifespan change is relative difference in mean lifespan between *mrvl-1*-overexpressing animals and controls. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.4. Gene expression changes in C. elegans exposed to mrvl-1 RNAi.

Genes differentially expressed during *mrvl-1* RNAi knockdown. Data from six biological replicates were compiled to show genes both upregulated and downregulated upon *mrvl-1* knockdown. Some genes are represented more than once on the array platform and thus occur multiple times in our list. "Num. Sig." refers to number of biological replicates in which gene expression was significantly changed. "Avg. fold change" refers to average fold up- or down-regulation of each gene upon *mrvl-1* RNAi treatment compared to empty-vector-fed controls.

Table 4.5. mrvl-1 RNAi affects survival after PA14 pathogen exposure.

Survival of animals fed bacteria expressing dsRNA for *mrvl-1*. Each strain was exposed to RNAi bacteria for two generations before being transferred, at day 2 of adulthood, to plates seeded with the pathogenic bacterial strain PA14. Number of animals refers to number of observed deaths/total number of animals subjected to RNAi treatment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. Control animals were fed bacteria containing an empty vector for two generations before being transferred to PA14 plates at day 2 of adulthood. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.6. mrvl-1 overexpression affects survival after PA14 exposure.

Survival of animals expressing a *mrvl-1::mrvl-1::mCherry* construct (overexpression lifespan). Strains were allowed to develop on OP50-seeded plates until day 1 of

adulthood. At this point, animals were transferred to plates seeded with the pathogenic bacterial strain PA14. Number of animals refers to number of observed deaths/total number of animals in experiment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. wt survival refers to mean survival of corresponding non-transgenic strains. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.7. mrvl-1 overexpression does not extend lifespan when animals are grown on UV-killed OP50 bacteria.

Lifespan of animals expressing a *mrvl-1::mrvl-1::mCherry* construct (overexpression lifespan). Animals were grown on either live OP50 bacteria or bacteria that had been killed by 30 minutes of exposure to ultra-violet (UV) light at 500 x 100 $\mu J/cm^2$. Number of animals refers to number of observed deaths/total number of animals in experiment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. wt lifespan refers to mean lifespan of corresponding non-transgenic strains. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.8. Effect of tissue-specific overexpression of mrvl-1 on lifespan.

Lifespan of animals expressing a *mrvl-1::mrvl-1::mCherry* construct (overexpression lifespan) in a tissue-specific fashion. Number of animals refers to number of observed

deaths/total number of animals in experiment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. wt lifespan refers to mean lifespan of corresponding non-transgenic strains, in this case, either N2 or *eat-2(ad1116)*.. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Table 4.9. Epistatic analysis of mrvl-1 and known lifespan genes.

Lifespan of animals expressing a *mrvl-1::mrvl-1::mCherry* construct and exposed to specific RNAi (overexpression lifespan). Animals were fed bacteria expressing dsRNA for either *daf-16*, *hsf-1*, or *bec-1* from the time of hatching. For *pha-4* and *skn-1* knockdown, animals were grown on bacteria containing an empty vector cassette until the L4 stage. At this point, animals were switched to plates seeded with bacteria expressing dsRNA for either *pha-4* or *skn-1*. Overexpression lifespan refers to mean lifespan of strain expressing *muEx430* transgene while wt lifespan refers to mean lifespan of corresponding non-transgenic strain, in this case, either N2 or *eat-2(ad1116)*. Number of animals refers to number of observed deaths/total number of animals in experiment. The difference between these numbers represents the number of animals censored during the experiment due to bagging, crawling off plates or rupturing through the vulva. wt lifespan refers to mean lifespan of corresponding non-transgenic strains. *P* values were calculated by pair-wise comparisons to the control of the experiment by using Mantel-Cox logrank test.

Figure 4.1. RNAi knockdown of *mrvl-1* shortens lifespan and accelerates tissue aging.

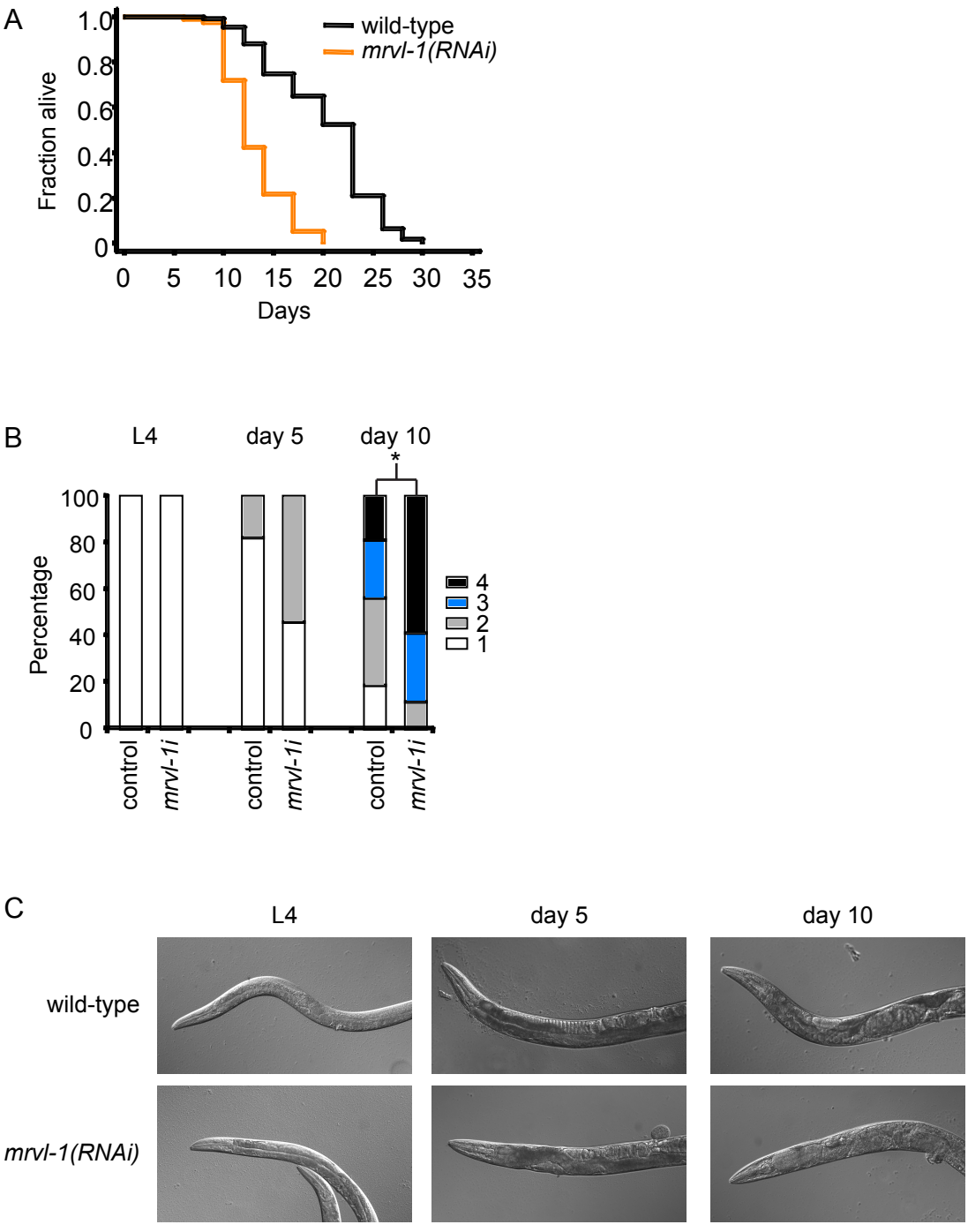

Figure 4.2. *mrvl-1* overexpression extends lifespan.

