A novel function for MicroRNA-1: Regulation of Protein Quality Control

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The idea is to die young as late as possible.

- Ashley Montagu

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Abstract

Ageing results in a progressive decline in all organ systems throughout the body. This is particularly evident in the musculature, where it can manifest as sarcopenia, the loss of muscle mass and strength. At the molecular level, ageing is often accompanied by a decline in muscle structure and function, as well as alterations in muscle proteostasis and metabolism. MicroRNAs (miRNAs) are small 22-26 nucleotide RNAs that bind with complementarity through their seed sequence to target mRNAs to downregulate gene expression. They can work as molecular switches or fine tune gene regulation through feedback, and typically have multiple targets, thereby coordinating cellular programs. As miRNAs could serve as therapeutic targets and are shown to be regulated with age, it would be of interest to investigate whether miRNAs can influence ageing parameters. *Mir-1* is a muscle enriched microRNA highly conserved in evolution and is essential to mammalian muscle and heart function. However, elevated *mir-1* levels are associated with muscle dysfunction and disease, suggesting a possible pleiotropic effect of *mir-1* during the ageing process. *Mir-1* mouse knockouts, however, are lethal and therefore difficult to study. By contrast, *C. elegans mir-1* deletion mutants are viable, making it an ideal model organism to study the function of *mir-1* in muscle ageing.

In this work I focused on the potential role of *mir-1* in regulating muscle function, proteostasis and organismal ageing. I found that *mir-1* null mutations alleviate some of the known ageing-related symptoms: *mir-1* null animals have increased motility, reduced aggregate formation under proteotoxic challenge, and show enhanced autophagy and lysosomal function. I subsequently identified lysosomal v-ATPase subunits and *daf-16* as strong candidates for mediating the effect of *mir-1* through proteomics- and bioinformatics-based screens. Follow-up experiments revealed that *mir-1* downregulates VHA-13, a subunit of the lysosomal v-ATPase, in the muscle of *C. elegans*. It is likely that *mir-1* directly suppresses *vha-13* mRNA translation through its binding to homologous sequences in the 3´UTR. Interestingly, I also identified *mir-1*-dependent effects outside of the muscle, such as increased lysosomal acidification in the gut of *mir-1* mutants. This suggests potential non-tissue autonomous regulation by *mir-1*. In agreement with this observation I provided evidence that *mir-1* is present in the circulation of Drosophila and possibly reaches target tissues. Elucidating, whether and how *mir-1* could affect gene expression in tissues other than its site of expression could open up an interesting new field of research.

In summary, this study reveals that muscle expressed *mir-1* impacts organismal function, and implies both cell autonomous and non-autonomous control.

Zusammenfassung

Altern ist ein komplexer Prozess, bei dem die Funktion aller Organsysteme des Körpers zunehmend beeinträchtigt werden. Solche altersbedingten Funktionsverluste lassen sich in der Muskulatur besonders gut beobachten, wo sie sich als Sarkopenie, also dem vorschreitenden Abbau von Muskelmasse und dem Abnehmen der Muskelkraft, manifestieren. Auf dem zellulären und molekularen Niveau geht dies oft mit Beeinträchtigung der Muskelstruktur und -funktion einher, und auch mit der Veränderung der Eiweisshomöostase (Proteostase) und des Stoffwechsels. Trotz dieser oft umfangreichen Umgestaltung zeigt die Muskultur jedoch auch noch im hohen Alter ein hohes Maß an Plastizität und kann positiv auf Training und Belastung reagieren und sich regenerieren. Darüber hinaus sind die positiven Effekte des Muskeltrainings häufig auch in anderen Organsystemen zu sehen, was darauf hinweist das die Muskulatur eventuell auch andere Gewebe in einer nicht-zellautonomen Weise beeinflusst. Trotz dieser interessanten Rolle, welche die Muskulatur im Alterungsprozesses innehat, ist es jedoch unklar welche molekularen Mechanismen für die Veränderungen verantwortlich sind. MicroRNAs (miRNAs) sind kleine RNA Moleküle die durch Sequenzkomplementarität an mRNAs binden und dadurch die Exprimierung der entsprechenden Gene senken. miRNAs können als molekulare Schalter fungieren, haben oft mehrere Zielgene, und können diese durch Rückkopplungsmechanismen präzise regulieren, wodurch sie zur Koordination von zellulären Programmen gut geeignet sind. Da miRNAs durch Pharmazeutika reguliert werden können und ihre Exprimierung sich im Altern quantitativ verändert, wollten wir wissen ob diese Moleküle auch den Alterungsvorgang beeinflussen. *Mir-1* ist eine miRNA die in der Muskulatur angereichert ist, evolutionär konserviert ist und in Säugetieren für Muskel- und Herzfunktion unerlässlich ist. Andererseits sind erhöhte Mengen an *mir-1* mit Muskelfehlfunktionen assoziiert, was auf eine pleiotrope Wirkung von *mir-1* im Altern hinweist. Knockout-Mäuse bei denen das *mir-1* Gen fehlt, sind nicht lebensfähig, und dadurch schwer zu untersuchen. *C. elegans* Fadenwürmer mit einer *mir-1* Deletion sind jedoch lebensfähig, und sind dadurch ein gutes Model um die Rolle von *mir-1* im alternden Muskel zu studieren.