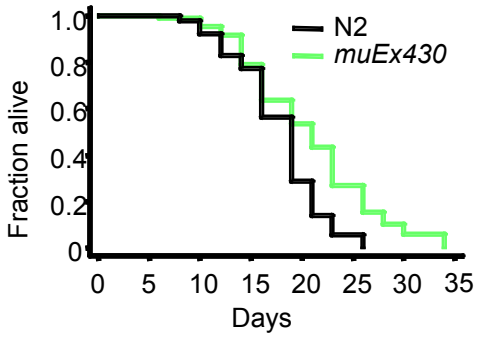

Figure 4.3. *mrvl-1* is partially localized to the Golgi apparatus

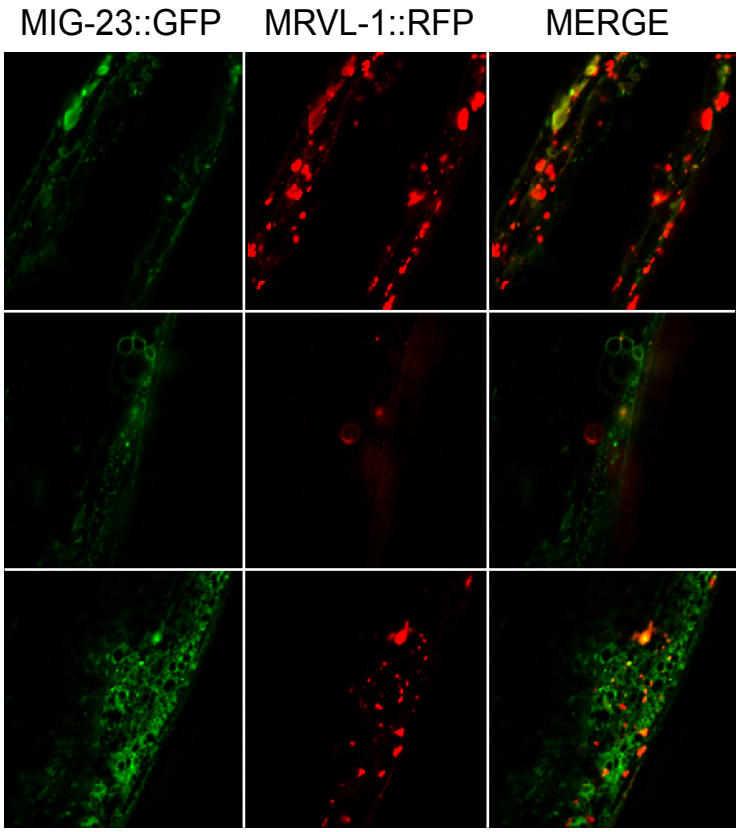

Figure 4.4. *mrvl-1* activity is important when bacteria are pathogenic

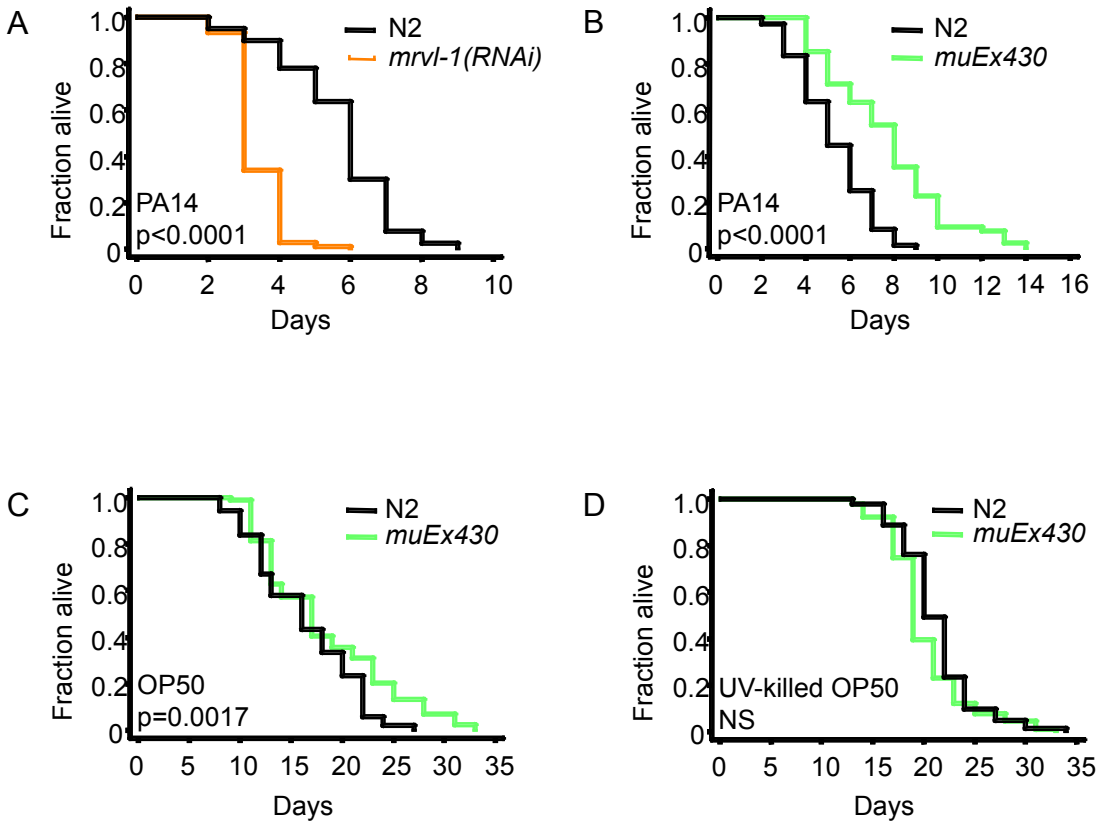

Figure 4.5. Epistatic analysis of *mrvl-1* and known longevity genes

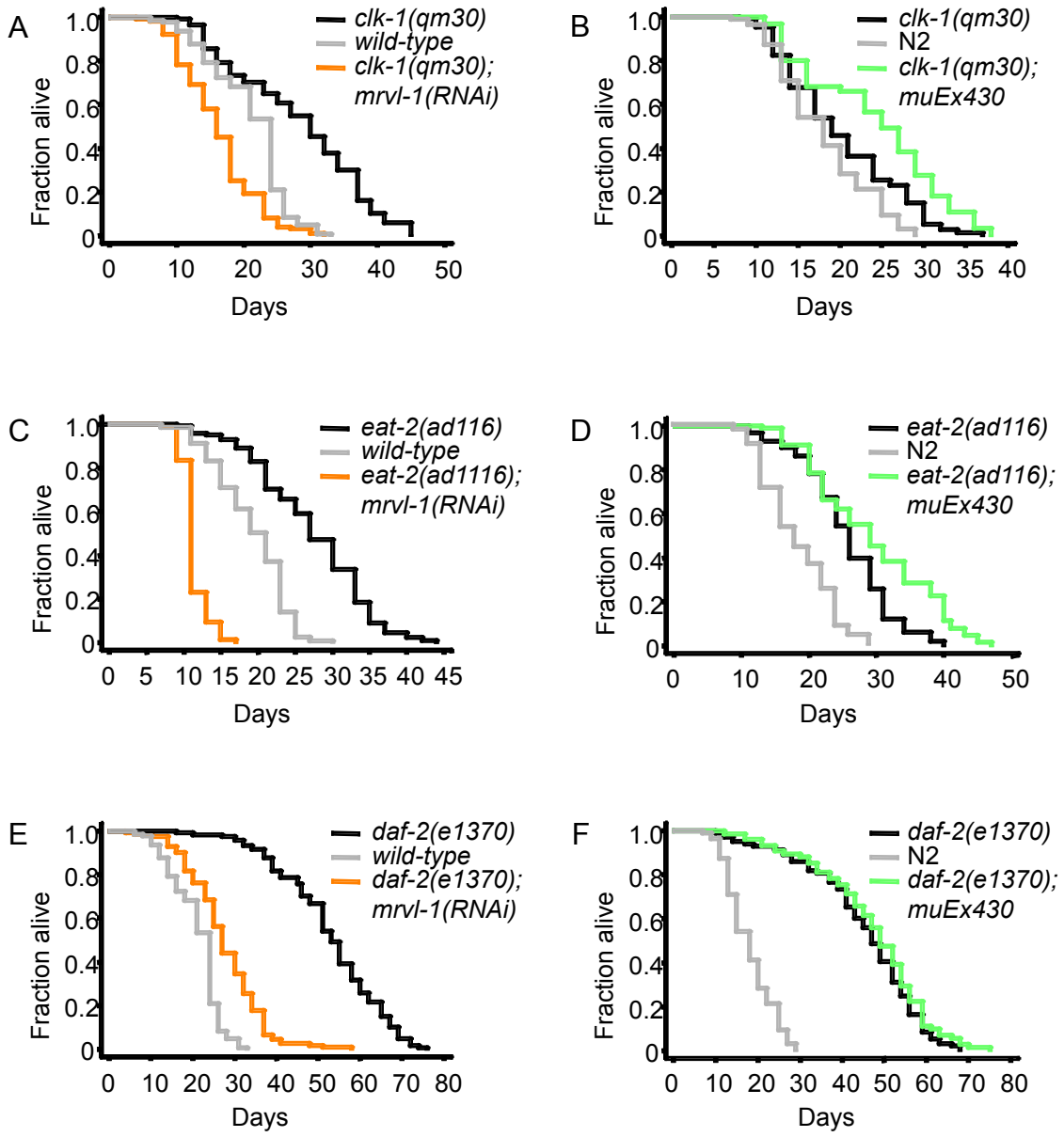

Figure 4.6. Effect of *mrvl-1* knockdown and overexpression on pathogen resistance of known longevity mutants

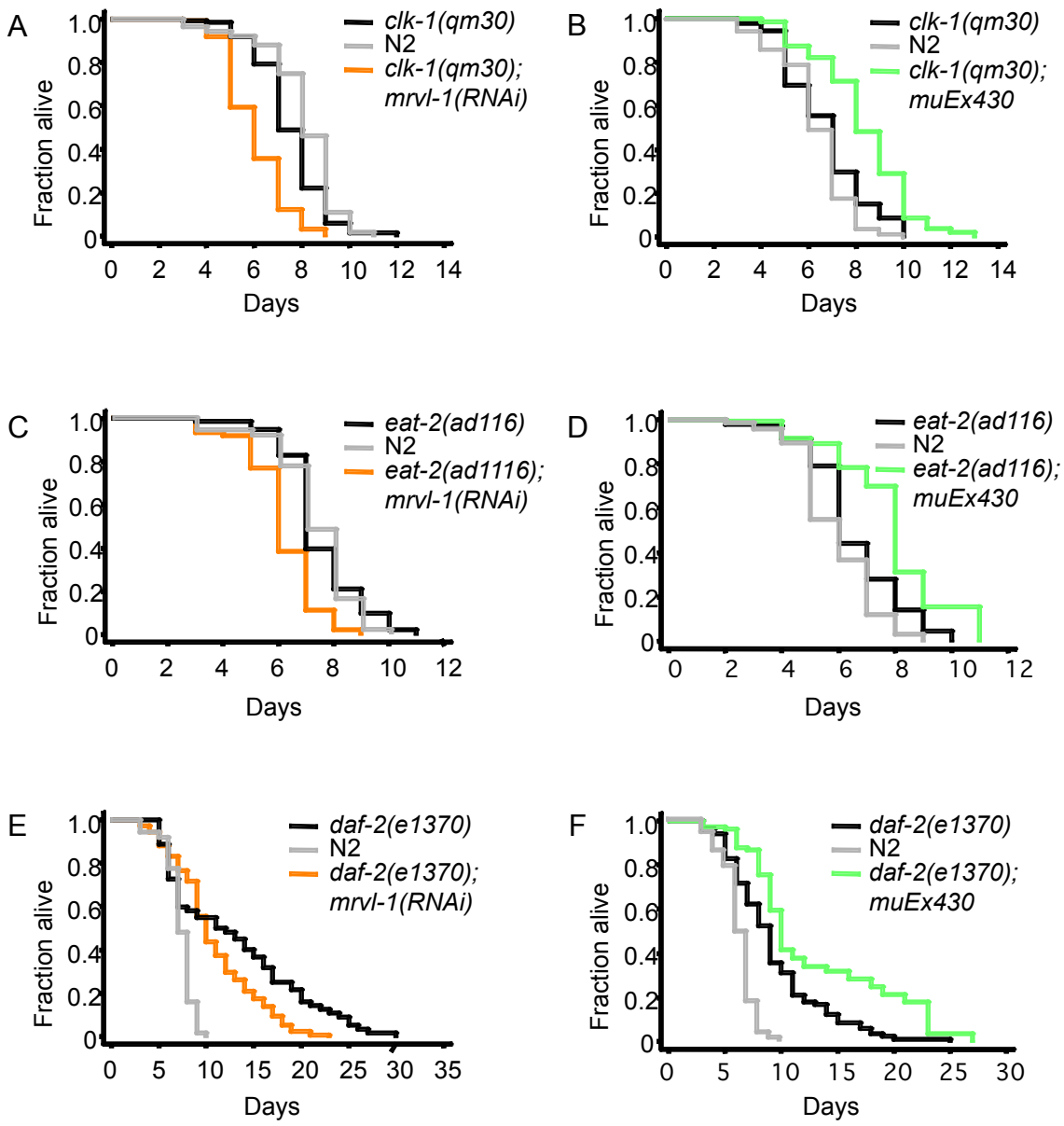

Figure 4.7. *mrvl-1* RNAi causes progeria in an *eat-2(ad1116)* background.