Fokus der hier vorgelegten Arbeit war es die mögliche Rolle von *mir-1* bei der Regulierung von Muskelfunktion, Proteostase und Alterungsprozessesen zu

entschlüsseln. Dabei konnte ich nachweisen, dass mehrere altersbedingten Symptome bei Fadenwürmern ohne funktionsfähiges *mir-1* reduziert zu beobachten sind. So haben diese Tiere zum Beispiel erhöhte Beweglichkeit, formen weniger Aggregate bei proteotixischem Stress, und zeigen gesteigerte autophagische und lysosomale Aktivität. Mittels Proteomanalyse und Bioinformatik konnte ich ausserdem mehrere lysosomalen v-ATPase Untereinheiten und *daf-16* als Kandidaten identifizieren, die den Effekt von *mir-1* vermitteln könnten. In Folgeexperimenten konnte ich belegen, dass *mir-1* die Menge der lysosomalen v-ATPase Untereinheit VHA-13 im Muskel von *C. elegans* reduziert. Des Weiteren konnte ich zeigen, dass diese Regulierung wahrscheinlich durch das direkte Binden von *mir-1* an Zielsequenzen in dem 3‘ untranslatiertem Bereich von vha-13 erfolgt. Interessanterweise zeigten meine Experimente auch *mir-1*-abhängige Regulierung außerhalb der Muskulatur, so zum Beispiel war erhöhte Versäuerung der Lysosomen im Darm von *mir-1* Mutanten zu messen. Dies deutet darauf hin, dass *mir-1* eventuell auch nicht-zellautonome Funktionen ausüben könnte. In Übereinstimmung mit dieser Hypothese konnte ich *mir-1* Moleküle in der Hämolymphe von *Drosophila melanogaster* nachweisen.

Zusammenfassend konnte ich belegen, dass die in der Muskulatur exprimierte *mir-1* microRNA mehrere Merkmale des Alterungsvorgangs beeinflusst, und konnte sowohl Gene identifizieren, welche diesen Effekt vermitteln könnten, als auch eine mögliche nicht-zellautonome Funktion von *mir-1* nachweisen.

Abbreviations

AD	Alzheimer´s disease
AGO 2	argonaute 2
AMPK	AMP-activated proteinkinase
Aß	amyloid-beta
ATG	autophagy-related
ATP	adenosine triphosphate
ATPase	adenosintriphosphatase
BDNF	brain-derived neutrophic factor
bp	basepair
C. elegans	*Caenorhabditis elegans*
cDNA	complementary DNA
CMA	chaperone-mediated autophagy
CR	caloric restriction
CRISPR	clustered regularly interspaced short palindromic repeats
DAF	abnormal dauer formation
DAF-2	*C. elegans* homolog of mammalian IRS-1R
DAF-16	*C. elegans* homolog of mammalian FOXO
Drosophila	*Drosophila melanogaster*
dsRNA	double-stranded RNA
ER	endoplasmatic reticulum
ETC	electron transport chain
FOXO	forkhead box class O
GFP	green fluorescent protein
HD	Huntington´s disease
HDL	high-density lipoproteins
HLH-30	helix loop helix 30
HSP-70	heat-shock protein 70
HSR	heat shock response
IGF-1	insulin-like growth factor 1
IRS-1	insulin-receptor-substrate 1
KD	Knock-down
KO	knock-out
LAMP2a	lysosome-associated membrane protein 2a
Lc3	light chain 3
LIR	LC3-interacting regions
LMP-1	LAMP (lysosome-associated membrane protein) homolog
MEF-2	myogenic transcription factor 2
mir-1	*MicroRNA-1*

miRNA	MicroRNA
mRNA	messenger RNA
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
OE	overexpression
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde DAPI 4′,6-diamidino-2-phenylindol
poly Ub	polyubiquitin
polyQ	polyglutamine
PQC	protein Quality Control
PTM	posttranslational modification
PTM	posttranslational modification
RISC	RNA-induced silencing complex
RNAi	RNA interference
RT	room temperature
RT-qPCR	quantitative real-time PCR
S. cervisiae	*Saccharomyces cervisiae*
SDS	sodium dodecyl sulfate
siRNA	small interference RNA
snoRNA	small nucleolar RNA
ssRNA	single-stranded RNA
TFEB	transcription factor EB
UB	ubiquitin
Unc	uncoordinated
UPR	unfolded protein response
UPR	unfolded protein response
UPRER	unfolded protein response endoplasmatic reticulum
UPRmt	unfolded protein response mitochondrial
UPS	ubiquitin–proteasome system
UTR	untranslated region
v-ATPase	vacuolar ATPase
VHA	Vacuolar H ATPase
XBP-1	X-box binding protein 1
XBP1s	spliced XBP1
YFP	yellow gluorescent protein
Zyx-1	zyxin-1
α	alpha
β	beta
°	degree
µ	micro

1 Introduction

1.1 Ageing

1.1.1 Human Life Expectancy

Improvements in public health and medicine have led to a remarkable increase in human life expectancy across the world. For example, global average life expectancy increased by 5.5 years between 2000 and 2016, the fastest increase since the 1960s (WHO, Global Health Observatory (GHO) data, 2018). Furthermore, by 2050, one in six people in the world will be over the age of 65 (16%), as compared to one in 11 in 2019 (9%) (World Population Prospects: The 2017 Revision, United Nations).

The down side of this achievement is that many of the elderly experience age-associated disease. The healthy life expectancy (HALE) is calculated by the WHO to be 71.6 years (WHO, Global Health Observatory (GHO) data, 2018), which gives a period of around 10 years in which the elderly person will encounter various age-related diseases. This has been accompanied by demographic changes in which an ever-greater proportion of the population is elderly (World Population Prospects: The 2015 Revision, United Nations). These developments are not only a burden for individuals and their families, but also put increasing pressure on the health care sector and governments. In particular, age-associated neurodegenerative diseases like Alzheimer´s disease (AD) currently have no viable treatment (Bulck et al. 2019). Current worldwide number of patients is estimated at 46.8 million patients living with dementia in 2015, with projected increases up to 131.5 million patients by 2050 (World Alzheimer Report 2019, Alzheimer´s Disease International). This is a serious issue that must be dealt with now, and research on ageing and dementia is essential to increase healthspan of the growing population of elderly people.