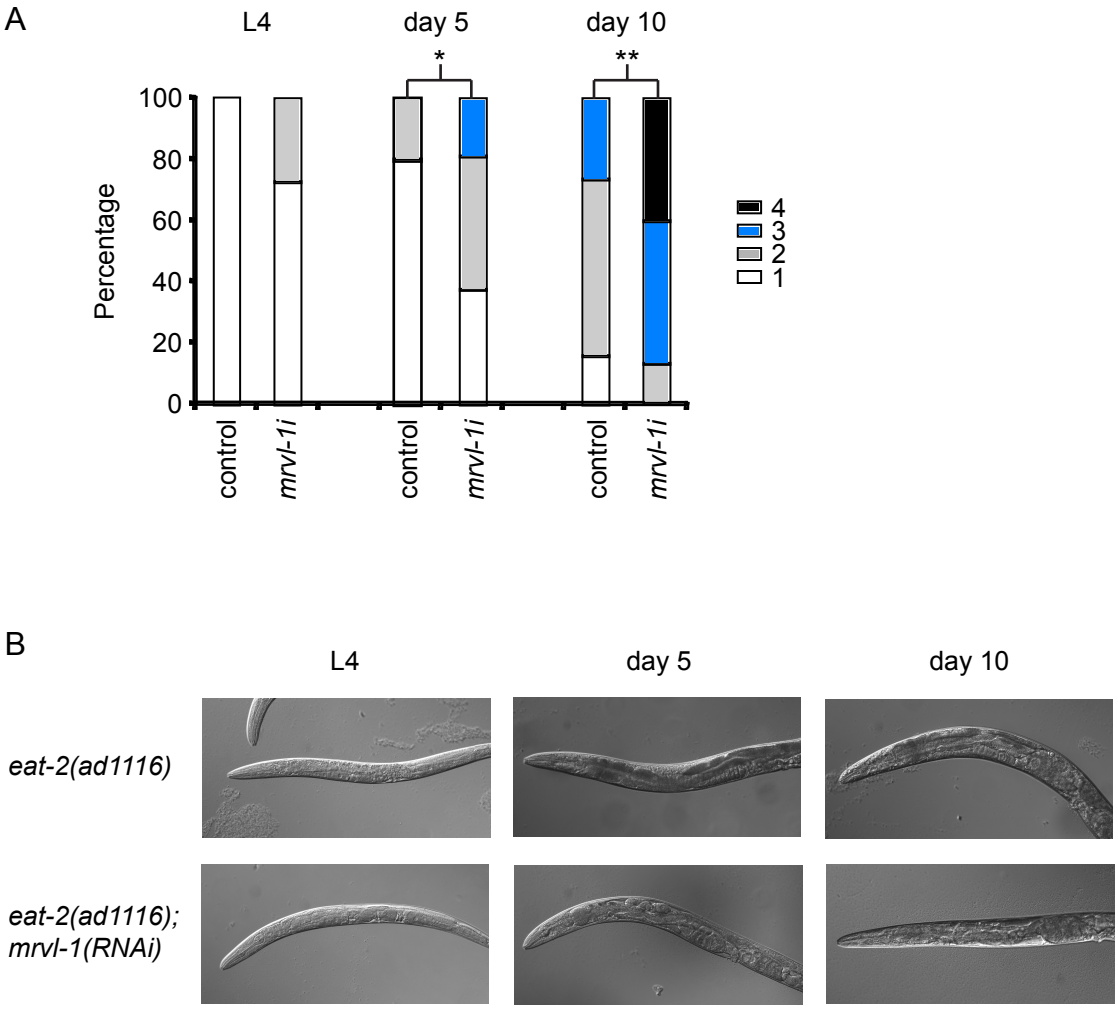

Figure 4.8. *mrvl-1* activity is more important when bacteria are potentially pathogenic.

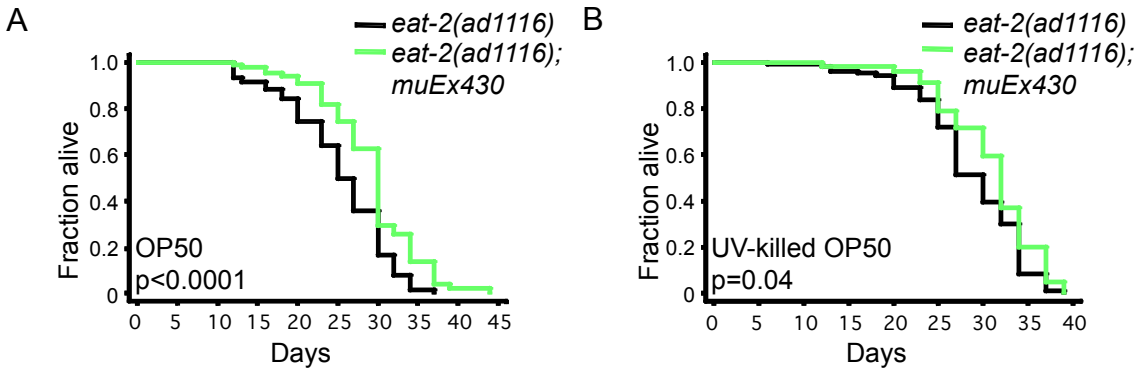

Table 4.1. Knockdown of mrvl-1 by RNAi decreases mean lifespan.

Strain	Temp.	Exp.	*mrvl-1* RNAi lifespan (days)	Number of RNAi animals	Vector-only control lifespan (days)	Number of control animals	% lifespan change	*P* value
N2	20°C	1	19.4	96/150	20.5	98/150	-5.3	0.0137
		2	13.0	78/115	20.2	125/155	-35.6	<0.0001
		3	12.6	74/150	19.2	122/150	-34.2	<0.0001
NL2099 *rrf-3(pk1426)*	20°C with 25°C pulse	4	11.6	68/75	17.1	71/74	-32.4	<0.0001
		5	11.8	88/90	20.0	83/90	-41.0	<0.0001
		6	11.7	123/150	21.0	144/150	-44.1	<0.0001
	20°C	7	20.4	131/150	21.04	120/150	-3.3	0.0061
		6	10.6	50/120	22.786	73/90	-53.3	<0.0001
CF1908 *eat-2(ad1116)*	20°C	1	20.6	80/150	23.8	94/150	-13.7	<0.0001
		2	10.9	43/94	23.0	75/94	-52.4	<0.0001
		3	11.3	77/150	27.2	91/150	-58.3	<0.0001
CF1850 *eat-2(ad1116) rrf-3(pk1426)*	20°C with 25°C pulse	4	9.0	71/75	17.7	59/75	-49.1	<0.0001
		5	7.3	77/90	25.4	77/90	-71.3	<0.0001
		6	11.1	109/150	25.5	128/150	-56.7	<0.0001
CF2485 *rrf-3(pk1426); clk-1(qm30)*	20°C with 25°C pulse	5	13.4	77/90	21.3	48/75	-37.1	<0.0001
		6	16.2	100/150	28.3	117/150	-42.6	<0.0001
CF1814 *rrf-3(pk1426); daf-2(e1370)*	20°C	7	38.4	122/150	52.5	24/36	-26.9	<0.0001
		6	27.5	107/150	52.8	114/150	-47.9	<0.0001
CF2481 *rrf-3(pk1426); glp-1(e2141)*	20°C with 25°C pulse	5	9.9	79/90	23.1	71/90	-57.0	<0.0001
		6	14.0	147/150	22.1	131/150	-36.5	<0.0001

Table 4.2. mrvl-1 RNAi knockdown causes progeria.

	wild-type				***mrvl-1i***			
Age	n=	mean	median	range	n=	mean	median	range
L4	10	1	1	1-1	17	1	1	1-1
Day 5	17	1.18	1	1-2	13	1.54	2	1-2
Day 10	16	2.44	2	1-4	17	3.47	4	2-4

	eat-2(ad1116)				***eat-2(ad1116); mrvl-1i***			
Age	n=	mean	median	range	n=	mean	median	range
L4	11	1	1	1-1	11	1.27	1	1-2
Day 5	15	1.2	2	1-2	16	1.81	2	1-3
Day 10	19	2.1	2	1-3	15	3.27	3	2-4

Table 4.3. Lifespan analysis of mrvl-1 overexpression.

Strain	overexpression lifespan (days)	Number of overexpression animals	wt lifespan (days)	Number of wt animals	% lifespan change	*P* value
CF3012 *muEx430*	21.7	78/126	18.8	86/109	15.3	<0.0001
	21.7	65/148	20.2	125/155	7.4	0.013
	24.5	82/150	19.0	102/150	29.3	<0.0001
	21.2	108/150	19.9	136/150	6.7	0.0068
	20.7	104/150	18.4	90/105	12.3	0.002
	21.2	53/150	20.1	89/120	5.4	0.0014
CF3487 *clk-1(qm30); muEx430*	24.4	62/150	19.8	74/135	23.3	0.0029
	24.2	85/150	20.1	79/150	20.3	0.0002
CF3073 *eat-2(ad1116); muEx430*	27.7	60/128	23.6	64/105	17.7	0.0005
	31.0	52/102	23.0	75/94	34.9	<0.0001
	32.6	43/140	27.2	91/150	19.7	0.0002
	29.3	52/150	26.4	112/150	10.6	0.0095
	26.6	60/150	22.7	99/150	17.0	0.0009
	29.5	72/150	25.6	101/150	15.0	0.0001
	28.3	51/150	23.5	94/150	20.3	<0.0001
	27.8	64/150	22.7	105/150	22.2	<0.0001
CF3469 *daf-2(e1370); muEx430*	52.1	81/150	49.4	125/150	5.6	0.0319
	47.4	72/150	45.3	97/150	4.7	NS
	44.4	56/120	46.0	89/135	-3.4	NS
CF3483 *glp-1(e2141); muEx430*	26.8	101/149	22.1	149/150	21.4	<0.0001
	24.3	102/150	22.8	146/150	6.9	NS

Table 4.4. Gene expression changes in C. elegans exposed to mrvl-1 RNAi.