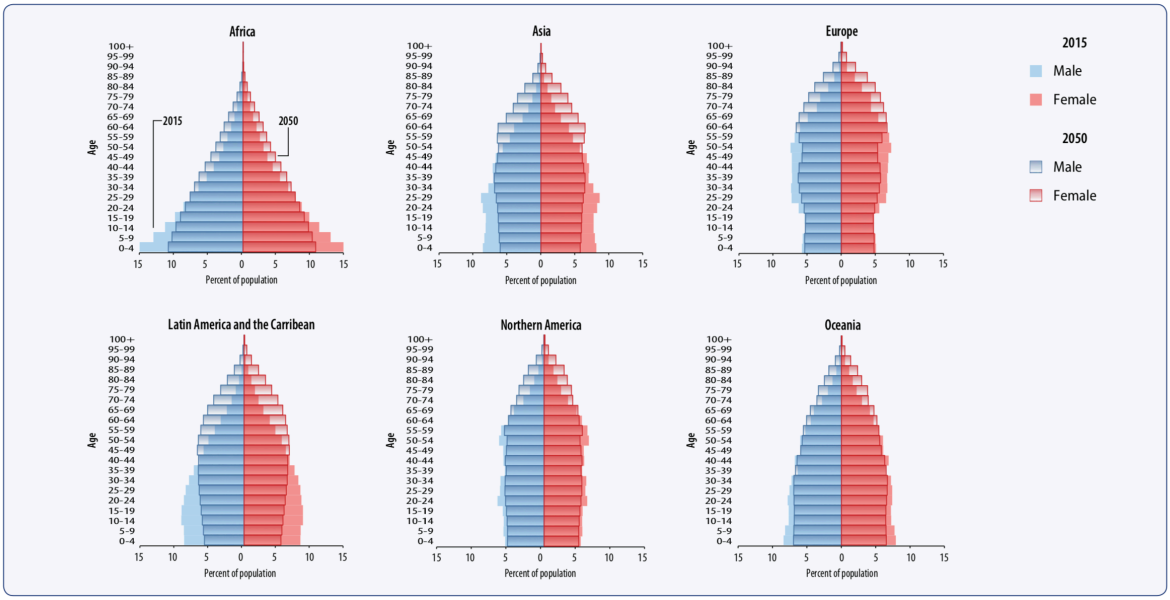


Figure 1. Global ageing will accelerate in coming decades. In 2015, 12 per cent of the global population, or 901 million people, were aged 60 or over. The number of older persons is growing at an annual rate of 3.3 per cent, faster than any other age group. Due to a projected overall reduction in fertility, population ageing will continue at high levels globally, and by 2050, 22 per cent of the total population, or 2.1 billion persons, will be aged 60 or over. Currently, Europe has the highest percentage of population aged 60 or over (24 per cent), but rapid ageing will occur in other parts of the world as well. All major areas of the world, except for Africa, will have nearly a quarter or more of their populations aged 60 or over by 2050. Data source. United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Prospects: The 2015 Revision.

1.1.2 Ageing

Ageing is a complex process and is usually defined as a gradual loss of physiological functions in combination with reduced fertility and increased mortality. Understanding the forces of why we age and how we can modulate the ageing process is an important field of research as ageing is accompanied by decreased fitness and by a strongly increased risk for severe diseases. Fitness is under evolutionary selection as it determines the changes to reproduce. However, accumulation of late-acting mutations could be under the so-called ‘selection shadow’ and are not selected against. Furthermore, pleiotropic genes that benefit organisms early in life will be favored by selection even if they might be deleterious post-reproductively (Kirkwood and Austad 2000). Even though environmental factors can influence the ageing process, I will highlight the genetic basis underlying longevity and the ageing process in the following paragraph.

López-Otín and colleagues ascribed the decline of physiological functions that occur during ageing into specific molecular hallmarks of ageing (López-Otín et al. 2013). These hallmarks include genomic instability, telomere attrition, epigenetic alterations,

loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. The underlying cause of each hallmark in particular and the ageing process in general is hypothesized to be the time-dependent accumulation of molecular damage. The interconnectedness of the hallmarks and their individual contribution to the ageing phenotype is still under investigation. Obviously, interventions that enhance health and lifespan are the most informative about the ageing process, and much of the current ageing research aims to show that amelioration of single hallmarks or the combination of hallmarks improves lifespan.

Interestingly, single-gene mutations were discovered who could stave off the ageing process and delay many of the hallmarks of ageing at once.

Figure 2. The hallmarks of ageing. These hallmarks shown in this scheme include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (López-Otín et al. 2013).

1.1.3 Insulin-IGF-signaling

One well-established example is the missense mutation in the *daf-2* insulin receptor in *C. elegans* which extended animal lifespan by 200% (Kenyon et al. 1993). Importantly, *daf-2*, not only extends life span but also delays the onset of age-associated functional decline and confers resistance to pathogens (Halaschek-Wiener et al. 2005). Later it was found that this same signalling pathway regulates life span across taxa (Clancy et al. 2001) (Tatar et al. 2001) (Blüher et al. 2013) (Bonafè et al. 2003). Interestingly, this lifespan extension by *daf-2* mutation can be fully suppressed by inhibition of the Fork head transcription factor DAF-16 (Kenyon et

al. 1993) (Ogg et al. 1997) (Larsen et al. 1995). Furthermore, several longevity pathways like mTOR and AMPK signaling impinge on DAF-16 (Robida-Stubbs et al. 2012) (Greer et al. 2008), making DAF-16 a key transcription factor that integrates different signals from these pathways to modulate aging, and longevity via shuttling from cytoplasm to nucleus. DAF-16 participates in a wide range of important cellular processes such as cell cycle arrest, apoptosis, and metabolism besides its function in stress resistance and longevity (A. D and Kc 2004). Understanding how these longevity genes orchestrate is of paramount importance to understand the process of ageing on an organismal, but also on tissue level.

1.1.4 Muscle ageing

Ageing is a segmental process and not all tissues undergo ageing at the same rate. In particular, muscle ageing is associated with gradual subcellular changes and the effect of functional loss of muscle tissue is impacting physical performance and overall health, and is observed in ageing animals across taxa. Recently, a single gene was discovered in *C. elegans*, called *unc-120*, which delays muscle ageing and thereby greatly improves health span of the worms. Interestingly, this gene does not extend maximum lifespan, uncoupling healthspan from lifespan (Mergoud, Molin, and Solari 2014).