Downregulated Genes

Gene ID	Gene Name	q-value (%)	Num. Sig.	Avg. fold change
WBGene00007872	C32H11.9	0	6	-7.11
WBGene00009710	F44G3.10	0	6	-6.98
WBGene00045457	F33H12.7	0	6	-6.70
WBGene00004172	Y75B8A.27	0	6	-6.64
WBGene00045457	F33H12.7	0	6	-6.40
WBGene00014689	C28D4.11	0	6	-6.15
WBGene00018601	F48C1.9	0	6	-5.89
WBGene00045457	F33H12.7	0	6	-5.77
WBGene00018997	F57B9.3	0	6	-5.77
WBGene00012571	Y37H2A.11	0	6	-5.75
#N/A	EB995490	0	6	-5.72
WBGene00018997	F57B9.3	0	6	-5.69
WBGene00022572	ZC239.14	0	6	-5.64
WBGene00022570	ZC239.12	0	6	-5.62
WBGene00011844	T19C9.8	0	6	-5.53
WBGene00007097	B0024.4	0	6	-5.52
WBGene00006627	C02F5.8	0	6	-5.47
#N/A	Y51A2B.1	0	6	-5.46
WBGene00022570	ZC239.12	0	6	-5.38
WBGene00006628	C02F5.11	0	6	-5.38
WBGene00008988	F20G2.5	0	6	-5.32
WBGene00015602	C08E3.10a	0	6	-5.30
#N/A	CV125252	0	6	-5.28
WBGene00015602	C08E3.10a	0	6	-5.26
WBGene00015598	C08E3.6	0	6	-5.23
WBGene00017705	F22E5.6	0	6	-5.22
WBGene00008988	F20G2.5	0	6	-5.16
WBGene00015602	C08E3.10	0	6	-5.13
WBGene00016785	C49G7.7	0	6	-5.08
WBGene00015152	B0348.2	0	6	-5.04
WBGene00008988	F20G2.5	0	6	-5.00
WBGene00007440	C08E8.4	0	6	-4.97
WBGene00005005	K04A8.8	0	6	-4.97
#N/A	EB995490	0	6	-4.93
#N/A	EB995490	0	6	-4.87
WBGene00008988	F20G2.5	0	6	-4.85
WBGene00000190	F45E4.1	0	6	-4.75
WBGene00044206	T26H5.9	0	6	-4.69
WBGene00044206	T26H5.9	0	6	-4.67

WBGene00044206	T26H5.9	0	6	-4.65
WBGene00044206	T26H5.9	0	6	-4.65
WBGene00006628	C02F5.11	0	6	-4.64
WBGene00008988	F20G2.5	0	6	-4.63
WBGene00018997	F57B9.3	0	6	-4.59
WBGene00012757	Y41C4A.11	0	6	-4.59
WBGene00044206	T26H5.9	0	6	-4.59
WBGene00045411	C25F9.11	0	6	-4.53
WBGene00045411	C25F9.11	0	6	-4.51
WBGene00003092	Y22F5A.6	0	6	-4.49
WBGene00015598	C08E3.6	0	6	-4.48
WBGene00000190	F45E4.1	0	6	-4.44
WBGene00008483	E04D5.4	0	6	-4.35
WBGene00015046	B0213.17	0	6	-4.32
WBGene00008485	F01D4.1	0	6	-4.30
WBGene00005833	C25F9.7	0	6	-4.29
WBGene00044238	C30H6.12	0	6	-4.28
WBGene00008105	C45B11.2	0	6	-4.28
WBGene00008988	F20G2.5	0	6	-4.22
WBGene00044238	C30H6.12	0	6	-4.22
#N/A	EC008547	0	6	-4.21
WBGene00009429	F35E12.5	0	6	-4.18
WBGene00012683	Y39B6A.24.2	0	6	-4.16
WBGene00044238	C30H6.12	0	6	-4.15
WBGene00000556	R09B5.3	0	6	-4.14
WBGene00000556	R09B5.3	0	6	-4.13
WBGene00000556	R09B5.3	0	6	-4.11
WBGene00012910	Y46G5A.20	0	6	-4.08
WBGene00012910	Y46G5A.20	0	6	-4.08
WBGene00014132	ZK896.1	0	6	-4.08
WBGene00020774	T24E12.5	0	6	-4.07
WBGene00000556	R09B5.3	0	6	-4.05
WBGene00000556	R09B5.3	0	6	-4.05
WBGene00009964	F53B6.9	0	6	-4.04
WBGene00012961	Y47H10A.5	0	6	-4.04
WBGene00012910	Y46G5A.20	0	6	-4.02
WBGene00020774	T24E12.5	0	6	-4.01
WBGene00011190	R10D12.9.1	0	6	-4.00
WBGene00008483	E04D5.4	0	6	-3.98
WBGene00012910	Y46G5A.20	0	6	-3.98
WBGene00020760	T24C4.4	0	6	-3.97
WBGene00012910	Y46G5A.20	0	6	-3.96
WBGene00005616	F22B8.1	0	6	-3.93

WBGene00012910	Y46G5A.20	0	6	-3.91
WBGene00011190	R10D12.9.1	0	6	-3.89
WBGene00007506	C10C5.2	0	6	-3.88
WBGene00000556	R09B5.3.2	0	6	-3.87
WBGene00044238	C30H6.12	0	6	-3.87
WBGene00015597	C08E3.5	0	6	-3.78
WBGene00020325	T07H3.2	0	6	-3.77
WBGene00005548	F33H12.5	0	6	-3.76
WBGene00015598	C08E3.6	0	6	-3.75
WBGene00016845	C50F7.5	0	6	-3.74
WBGene00011979	T24B8.5	0	6	-3.73
WBGene00044644	B0205.13	0	6	-3.72
WBGene00011979	T24B8.5	0	6	-3.71
WBGene00008105	C45B11.2	0	6	-3.70
WBGene00002090	ZK1251.2	0	6	-3.69
WBGene00009517	F38A1.4	0	6	-3.67
WBGene00044644	B0205.13	0	6	-3.67
WBGene00011979	T24B8.5	0	6	-3.63
WBGene00045415	Y43F8B.15	0	6	-3.63
WBGene00011979	T24B8.5	0	6	-3.62
WBGene00021978	Y58A7A.4	0	6	-3.61
WBGene00019744	M02H5.8	0	6	-3.60
WBGene00013900	ZC443.5.2	0	6	-3.59
WBGene00015933	C17H12.8	0	6	-3.58
WBGene00011979	T24B8.5	0	6	-3.57
WBGene00044644	B0205.13	0	6	-3.56
WBGene00011979	T24B8.5	0	6	-3.56
WBGene00020579	T19D12.4b	0	6	-3.55
WBGene00009957	F53B2.8	0	6	-3.55
WBGene00008850	F15B9.6	0	6	-3.51
WBGene00044238	C30H6.12	0	6	-3.50
WBGene00016783	C49G7.5	0	6	-3.50
WBGene00006464	T07H3.1	0	6	-3.49
#N/A	D26890	0	6	-3.48
WBGene00006429	M176.1	0	6	-3.47
WBGene00044783	T26H5.10	0	6	-3.46
WBGene00001786	F35E8.8	0	6	-3.45
WBGene00010655	K08D8.1	0	6	-3.45
#N/A	BJ781994	0	6	-3.42
WBGene00006208	Y9C9A.3	0	5	-3.41
WBGene00044349	Y71G12B.32	0	6	-3.40
WBGene00022375	Y94H6A.2	0	6	-3.39
WBGene00003734	F58E1.6a	0	6	-3.36

WBGene00000783	T10H4.12	0	6	-3.35
WBGene00009969	F53B7.7	0	6	-3.35
WBGene00009520	F38A1.7	0	6	-3.35
WBGene00000558	R09B5.9	0	6	-3.34
WBGene00015599	C08E3.7	0	6	-3.34
WBGene00015216	B0496.7	0	6	-3.34
WBGene00012959	Y47H10A.3	0	5	-3.33
WBGene00008301	C54D10.7	0	6	-3.32
WBGene00044783	T26H5.10	0	6	-3.32
#N/A	TC142805	0	6	-3.32
#N/A	EC029033	0	6	-3.27
WBGene00008632	F10A3.2	0	6	-3.24
WBGene00020579	T19D12.4c	0	6	-3.21
#N/A	BJ779622	0	6	-3.21
WBGene00010655	K08D8.1	0	6	-3.20
WBGene00007660	C17H1.7	0	6	-3.20
WBGene00044349	Y71G12B.32	0	6	-3.20
WBGene00003096	C02A12.4.1	0	6	-3.15
WBGene00019660	K11H12.4	0	6	-3.14
WBGene00044212	Y68A4A.13	0	6	-3.14
WBGene00018353	F42G2.4a	0	6	-3.13
WBGene00044783	T26H5.10	0	6	-3.12
WBGene00021121	W09G12.7	0	6	-3.12
WBGene00008602	F09B9.1	0	6	-3.11
WBGene00009431	F35E12.7	0	6	-3.10
#N/A	BJ779622	0	6	-3.09
WBGene00007875	C32H11.12	0	6	-3.09
WBGene00045067	F07C6.6	0	6	-3.09
WBGene00012069	T26H5.4	0	6	-3.08
WBGene00017308	F09F9.3	0	6	-3.08
#N/A	EC029033	0	6	-3.08
WBGene00017705	F22E5.6	0	6	-3.07
WBGene00019564	K09D9.1	0	6	-3.07
WBGene00045415	Y43F8B.15	0	6	-3.06
WBGene00006539	T04H1.9	0	6	-3.05
WBGene00021178	Y9C9A.8	0	6	-3.05
WBGene00001158	F01G10.3	0	6	-3.04
WBGene00018970	F56D6.1	0	6	-3.03
WBGene00045338	M01B2.13	0	5	-3.03
WBGene00018971	F56D6.2	0	6	-3.03
WBGene00003879	F57H12.3	0	6	-3.03
WBGene00021339	Y34F4.4	0	6	-3.02
WBGene00015599	C08E3.7	0	6	-3.02

#N/A	AV181233	0	6	-3.02
#N/A	EC007999	0	6	-3.00
WBGene00011677	T10B9.8	0	6	-2.99
WBGene00021517	Y41D4B.15	0	6	-2.99
#N/A	TC142803	0	6	-2.99
#N/A	BJ813847	0	6	-2.99
WBGene00019021	F57H12.6	0	6	-2.98
WBGene00015932	C17H12.6	0	6	-2.98
WBGene00015178	B0416.2	0	6	-2.97
WBGene00008602	F09B9.1	0	6	-2.97
WBGene00044457	C18H7.11	0	6	-2.96
WBGene00010655	K08D8.1	0	6	-2.95
WBGene00013837	ZC47.4	0	6	-2.94
WBGene00002271	R07B1.10.2	0	6	-2.94
#N/A	TC142803	0	6	-2.94
WBGene00008633	F10A3.3	0	6	-2.93
#N/A	EC029033	0	5	-2.92
WBGene00007153	B0365.6.3	0	6	-2.92
#N/A	TC142801	0	6	-2.91
WBGene00010655	K08D8.1	0	6	-2.90
WBGene00015537	C06E4.8	0	6	-2.90
WBGene00011517	T06C12.10	0	6	-2.89
WBGene00019619	K10C2.3	0	6	-2.89
WBGene00015947	C18A11.1	0	6	-2.88
WBGene00009709	F44G3.8	0	6	-2.88
WBGene00000522	C09F12.1	0	6	-2.87
WBGene00045412	C25F9.12	0	6	-2.86
WBGene00008199	C49C3.9	0	6	-2.85
WBGene00007153	B0365.6.3	0	6	-2.85
WBGene00011672	T10B9.2	0	6	-2.84
#N/A	TC146955	0	6	-2.84
WBGene00010005	F53H2.2	0	6	-2.82
WBGene00004987	T08A9.12	0	6	-2.82
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WBGene00022142	Y71G12B.2	0	6	-2.82
WBGene00016302	C32B5.9	0	6	-2.82
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#N/A	EB993401	0	6	-2.80
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WBGene00012190	W02A2.5	0	6	-2.77
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#N/A	EC008547	0	6	-2.74
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#N/A	BJ152146	0	6	-2.43
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#N/A	D26890	0	5	-2.39
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WBGene00003167	F01D4.6b	0	6	-2.36
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WBGene00017586	F19B10.4	0	6	-2.35
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WBGene00007682	C18D11.6	0	6	-2.34
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WBGene00077525	C41G7.8	0	6	-2.18
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WBGene00003702	Y70C5C.6a	0	6	-2.14
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WBGene00022847	ZK1055.6c	0	6	-2.11
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WBGene00077554	M01G12.16	0	6	-2.04