In fact, staying active throughout life increases general health and improves healthy ageing. Good functioning of skeletal, as well as cardiac muscle, is essential for proper movement and maintaining activity during ageing. Sarcopenia is among the most prevalent age-associated muscle disease. Muscle strength slowly declines every decade until the age of 70, where after muscle strength falls off precipitously (Siparsky, Kirkendall, and Garrett 2014). One reason for this decline in muscle mass could be the general decrease of muscle proteins with ageing (Mergoud, Molin, and Solari 2014) (Ayyadevara et al. 2016). Muscle tissue undergoes constant remodeling during exercise and needs to compensate for high levels of oxidative stress as well as tissue damage. Muscle health is therefore expected to be highly reliant on processes such as protein synthesis, folding, and degradation, i.e. proteostasis (see below), and reflects a lifetime of continuous mechanical and metabolic stress. Thus, the age-dependent decline in proteostasis (as described in the "hallmarks of ageing"), unfortunately leads to increased accumulation of misfolded proteins in skeletal, as well as cardiac muscle, causing functional decline (Ayyadevara et al. 2016). It is widely accepted that physical activity and moderate exercise are essential for muscle health and healthy ageing (Harber et al. 2009) (Nascimento et al. 2019) .

By inference, exercise leads to improvements in muscle proteostasis (Campos et al. 2018) (Masiero et al. 2009) (Park 2019). Therefore, it is important to understand how to maintain muscle strength into older age, and find novel interventions to improve proteostasis and prevent protein aggregation in the ageing muscle.

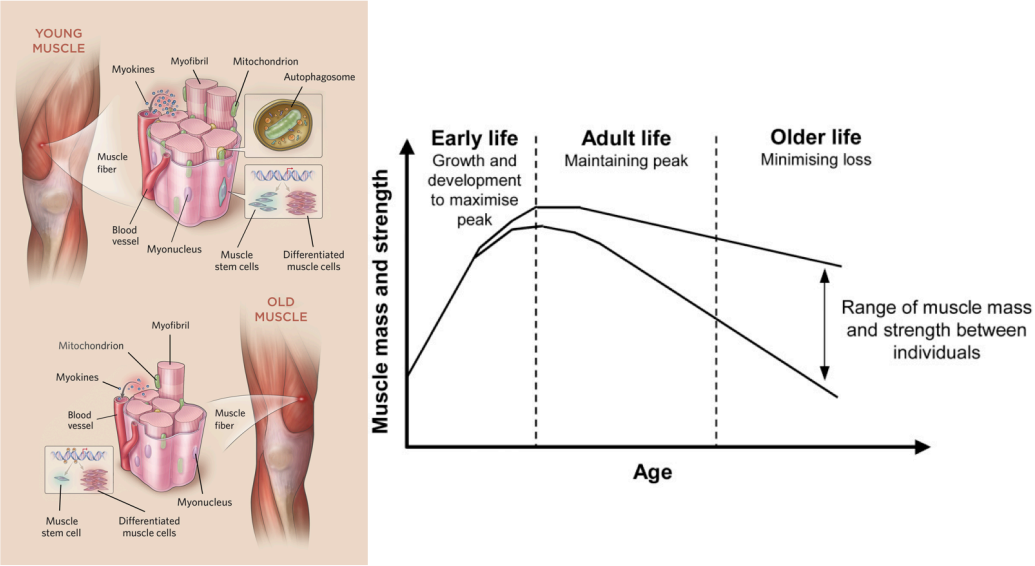


Figure 3. Muscle functional decline during ageing can be modulated by exercise. Muscle ageing is associated with a loss of muscle stem cells (satellite cells), decrease in mitochondrial number and abnormal morphology and function, lower levels of autophagy and a decrease in myokine secretion (left picture). Data source: https://myfusimotors.com/2018/12/27/how-muscle-age/. Although muscle function declines with age, muscle function can vary greatly between individuals and can be modulated by exercise (right) (Sayer et al. 2013).

1.1.5 *C. elegans* as model organism for ageing research

The genetics of ageing have proven instrumental in establishing a cause and effect relationship between gene function and life span. Short-lived model organisms such as *C. elegans*, Drosophila melanogaster, yeast, and mouse, have contributed enormously to our current understanding of the biology of ageing because we can see how genes impact longevity. The nematode *C. elegans* has proven to be aparticularly important organism to study ageing, since it provided for example, the very first single gene mutants that extend animal longevity (Klass 1977).