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WBGene00044120	C36B1.13	0	6	-2.01
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#N/A	NM_073448	0	5	-1.86
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#N/A	TC134297	0	4	-1.83
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#N/A	BJ152146	0	6	-1.80
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#N/A	AF304122	0	6	-1.75
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#N/A	TC149534	0	6	-1.72
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WBGene00016379	C33H5.13	0	6	-1.71
WBGene00044663	F26G1.10	0	4	-1.71
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WBGene00018725	F53A9.2	0	6	-1.67
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WBGene00019404	K05B2.4	0	6	-1.65
WBGene00008831	F14H3.12	0	6	-1.65
WBGene00018725	F53A9.2	0	6	-1.65
WBGene00044646	B0205.14	0	6	-1.65
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WBGene00044681	T24C4.8	0	6	-1.60
WBGene00009796	F46G10.1b	0	6	-1.60
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#N/A	BJ152146	0	6	-1.60
WBGene00044681	T24C4.8	0	6	-1.60
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WBGene00010851	M04C3.2	0	6	-1.58
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WBGene00044381	K10G6.5	0	6	-1.58
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WBGene00011957	T23F11.6	0	6	-1.57
WBGene00007973	C36B1.6	0	6	-1.57
WBGene00018333	F42A9.4	0	6	-1.57
WBGene00015955	C18B2.4.2	0	6	-1.57

WBGene00013119	Y51H4A.25a	0	6	-1.57
WBGene00007973	C36B1.6	0	6	-1.57
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WBGene00013009	Y48E1B.11	0	6	-1.56
WBGene00016218	C29F9.3a.1	0	6	-1.56
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#N/A	BJ152146	0	6	-1.55
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WBGene00013012	Y48E1B.15	0	6	-1.53
WBGene00018645	F49F1.5.1	0	6	-1.53
WBGene00006680	C52B9.6	0	6	-1.52
WBGene00010296	F59A1.10	0	6	-1.52
WBGene00009927	F52B11.5	0	6	-1.52
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WBGene00017727	F22H10.3	0	6	-1.51
#N/A	BJ770392	0	6	-1.51
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WBGene00012135	T28F3.9	0	6	-1.50
WBGene00022333	Y82E9BL.17	0	6	-1.50
WBGene00017727	F22H10.3	0	6	-1.50
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WBGene00009898	F49E12.2	0	6	-1.49
WBGene00012621	Y38H6C.8	0	6	-1.49
WBGene00018694	F52E1.5	0	6	-1.48
WBGene00010605	K06G5.1.2	0	6	-1.48
WBGene00003143	C34B4.1.1	0	6	-1.48
WBGene00018260	F41B5.2	0	6	-1.48
WBGene00011520	T06C12.13	0	6	-1.48
WBGene00010850	M04C3.1a	0	6	-1.47
WBGene00020449	T12B5.2	0	6	-1.47
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#N/A	AU112243	0	6	-1.45
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#N/A	BJ770392	0	6	-1.44
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#N/A	F58F9.2	0	6	-1.44
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#N/A	BJ781994	0	6	-1.43
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WBGene00008372	D1054.5	0	6	-1.40
WBGene00016218	C29F9.3b	0	6	-1.40
WBGene00018874	F55C12.7.1	0	6	-1.40
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WBGene00017417	F13B6.2	0	6	-1.39
WBGene00008644	F10C2.3	0	6	-1.39
WBGene00017093	E02C12.8c	0	6	-1.39
WBGene00012624	Y38H6C.11	0	6	-1.39
WBGene00015716	C12D12.5	0	6	-1.39
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#N/A	AU112243	0	6	-1.39
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WBGene00015716	C12D12.5	0	6	-1.39
#N/A	AU112243	0	6	-1.38
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WBGene00012554	Y37D8A.16	0	6	-1.37
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WBGene00016218	C29F9.3a.2	0	6	-1.37
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#N/A	NP008188	0	6	-1.36
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#N/A	AU112243	0	6	-1.36
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WBGene00012824	Y43F8B.14	0	6	-1.35
WBGene00045053	K12C11.7	0	6	-1.34
WBGene00002058	F25E2.4.1	0	6	-1.34
WBGene00018755	F53C3.12.1	0	6	-1.34
WBGene00018145	F37C4.5a.2	0	6	-1.34
WBGene00044654	Y61B8A.4	0	6	-1.33
WBGene00044696	F52E1.14	0	6	-1.33
WBGene00011090	R07B7.6	0	6	-1.33
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#N/A	AU112243	0	6	-1.32
WBGene00012554	Y37D8A.16	0	6	-1.32
WBGene00045053	K12C11.7	0	6	-1.32
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WBGene00018145	F37C4.5a.2	0	6	-1.31
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WBGene00003095	F58B3.3	0	6	-1.30
WBGene00013197	Y54E5A.1	0	6	-1.30
WBGene00017478	F15A8.6	0	6	-1.30
WBGene00045053	K12C11.7	0	6	-1.30
WBGene00018470	F45E4.5	0	6	-1.30
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WBGene00045392	F26D11.12	0	6	-1.29
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WBGene00008575	F08E10.7	0	4	-1.29
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WBGene00007807	C29F3.7	0	6	-1.27
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WBGene00044660	F44G3.13	0	5	-1.26
WBGene00009840	F47H4.9	0	6	-1.26
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WBGene00044696	F52E1.14	0	6	-1.26
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WBGene00015716	C12D12.5	0	6	-1.25
WBGene00003640	C06C6.5b.1	0	6	-1.25
WBGene00010127	F55G11.7	0	5	-1.25
WBGene00011090	R07B7.6	0	6	-1.25
WBGene00018470	F45E4.5	0	6	-1.25
WBGene00003640	C06C6.5b.1	0	6	-1.24
WBGene00016004	C18H9.6	0	6	-1.24
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WBGene00019674	K12C11.3	0	6	-1.24
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WBGene00016719	C46F9.3	0	6	-1.23
WBGene00022484	Y119D3B.6	0	6	-1.23
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#N/A	TC141088	0	6	-1.21
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WBGene00002273	W01A11.4	0	6	-1.21
WBGene00009394	F35C5.6.2	0	6	-1.21
WBGene00008917	F17C11.6	0	6	-1.21
WBGene00016218	C29F9.3a.1	0	6	-1.20
WBGene00003524	T23H4.3	0	6	-1.20
WBGene00018470	F45E4.5	0	6	-1.20

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WBGene00018470	F45E4.5	0	6	-1.19
WBGene00020245	T05B11.1	0	6	-1.19
WBGene00010873	M05B5.6.1	0	6	-1.19
WBGene00018470	F45E4.5	0	6	-1.19
WBGene00044073	C34H4.3	0	6	-1.18
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#N/A	D65699	0	5	-1.18
WBGene00044120	C36B1.13	0	6	-1.17
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WBGene00018470	F45E4.5	0	6	-1.16
WBGene00044607	K02A11.4	0	6	-1.16
WBGene00017093	E02C12.8c	0	6	-1.16
WBGene00009394	F35C5.6.1	0	6	-1.15
WBGene00009023	F21G4.1.3	0	6	-1.15
WBGene00017744	F23F1.3	0	6	-1.15
WBGene00017727	F22H10.3.1	0	6	-1.15
WBGene00044469	T26C12.6	0	6	-1.15
WBGene00012058	T26F2.2	0	6	-1.15
#N/A	BJ150041	0	6	-1.14
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WBGene00021556	Y45G5AM.3	0	6	-1.13
#N/A	BJ776166	0	6	-1.13
WBGene00008836	F15A2.7	0	5	-1.13
#N/A	BJ150041	0	6	-1.13
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WBGene00018346	F42C5.4	0	6	-1.12
WBGene00018384	F43C11.7	0	6	-1.12
WBGene00010852	M04C3.3	0	6	-1.12
WBGene00020329	T07H3.6	0	6	-1.11
WBGene00015946	C18A3.10	0	6	-1.11
WBGene00004859	W06B3.2a	0	6	-1.11
WBGene00010047	F54D1.6.2	0	6	-1.11
WBGene00011673	T10B9.3	0	6	-1.11
WBGene00015984	C18G1.6	0	6	-1.10

WBGene00007073	AC3.8	0	6	-1.10
WBGene00020358	T08E11.2	0	6	-1.10
WBGene00044607	K02A11.4	0	6	-1.09
WBGene00003958	F23B2.11.2	0	6	-1.09
WBGene00016717	C46F9.1	0	6	-1.09
WBGene00004859	W06B3.2d.1	0	6	-1.09
WBGene00011798	T16G1.4	0	6	-1.08
WBGene00010659	K08D8.5	0	6	-1.07
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WBGene00044478	K06B9.6	0	6	-1.07
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WBGene00003644	F36D3.2	0	6	-1.05
WBGene00022486	Y119D3B.8	0	6	-1.04
WBGene00045507	F10A3.17	0	6	-1.04
WBGene00021927	Y55F3AM.10	0	6	-1.04
WBGene00020004	R11E3.2	0	6	-1.03
WBGene00018225	F40B5.1	0	6	-1.03
WBGene00020851	T27B7.5	0	6	-1.03
WBGene00014836	T09F5.12	0	6	-1.03
WBGene00002187	T07A9.3	0	6	-1.02
WBGene00044478	K06B9.6	0	6	-1.02
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#N/A	BJ150041	0	6	-1.01
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#N/A	TC149052	0	6	-1.01
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WBGene00044607	K02A11.4	0	6	-0.99
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WBGene00008498	F01D5.8	0	6	-0.98
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WBGene00017448	F14B8.6	0	6	-0.98
WBGene00003705	T27B7.4	0	6	-0.97
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#N/A	TC141088	0	6	-0.97