C. elegans is a small soil dwelling nematode established as a genetic model organism by Sydney Brenner in 1974 (Brenner 1974). It is only 1mm in length, transparent, and comprised of only 959 somatic cells, thus facilitating its cellular development to be traced in entirety from embryo to adult (Sulston et al. 1983). Moreover its neural connectivity has been determined by EM reconstruction,

providing the first connectome of any metazoan (White et al. 1986). Because of its cellular simplicity, fluorescently tagged proteins (e.g. green fluorescent protein) as vital cellular fusion proteins were pioneered in the worm by Chalfie (Chalfie et al. 1994), and are easily visualized in the live animal under the microscope. Protein localization and changes of expression over time can be monitored in high cellular precision. *C. elegans* also has facile genetics and its genome is sequenced, enabling genetic screens, transgenesis, and genome engineering. The genome of *C. elegans* contains about 22000 genes (Consortium 1998), encoding molecular pathways that are highly conserved in evolution. Remarkably, it was first discovered in *C. elegans* by Andy Fire that gene knock down can be achieved by feeding double stranded RNA expressing bacteria (RNAi) (Fire et al. 1998). Whole genome RNAi libraries (Boutros and Ahringer 2008) as well mutagenesis and genome sequencing have been used in unbiased genetic screens for various physiologic processes. Highlights include the discovery of the first genes involved in regulating apoptosis (Ellis, Jacobson, and Horvitz 1991), the systematic cloning of the genome (Coulson et al. 1986), the discovery of microRNAs (miRNAs) (R. C. Lee, Feinbaum, and Ambros 1993) and ageing. Furthermore, *C. elegans* exhibits visible signs of tissue deterioration associated with the process of ageing. These phenotypic changes, as pharyngeal sarcopenia, loss of motility, bacterial proliferation and changes of gut morphology (Herndon et al. 2002) (Chow et al. 2008) (G. D et al. 2002) (McGee et al. 2011), can be easily measured in the transparent worm. In addition to naturally occurring age-associated changes, many diseases can be modelled in *C. elegans*. Transgenic expression of human Aβ or polyglutamine stretches resembles Alzheimer´s or Huntington´s disease, respectively (Link 2001) (Morley et al. 2002) (Alexander, Marfil, and Li 2014). These fascinating aspects of *C. elegans*, together with its short lifespan (three weeks) and the fact that it can be grown in large numbers, makes it ideally suited for studying ageing.

1.1.6 Age-dependent protein misfolding diseases in humans...

Ageing is a major risk factor for disease. Neurodegenerative diseases like Alzheimer´s (AD) and Huntington´s disease (HD) are tightly linked to advanced age (Niccoli and Partridge 2012). Although progressive diseases of the nervous system, such as AD and HD have distinctive pathological symptoms, they share a key characteristic: the gradual aggregation and accumulation of misfolded proteins in the brain. In the case of HD, a gene mutation in the Huntington gene is causing the protein to misfold and aggregate. Also in AD, rare hereditary mutations can cause

the disease, which usually manifests much earlier in life than the sporadic cases (Fidani et al. 2003). Although the cause of the sporadic cases of AD are difficult to determine due to their multifactorial origin, mitochondrial dysfunction and production of oxidative stress were identified to be risk factors for the manifestation of disease (Zetterberg and Mattsson 2014).

While young individuals are more protected against the risk factors, the deregulation and decline of cellular proteostasis mechanisms in older individuals can no longer sustain a healthy proteome. As discussed in the previous chapter, the decline in defense mechanism against misfolded proteins, like autophagy, could be one of the reasons why the incidence of AD doubles every five years after 65 years of age, so that AD affects 30%–50% of all people by the age of 85 years (Isik 2010). As mentioned before, AD is an enormous psychological burden for the family of the diseased person and a financial burden for the economy.

It is therefore of crucial importance to understand the disease and find suitable interventions to treat or even better, prevent AD.