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WBGene00044201	H39E23.3	0.058	5	-4.12
#N/A	TC144204	0.058	5	-4.05
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WBGene00044201	H39E23.3	0.058	5	-3.56
WBGene00001158	F01G10.3	0.058	6	-3.12
WBGene00014770	F54F3.2	0.058	5	-2.75
#N/A	A_12_P107257	0.058	5	-2.64
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WBGene00007829	C31A11.1	0.058	6	-2.33
WBGene00011831	T19B10.2.2	0.058	6	-2.28
#N/A	C55792	0.058	4	-2.15
#N/A	D34216	0.058	6	-2.14
WBGene00044656	T06E6.14	0.058	5	-2.08
WBGene00044901	Y41G9A.10	0.058	6	-2.06
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#N/A	BJ812030	0.058	6	-1.81
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WBGene00018725	F53A9.2	0.058	6	-1.76
WBGene00016660	C45B2.3	0.058	6	-1.71
WBGene00015338	C02E7.1	0.058	6	-1.68
#N/A	BJ779970	0.058	5	-1.68
WBGene00017127	E04F6.8	0.058	6	-1.65
#N/A	EC030746	0.058	5	-1.61
WBGene00015759	C14C6.5	0.058	6	-1.61
WBGene00014732	F09F3.8	0.058	5	-1.61
WBGene00017408	F12E12.7	0.058	6	-1.55
WBGene00005685	F36G9.5	0.058	5	-1.51
#N/A	BJ814048	0.058	4	-1.46
WBGene00044890	F40F12.10	0.058	6	-1.44
WBGene00011800	T16G1.6	0.058	6	-1.43
WBGene00016052	C24B9.9.1	0.058	6	-1.42
WBGene00001476	K08C7.2.2	0.058	6	-1.40
#N/A	BJ814048	0.058	6	-1.40
#N/A	EC030832	0.058	6	-1.39
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WBGene00007414	C07E3.3	0.058	6	-1.38
WBGene00010150	F56D5.6	0.058	6	-1.38
WBGene00017127	E04F6.8.2	0.058	6	-1.37
WBGene00018145	F37C4.5a.2	0.058	6	-1.32
WBGene00044381	K10G6.5	0.058	6	-1.31
#N/A	EC030832	0.058	6	-1.31
WBGene00022736	ZK418.7	0.058	6	-1.31
WBGene00016599	C42D8.1	0.058	6	-1.31
WBGene00021079	W08A12.2	0.058	6	-1.30
WBGene00044442	T08A9.13	0.058	6	-1.30
WBGene00018145	F37C4.5a.3	0.058	6	-1.29
WBGene00010475	K01F9.2	0.058	6	-1.28
WBGene00018145	F37C4.5a.2	0.058	6	-1.28
WBGene00006923	F08B1.1a.2	0.058	6	-1.28
WBGene00015382	C03B1.14	0.058	6	-1.27
WBGene00006923	F08B1.1a.2	0.058	6	-1.25
WBGene00044492	Y54G2A.49	0.058	6	-1.24
WBGene00001787	Y53F4B.33	0.058	6	-1.23
WBGene00044510	K07H8.11	0.058	5	-1.23
WBGene00018724	F53A9.1	0.058	6	-1.23
WBGene00016332	C32F10.4.2	0.058	6	-1.20
WBGene00019115	F59E11.10	0.058	6	-1.19
WBGene00009512	F37H8.3	0.058	6	-1.18
#N/A	BJ141832	0.058	5	-1.18
WBGene00022485	Y119D3B.7	0.058	6	-1.17
WBGene00003607	F33D4.1b	0.058	6	-1.16
WBGene00009394	F35C5.6.2	0.058	6	-1.16
WBGene00009394	F35C5.6.2	0.058	6	-1.16
WBGene00018470	F45E4.5	0.058	6	-1.16
WBGene00009093	F23H12.3	0.058	6	-1.15
WBGene00018931	F56B3.7	0.058	6	-1.14
WBGene00008830	F14H3.11	0.058	6	-1.13
WBGene00044488	Y54G2A.45	0.058	6	-1.13
WBGene00010659	K08D8.5	0.058	6	-1.12
WBGene00009396	F35C5.8.1	0.058	6	-1.11
WBGene00006798	F56A8.7a	0.058	6	-1.11
WBGene00015479	C05D10.3	0.058	6	-1.11
WBGene00045212	Y110A7A.21	0.058	6	-1.10
WBGene00008830	F14H3.11	0.058	6	-1.10
WBGene00014836	T09F5.12	0.058	6	-1.09
WBGene00045397	Y54G2A.52	0.058	6	-1.08
#N/A	A_12_P119038	0.058	6	-1.08
WBGene00013037	Y49E10.18	0.058	6	-1.07

WBGene00002188	ZC416.4	0.058	6	-1.07
WBGene00010659	K08D8.5	0.058	6	-1.06
WBGene00017510	F16B4.9	0.058	6	-1.06
WBGene00009839	F47H4.8	0.058	5	-1.06
WBGene00017258	F08F1.4b.2	0.058	6	-1.06
WBGene00017258	F08F1.4b.2	0.058	6	-1.05
WBGene00007392	C06H5.1	0.058	6	-1.05
#N/A	TC141088	0.058	5	-1.05
WBGene00013037	Y49E10.18	0.058	6	-1.04
WBGene00013037	Y49E10.18	0.058	6	-1.03
#N/A	BJ150041	0.058	6	-1.03
WBGene00014836	T09F5.12	0.058	6	-1.02
WBGene00022327	Y82E9BL.11	0.058	5	-1.02
WBGene00011856	T20D3.2	0.058	6	-1.02
WBGene00013037	Y49E10.18	0.058	6	-1.02
WBGene00007937	C34E11.4	0.058	6	-1.01
WBGene00004859	W06B3.2b	0.058	6	-1.00
WBGene00018600	F48C1.8	0.058	5	-1.00
#N/A	BJ150041	0.058	6	-1.00
WBGene00010899	M28.8	0.058	6	-1.00
WBGene00013037	Y49E10.18	0.058	6	-1.00
WBGene00019525	K08D9.4	0.058	6	-0.99
WBGene00017131	E04F6.15	0.058	6	-0.99
WBGene00015219	B0507.2	0.058	6	-0.98
WBGene00012026	T25E12.11	0.058	5	-0.97
WBGene00009984	F53F1.6	0.058	5	-0.97
WBGene00017258	F08F1.4b.2	0.058	6	-0.96
WBGene00016097	C25E10.8.1	0.058	6	-0.96
#N/A	TC141088	0.058	6	-0.95
WBGene00044201	H39E23.3	0.095	5	-3.83
WBGene00044201	H39E23.3	0.095	5	-3.50
WBGene00021865	Y54F10BM.11	0.095	5	-2.47
WBGene00006650	F09E10.11b	0.095	6	-2.31
WBGene00045338	M01B2.13	0.095	6	-2.28
#N/A	AU211339	0.095	6	-2.27
#N/A	EB995655	0.095	6	-2.23
#N/A	D34216	0.095	6	-2.12
WBGene00023423	F43C1.7	0.095	6	-2.03
WBGene00014173	ZK970.7	0.095	6	-1.95
WBGene00021435	Y39A3A.4	0.095	6	-1.93
WBGene00008295	C54C8.9	0.095	5	-1.86
WBGene00045009	M117.8	0.095	5	-1.86
#N/A	C55792	0.095	6	-1.79

WBGene00044646	B0205.14	0.095	6	-1.74
WBGene00004172	Y75B8A.27	0.095	6	-1.66
WBGene00015759	C14C6.5	0.095	6	-1.56
WBGene00010659	K08D8.5	0.095	6	-1.49
WBGene00022736	ZK418.7	0.095	6	-1.48
WBGene00022736	ZK418.7	0.095	6	-1.44
WBGene00009487	F36G9.11	0.095	6	-1.44
WBGene00015617	C08G5.5	0.095	6	-1.40
WBGene00017127	E04F6.8.2	0.095	6	-1.40
WBGene00012668	Y39B6A.5	0.095	6	-1.40
WBGene00021164	Y5H2B.1	0.095	6	-1.39
WBGene00008992	F21A3.2	0.095	6	-1.39
WBGene00044442	T08A9.13	0.095	5	-1.38
WBGene00011800	T16G1.6	0.095	5	-1.35
WBGene00022382	Y94H6A.10	0.095	6	-1.33
WBGene00015295	C01C10.3	0.095	6	-1.31
WBGene00018706	F52F10.2	0.095	5	-1.30
WBGene00010659	K08D8.5	0.095	5	-1.27
WBGene00021500	Y40C7B.4	0.095	6	-1.27
#N/A	D34216	0.095	6	-1.25
WBGene00008739	F13D12.3	0.095	5	-1.22
WBGene00006923	F08B1.1a.2	0.095	6	-1.22
WBGene00006798	F56A8.7b.1	0.095	6	-1.18
WBGene00007393	C06H5.2	0.095	5	-1.18
WBGene00010086	F55B11.4.1	0.095	6	-1.17
WBGene00018237	F40F4.6	0.095	6	-1.17
WBGene00003959	Y116F11B.3.1	0.095	6	-1.16
WBGene00007864	C32H11.1	0.095	6	-1.13
WBGene00021340	Y34F4.5	0.095	6	-1.12
#N/A	TC141088	0.095	6	-1.12
WBGene00022487	Y119D3B.9	0.095	4	-1.10
WBGene00007689	C18E9.9.1	0.095	6	-1.09
WBGene00007322	C05B5.5	0.095	5	-1.07
WBGene00014836	T09F5.12	0.095	6	-1.06
WBGene00007497	C09G9.8	0.095	6	-1.06
WBGene00006987	EGAP1.3	0.095	6	-1.04
WBGene00020153	T01G6.6	0.095	6	-1.04
WBGene00021874	Y54G2A.9	0.095	6	-1.03
WBGene00010659	K08D8.5	0.095	5	-1.01
WBGene00015110	B0281.3	0.095	5	-1.00
WBGene00016097	C25E10.8.1	0.095	6	-1.00
WBGene00010328	F59C6.11	0.095	6	-1.00
WBGene00044492	Y54G2A.49	0.095	6	-1.00