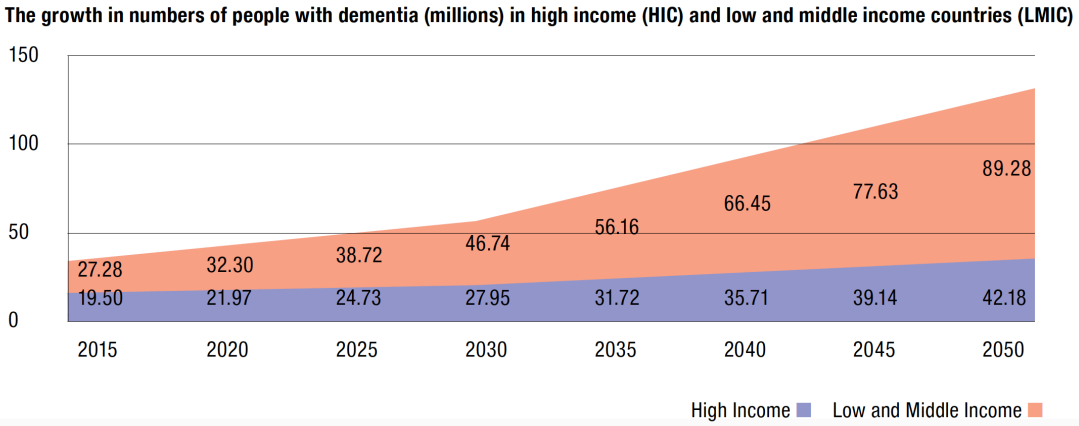


Figure 4. Strong increase in dementia in our ageing society. Prospective growth in numbers (in millions) of people with dementia in high income and low- and middle-income countries between the year 2015 and 2050. Data source: (World Alzheimer Report 2019, Alzheimer´s Disease International).

1.1.7 ... and how to model them in *C. elegans*

In 1995, Christopher D. Link cloned the human 42-amino acid ß-amyloid peptide (derived from human amyloid precursor protein cDNA) to produce muscle-specific deposits which were immunoreactive with anti-ß-amyloid polyclonal and monoclonal antibodies (Link 1995). Worms showed progressive paralysis and are used intensively in the field as a model to study modulation of proteotoxic disease. For Huntington´s disease, as well as some other diseases, it was shown that the basis for disease pathology was an extension of polyglutamine stretches. Richard I. Morimoto investigated the threshold of polyglutamine (polyQ) to cause proteotoxicity

in *C. elegans* by expressing varying numbers of polyQ stretches fused to a yellow-fluorescent protein (YFP) under different tissue-specific promotors. He observed that the number of polyQ stretches greatly influences the onset of aggregate formation. While Q82 aggregates already appear in embryos, Q35 foci appear at a much slower rate and can only be observed after 4-5 Days. Furthermore, he identified the long-lived mutant *age-1* as genetic modifier of protein aggregation (Morley et al. 2002). In the following years, delayed onset of proteotoxic aggregate formation was shown in several long-lived worm models and therefore, improved proteostasis was correlated with longevity. The intriguing advantage to use *C. elegans* as a model to study proteotoxic diseases is its short lifespan and its easy to manipulate genome. Huntington´s as well as Alheimer´s are diseases that are occurring at an advanced age when proteostasis mechanisms decline, which in worms only takes a few days. Furthermore, the beauty of RNAi feeding to get efficient gene knock-down makes *C. elegans* the perfect model organism to identify novel mediators of the proteostasis network.

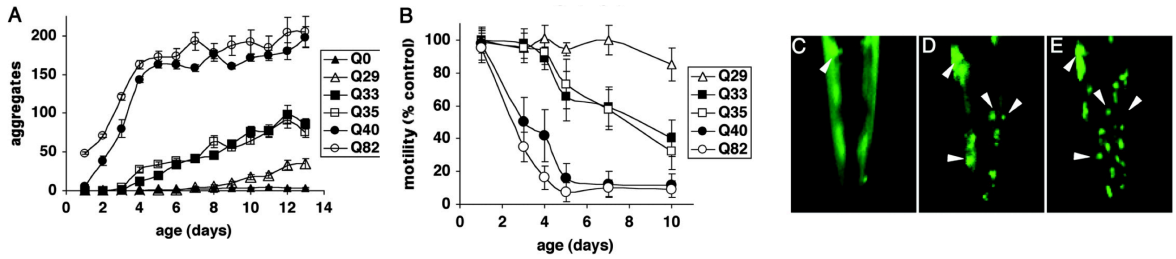


Figure 5. Influence of ageing on polyQ aggregation and toxicity. (A) Accumulation of aggregates in C. elegans expressing polyQ stretches of various length in the body wall muscle. **(B left)** Motility index as a function of age for the same cohorts of animals described in *A*. **(B right)** Epifluorescence micrographs of the head of an individual Q35 animal at 4 (*C*), 7 (*D*), and 10 (*E*) days of age, illustrating age-dependent accumulation of aggregates. Arrowheads indicate positions of the same aggregates on different days. In *E*, the animal is rotated slightly relative to its position in *D*. (adapted from (Morley et al. 2002))

1.2 MicroRNAs

1.2.1 MicroRNA Biogenesis and Function

MicroRNAs were first discovered in *C. elegans* by Victor Ambros and Gary Ruvkun. The first described microRNA (miRNA), *lin-4* (R. C. Lee, Feinbaum, and Ambros 1993), is a 22 base pair RNA that regulates the temporal patterning of seam cell lineage programs during larval development, and works by downregulating

expression of its target, the *lin-14* transcription factor by complementary binding to its 3'UTR . A few years later, the *let-7* miRNA family was identified to act, as *lin-4*, upstream of heterochronic genes to control late larval stage development (Reinhart et al. 2000).