WBGene00017695	F22B7.4	0.128	5	-2.65
WBGene00009226	F28G4.1	0.128	6	-2.31
WBGene00007131	B0284.1	0.128	5	-2.27
WBGene00003767	B0213.4	0.128	6	-2.02
WBGene00003769	B0213.6	0.128	6	-2.00
WBGene00003767	B0213.4	0.128	6	-2.00
#N/A	AU211339	0.128	6	-1.98
WBGene00007131	B0284.1	0.128	5	-1.98
WBGene00021999	Y59E9AR.4	0.128	5	-1.95
WBGene00003767	B0213.4	0.128	6	-1.92
WBGene00015913	C17F4.7.1	0.128	6	-1.90
WBGene00016187	C28G1.2	0.128	6	-1.86
#N/A	NM_073448	0.128	4	-1.86
WBGene00011321	T01C3.4	0.128	6	-1.76
WBGene00012728	Y39G8B.9	0.128	6	-1.73
WBGene00009914	F49H6.13	0.128	4	-1.73
#N/A	TC148136	0.128	5	-1.63
WBGene00021111	W09C3.3	0.128	5	-1.58
#N/A	BJ147796	0.128	6	-1.53
WBGene00016301	C32B5.8	0.128	5	-1.51
#N/A	D34216	0.128	5	-1.45
WBGene00001397	W06D12.3	0.128	6	-1.39
WBGene00007864	C32H11.1	0.128	5	-1.37
WBGene00020929	W02C12.2	0.128	6	-1.37
WBGene00021434	Y39A3A.3	0.128	5	-1.36
WBGene00017127	E04F6.8.2	0.128	6	-1.36
WBGene00018760	F53E10.4	0.128	6	-1.35
#N/A	EB993865	0.128	6	-1.34
WBGene00044166	C54C6.7	0.128	6	-1.34
WBGene00017659	F21C10.10.2	0.128	6	-1.34
WBGene00044442	T08A9.13	0.128	6	-1.33
WBGene00020618	T20D4.12	0.128	6	-1.33
WBGene00001397	W06D12.3	0.128	6	-1.32
WBGene00044442	T08A9.13	0.128	5	-1.31
WBGene00022314	Y77E11A.14	0.128	6	-1.30
WBGene00000372	T10B9.10	0.128	6	-1.28
WBGene00044492	Y54G2A.49	0.128	6	-1.27
WBGene00020929	W02C12.2	0.128	6	-1.27
WBGene00001397	W06D12.3	0.128	6	-1.26
WBGene00011595	T07G12.4	0.128	4	-1.22
WBGene00001397	W06D12.3	0.128	6	-1.22
WBGene00016662	C45B2.8	0.128	5	-1.22
WBGene00010658	K08D8.4c	0.128	5	-1.21

WBGene00020529	T15B7.17	0.128	6	-1.20
WBGene00007112	B0035.13	0.128	6	-1.19
WBGene00044492	Y54G2A.49	0.128	6	-1.17
WBGene00044148	T08D10.4	0.128	5	-1.17
WBGene00019261	H34I24.2	0.128	6	-1.16
WBGene00006798	F56A8.7a	0.128	6	-1.15
WBGene00044492	Y54G2A.49	0.128	6	-1.15
WBGene00019315	K02E7.11	0.128	5	-1.14
WBGene00021020	W04B5.3c.1	0.128	5	-1.14
#N/A	EB993865	0.128	5	-1.13
#N/A	TC149808	0.128	6	-1.11
WBGene00010658	K08D8.4c	0.128	5	-1.11
WBGene00004856	ZK370.2	0.128	6	-1.08
WBGene00010752	K10G4.3	0.128	5	-1.08
WBGene00008340	C55A6.11	0.128	6	-1.08
WBGene00019113	F59E11.7a	0.128	4	-1.07
WBGene00002257	W02D3.7	0.128	5	-1.07
#N/A	NP008188	0.128	6	-1.06
#N/A	CB396804	0.128	6	-1.06
WBGene00016870	C52B9.11	0.128	5	-1.06
WBGene00015110	B0281.3	0.128	5	-1.05
WBGene00014063	ZK673.9	0.128	6	-1.05
WBGene00010658	K08D8.4c	0.128	5	-1.04
WBGene00044287	F21H12.7	0.128	5	-1.03
WBGene00005141	Y22D7AR.8	0.128	5	-1.03
WBGene00016005	C18H9.8	0.128	5	-1.02
WBGene00022258	Y73C8B.1	0.128	6	-1.01
WBGene00022325	Y82E9BL.8	0.128	6	-1.01
WBGene00019525	K08D9.4	0.128	6	-1.01
#N/A	AV185061	0.128	6	-1.00
WBGene00003097	C17G10.5.2	0.128	6	-1.00
WBGene00008494	F01D5.3	0.128	5	-0.99
WBGene00021003	W03F9.9	0.128	5	-0.99
WBGene00011399	T03F6.3	0.128	6	-0.98
WBGene00017726	F22H10.2	0.128	6	-0.98
WBGene00045397	Y54G2A.52	0.128	5	-0.97
#N/A	T24H10.2	0.128	6	-0.97
WBGene00019957	R08E3.1a	0.128	6	-0.97
WBGene00044381	K10G6.5	0.128	5	-0.97
WBGene00022346	Y82E9BR.13	0.128	6	-0.96
#N/A	BJ137309	0.128	5	-0.96
WBGene00023021	F32A5.9	0.128	6	-0.96
WBGene00018274	F41C3.8	0.128	6	-0.95

Table 4.4. Gene expression changes in C. elegans exposed to mrvl-1 RNAi.

Upregulated Genes

Gene ID	Gene Name	q-value (%)	Num. Sig.	Avg. fold change
WBGene00010257	F58E6.8	0	6	3.24
WBGene00020182	T03D3.1	0	6	3.21
WBGene00044775	ZK1290.15	0	6	3.20
WBGene00000133	C05E11.4	0	6	3.17
WBGene00044775	ZK1290.15	0	6	3.09
WBGene00044775	ZK1290.15	0	6	3.07
WBGene00044775	ZK1290.15	0	6	3.01
WBGene00010257	F58E6.8	0	6	2.84
WBGene00004062	T10H9.5c.2	0	6	2.72
WBGene00004062	T10H9.5c.2	0	6	2.66
WBGene00013074	Y51A2D.5	0	6	2.56
WBGene00044775	ZK1290.15	0	6	2.52
WBGene00011846	T19H5.1	0	6	2.24
WBGene00016797	C50A2.3	0	6	2.09
WBGene00019917	R07C3.1	0	6	2.02
WBGene00020992	W03F8.2	0	6	2.02
WBGene00012680	Y39B6A.21	0	6	1.64
WBGene00010636	K07F5.8	0	6	1.63
#N/A	TC138618	0	6	1.58
WBGene00004130	F54E2.3c	0	6	1.56
WBGene00021072	W07B8.4	0	6	1.52
WBGene00004130	F54E2.3d	0	6	1.50
WBGene00010636	K07F5.8	0	6	1.41
WBGene00017680	F21F8.6	0	6	1.39
WBGene00021089	W08E12.8	0	6	1.28
WBGene00017680	F21F8.6	0	6	1.28
WBGene00008607	F09B12.3	0	6	1.27
WBGene00008125	C47A4.5	0	6	1.20
WBGene00022679	ZK180.5b.2	0	6	1.18
WBGene00000028	Y105C5A.4	0	6	1.16
WBGene00008038	C40H1.2	0	6	1.08
WBGene00021594	Y46E12BL.1	0	6	1.06
WBGene00008451	E01G6.3	0	6	1.05
WBGene00014998	AH9.1	0	6	0.99
WBGene00019421	K05F6.10	0.052	6	3.13
WBGene00019421	K05F6.10	0.052	6	2.98
WBGene00019421	K05F6.10	0.052	6	2.54
WBGene00013007	Y48E1B.8	0.052	6	2.48
WBGene00004062	T10H9.5c.1	0.052	6	2.28

WBGene00018138	F37B4.7	0.052	6	2.09
WBGene00005882	K12D9.11	0.052	5	2.09
WBGene00005954	K07C6.6	0.052	5	1.98
WBGene00013051	Y50E8A.8	0.052	4	1.73
WBGene00020528	T15B7.16	0.052	6	1.39
WBGene00018877	F55D10.1	0.052	6	1.30
WBGene00001155	T05G5.6.2	0.052	6	1.21
WBGene00004989	T08A9.8	0.052	6	1.20
WBGene00020311	T07D3.9b.1	0.052	6	1.09
WBGene00022391	Y95B8A.12	0.052	6	1.05
WBGene00014959	Y105C5A.7	0.052	6	1.03
WBGene00013575	Y76A2B.3	0.052	6	1.02
WBGene00020128	R193.2	0.052	6	0.97
WBGene00002095	C17C3.19	0.095	6	2.57
WBGene00020609	T20D4.3	0.095	6	2.27
WBGene00020326	T07H3.3	0.095	6	2.25
WBGene00016595	C42D4.2	0.095	6	2.22
WBGene00018138	F37B4.7	0.095	6	2.22
WBGene00018138	F37B4.7	0.095	6	2.09
WBGene00004062	T10H9.5b	0.095	6	2.07
WBGene00007358	C06A12.5	0.095	6	1.93
WBGene00006436	W06H8.8f	0.095	6	1.54
WBGene00006588	C14F5.3c	0.095	6	1.40
WBGene00020369	T08H10.1	0.095	6	1.32
WBGene00022679	ZK180.5c	0.095	6	1.01
WBGene00022585	ZC266.2	0.095	6	1.01
WBGene00013575	Y76A2B.3	0.095	6	0.98
WBGene00013575	Y76A2B.3	0.095	6	0.97
WBGene00015894	C17C3.12c.1	0.113	6	3.55
WBGene00010257	F58E6.8	0.113	6	3.06
WBGene00019421	K05F6.10	0.113	6	3.02
WBGene00018448	F45D11.1.2	0.113	6	2.34
WBGene00007947	C35A5.3	0.113	6	2.24
WBGene00000031	C03A7.14	0.113	4	2.20
#N/A	TC142059	0.113	6	2.17
WBGene00009824	F47G4.3	0.113	6	2.13
#N/A	F37B4.7.1	0.113	6	2.09
WBGene00007947	C35A5.3	0.113	6	2.07
WBGene00018138	F37B4.7	0.113	6	1.97
WBGene00000031	C03A7.14	0.113	4	1.94
WBGene00011340	T01G5.2	0.113	6	1.88
WBGene00006759	ZK617.1b	0.113	6	1.83
WBGene00000029	C03A7.7	0.113	6	1.81

WBGene00013073	Y51A2D.4.1	0.113	6	1.76
WBGene00011909	T22A3.6	0.113	4	1.67
WBGene00008565	F08A8.2	0.113	6	1.66
WBGene00010154	F56F3.3	0.113	4	1.58
WBGene00013138	Y53C10A.10	0.113	5	1.58
WBGene00006759	ZK617.1b	0.113	6	1.55
WBGene00007422	C08B6.1	0.113	6	1.53
WBGene00006588	C14F5.3c	0.113	6	1.52
WBGene00015860	C16D9.5	0.113	6	1.52
WBGene00011443	T04F8.4	0.113	6	1.40
WBGene00008960	F19H6.5	0.113	6	1.39
WBGene00015093	B0261.5	0.113	5	1.32
WBGene00000785	W07B8.5.2	0.113	6	1.28
WBGene00006789	F11C3.3.1	0.113	6	1.27
WBGene00006787	ZC101.2e	0.113	6	1.26
WBGene00006820	C09D1.1e	0.113	6	1.26
WBGene00004130	F54E2.3d	0.113	6	1.25
WBGene00001863	F15G9.4b	0.113	6	1.25
WBGene00008707	F11E6.3.1	0.113	6	1.25
WBGene00006925	K09F5.2	0.113	6	1.24
WBGene00045316	C49F5.9	0.113	6	1.21
WBGene00020144	T01C8.3	0.113	6	1.18
WBGene00045316	C49F5.9	0.113	6	1.17
WBGene00001155	T05G5.6.2	0.113	6	1.17
WBGene00015435	C04E12.5	0.113	6	1.16
WBGene00001068	F16F9.2	0.113	6	1.14
WBGene00017201	F07C4.7	0.113	6	1.13
WBGene00008607	F09B12.3.4	0.113	6	1.12
WBGene00000667	W05B2.6	0.113	6	1.12
WBGene00016714	C46F2.1	0.113	6	1.12
WBGene00008570	F08A10.1b	0.113	6	1.10
WBGene00007533	C12C8.2a	0.113	6	1.07
WBGene00011364	T02B5.3	0.113	6	1.04
WBGene00020022	R12C12.1b	0.113	6	1.03
#N/A	TC149977	0.113	6	0.99
WBGene00000668	W05B2.5	0.113	6	0.99
WBGene00003515	K12F2.1	0.113	6	0.99
WBGene00011436	T04F3.1	0.113	6	0.99
#N/A	K03C7.1	0.113	6	0.98
WBGene00020732	T23E7.2a	0.113	6	0.97
WBGene00017979	F32B5.6b	0.113	6	0.97
WBGene00023246	C33E10.4	0.113	6	0.96
WBGene00013575	Y76A2B.3	0.113	6	0.95

Table 4.5. mrvl-1 RNAi affects survival after PA14 pathogen exposure.

Strain	Exp.	*mrvl-1* RNAi survival (days)	Number of RNAi animals	Vector-only control survival (days)	Number of control animals	% survival change	*P* value
N2	1*	3.3	113/150	5.7	91/150	-41.6	<0.0001
	2	5.8	121/150	6.7	114/150	-13.7	<0.0001
	3	5.8	140/150	7.2	118/150	-20.6	<0.0001
	4	6.5	143/150	8.1	120/150	-19.5	<0.0001
eat-2(ad1116)	1*	4.3	88/150	4.5	69/150	-4.9	NS
	2	5.4	84/150	6.5	51/150	-17.0	<0.0001
	3	6.1	111/150	7.5	74/150	-17.8	<0.0001
clk-1(qm30)	3	6.0	130/150	7.3	136/150	-18.0	<0.0001
	4	6.0	132/150	7.5	133/150	-19.7	<0.0001
daf-2(e1370)	3	10.8	119/150	12.9	68/150	-16.5	<0.0001
	4	12.9	97/150	12.2	72/150	6.2	NS
glp-1(e2141)	3	5.2	142/150	7.1	137/150	-27.8	<0.0001
	4	5.2	135/150	6.6	147/150	-21.3	<0.0001

* These experiments were performed without FUDR

Table 4.6. mrvl-1 overexpression affects survival after PA14 exposure.

Strain	Exp.	overexpression survival (days)	Number of animals	wt survival (days)	Number of wt animals	% survival change	*P* value
CF3012 *muEx430*	1*	7.6	75/150	5.2	81/105	45.1	<0.0001
	2*	8.7	75/150	5.5	91/150	56.8	<0.0001
	3	6.7	100/150	5.9	110/150	13.0	0.0002
	4	7.4	104/144	6.2	94/136	20.0	<0.0001
	5	7.8	82/150	6.3	136/150	23.2	<0.0001
CF3073 *eat-2(ad1116); muEx430*	1*	9.7	35/150	7.7	39/150	26.0	0.0025
	2*	7.1	35/150	4.9	62/150	44.6	0.0179
	3	7.9	24/150	6.6	60/150	20.4	0.0006
CF3487 *clk-1(qm30); muEx430*	4	8.3	94/150	6.8	132/150	22.1	<0.0001
	5	8.3	108/150	6.7	130/150	23.9	<0.0001
CF3469 *daf-2(e1370); muEx430*	4	14.8	13/83	8.9	65/108	66.6	0.0001
	5	12.9	57/150	9.3	109/150	38.3	<0.0001
CF3483 *glp-1(e2141); muEx430*	4	6.7	84/150	6.9	141/150	-2.9	NS
	5	7.7	68/150	5.5	134/150	39.6	<0.0001

* These experiments were performed without FUDR

Table 4.7. mrvl-1 overexpression does not extend lifespan when animals are grown on UV-killed OP50 bacteria.

Strain	OP50 Bacteria	Lifespan (days)	Number of animals	% lifespan change: *muEx430* vs. wt	*P* value	% lifespan change: UV-killed vs. live bacteria	*P* value
N2	UV-killed	21.2	82/150	-	-	31.0	<0.0001
	Live	16.2	101/150	-	-	-	-
CF3012 *muEx430*	UV-killed	20.1	91/150	-5.0	NS	10.8	NS
	Live	18.2	91/150	12.4	0.0017	-	-
CF1908 *eat-2(ad1116)*	UV-killed	28.6	87/138	-	-	13.4	<0.0001
	Live	25.2	85/150	-	-	-	-
CF3073 *eat-2(ad1116); muEx430*	UV-killed	30.9	41/122	8.1	0.0442	6.2	NS
	Live	29.1	54/150	15.5	<0.0001	-	-

Table 4.8. Effect of tissue-specific overexpression of mrvl-1 on lifespan.

Strain	overexpression lifespan (days)	Number of animals	wt lifespan (days)	Number of wt animals	% lifespan change	*P* value	Does *mrvl-1* act in this tissue to increase LS?
muEx525 pmyo-3::mrvl-1::mCherry **(muscle)**	20.9	90/150	19.0	102/150	10.1	0.0103	yes
	19.3	87/150	18.4	90/105	4.9	NS	no
muEx528 pgrl-21::mrvl-1::mCherry **(hypodermis)**	20.1	103/150	19.0	102/150	5.9	NS	no
	16.3	67/150	18.4	90/105	-11.5	0.0014	no
muEx529 pgrl-21::mrvl-1::mCherry **(hypodermis)**	19.7	107/150	19.0	102/150	4.0	NS	no
	18.4	111/150	18.4	90/105	-0.1	NS	no
muEx574 pvit-2::mrvl-1::mCherry **(intestine)**	17.9	89/150	16.5	92/120	8.8	NS	no
muEx575 pvit-2::mrvl-1::mCherry **(intestine)**	16.7	87/150	16.5	92/120	1.5	NS	no
muEx576 pF08A8.4::mrvl-1::mCherry **(intestine)**	18.1	89/150	16.5	92/120	9.7	0.0318	yes
muEx577 pF08A8.4::mrvl-1::mCherry **(intestine)**	18.4	88/150	16.5	92/120	11.4	0.0443	yes
eat-2(ad1116); muEx525 **(muscle)**	27.7	102/150	25.6	101/150	7.9	0.027	yes
	25.5	91/150	22.7	105/150	12.1	0.007	yes
eat-2(ad1116); muEx528 **(hypodermis)**	30.4	103/150	25.6	101/150	18.6	<0.0001	yes
	26.2	79/150	22.7	105/150	15.2	0.0004	yes
eat-2(ad1116); muEx529 **(hypodermis)**	27.7	90/150	25.6	101/150	7.9	0.0266	yes
	26.7	86/150	22.7	105/150	17.7	<0.0001	yes

Table 4.9. Epistatic analysis of mrvl-1 and known lifespan genes.

Strain/RNAi	overexpression lifespan (days)	Number of animals	wt lifespan (days)	Number of wt animals	% lifespan change	*P* value	Is this gene needed for *mrvl-1* to increase LS?
wild-type background							
control RNAi	21.2	108/150	19.9	136/150	6.7	0.0068	-
	21.2	53/150	20.1	89/120	5.4	0.0014	-
daf-16 RNAi	16.5	93/150	16.3	140/150	1.2	NS	yes
	17.9	74/150	16.8	124/150	6.4	0.0275	no
hsf-1 RNAi	12.9	105/150	12.1	124/150	6.7	0.0001	no
	12.0	115/150	12.1	133/150	-0.6	NS	yes
pha-4 RNAi	15.3	119/150	15.9	136/150	-3.6	0.0118	yes
	14.4	107/150	16.0	138/153	-10.0	<0.0001	yes
skn-1 RNAi	17.8	124/150	18.9	140/150	-5.6	0.023	yes
	15.9	86/150	16.7	135/150	-4.9	<0.0001	yes
bec-1 RNAi	16.9	109/150	17.9	121/150	-5.7	0.0015	yes
	18.1	78/150	19.2	68/150	-5.5	NS	yes
***eat-2(ad1116)* background**							
control RNAi	29.3	52/150	26.4	112/150	10.6	0.0095	-
	26.6	60/150	22.7	99/150	17.0	0.0009	-
	28.3	51/150	23.5	94/150	20.3	<0.0001	-
daf-16 RNAi	27.6	40/150	23.3	111/150	18.7	<0.0001	no
	23.6	58/155	24.1	97/150	-2.2	NS	yes
	23.7	40/150	20.6	92/150	15.3	0.0025	no
hsf-1 RNAi	9.9	76/150	9.9	71/150	-0.1	NS	yes
	9.4	97/150	9.1	87/150	3.0	NS	yes
pha-4 RNAi	21.0	61/150	20.7	126/150	1.1	NS	yes
	21.6	107/155	19.6	129/150	10.0	0.0034	no
skn-1 RNAi	20.0	58/150	20.6	129/150	-2.9	NS	yes
	18.1	122/165	18.6	135/150	-2.8	NS	yes
bec-1 RNAi	21.4	109/150	20.9	126/150	2.4	NS	yes
	19.8	90/150	19.2	114/150	3.4	NS	yes

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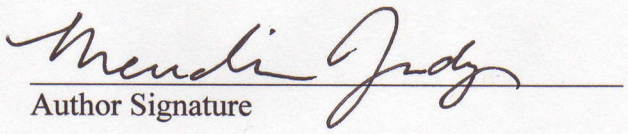

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