Several years later it became apparent that *C. elegans* microRNAs *lin-4* and *let-7*, were conserved in evolution and work by a similar mechanism in other species (Lagos-Quintana et al. 2001) (Washietl et al. 2005) (Kanamoto et al. 2006) (Caygill and Johnston 2008). Furthermore, the discovery that small non-coding RNAs are widespread and essential elements of gene regulation started a novel field of research focused on unraveling the functions and impact of the non-coding genome (Lagos-Quintana et al. 2001) (Zhou, Hu, and Lai 2010) .

Protein-coding regions represent less than 2% of the total genome, and relatively little is known about the regulatory function of the remaining 98% of the non-coding genome. Among the non-coding genome, several types of small RNAs in eukaryotes have evolved to suppress translation and silence expression. Small RNAs can be divided into different subgroups, depending on their biogenesis: small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs) and microRNAs (miRNAs) (Ghildiyal and Zamore 2009). While siRNAs are derived from double-stranded (dsRNA), and piRNAs are thought to be derived from single-stranded (ssRNA) precursors, miRNAs are encoded in the genome and are transcribed by RNA polymerase II (RNA Pol II) (Y. Lee et al. 2004; Ghildiyal and Zamore 2009). More than two-thirds of all human miRNAs are encoded in intronic regions of protein-coding genes as well as in long noncoding transcripts. However, miRNAs can also be encoded in exons or in found in chromosomal regions between two genes (intergenic) (Shukla, Singh, and Barik 2011).

The miRNA precursor transcripts, called pri-miRNAs, are long and typically contain 5′ and 3′ modifications identical to those present in mRNAs (Graves and Zeng 2012). This primary transcript is processed by the RNA enzyme, Drosha, to liberate a hairpin structured precursor, the pre-miRNA, of around 60–70 nucleotides (nt) in the nucleus. Export to the cytoplasm occurs through exportin-5 where the pre-miRNA is subsequently spliced by Dicer to produce a microRNA duplex of 22 basepairs (O'Brien et al. 2018). Only one strand associates with the Argonaute protein to produce the effector RNA-induced silencing complex (RISC), whereas the other strand is discarded. Which strand is chosen to be incorporated strongly depends on the relative thermodynamic stability of the two ends of the small RNA duplex (Ui-Tei et al. 2012). The strand with a relatively unstable terminus at the 5′ side is typically selected as the guide strand. Another important determinant seems to be

the starting nucleotide of the strand (Khvorova, Reynolds, and Jayasena 2003). MiRNAs guide the RISC complex to specifically recognize complementary sequences in the 3´UTR of messenger RNA (mRNA) and downregulate gene expression by one of the two posttranscriptional mechanisms: (1) translational repression and (2) mRNA cleavage (Gu and Kay 2010). The degree of miRNA–mRNA complementarity is a major determinant of the regulatory mechanism process (Wahid et al. 2010). In animals, extensive complementarity, resulting in consequent cleavage of the targeted message, occasionally occurs but is much more unusual (Filipowicz, Bhattacharyya, and Sonenberg 2008). This makes it difficult to develop genome-wide computational tools to predict true mRNA targets without too many false predictions (Bartel 2009). One major advance in predicting miRNA targets was the discovery that the complementarity of the 2-7th nucleotide (5´to 3´) of the miRNA, the so-called seed region, to the respective mRNA substantially improves prediction reliability. This also implied that this region was of biological importance for miRNA target recognition (Lewis et al. 2003). Although this finding improved bioinformatics target-prediction tools immensely, another caveat of identifying miRNA targets is that miRNAs regulate a broad diversity of biological processes. As miRNAs more often repress translation of mRNA targets, rather than degrade target transcripts, proteomic analyses are needed to observe the true effect of miRNA regulation. The group of Nikolaus Rajewsky measured genome-wide changes in protein synthesis using SILAC and found that miRNAs can fine-tune thousands of gene products (Selbach et al. 2008). Rajewsky could confirm the importance of the seed sequence in the 3´UTR to identify potential miRNA targets and he could further show that the fold-change of the protein changes was dependent on the number of seed sequences present in the target 3´UTR. In conclusion, a single miRNA can repress the production of hundreds of proteins, but the repression is typically relatively mild (Selbach et al. 2008).

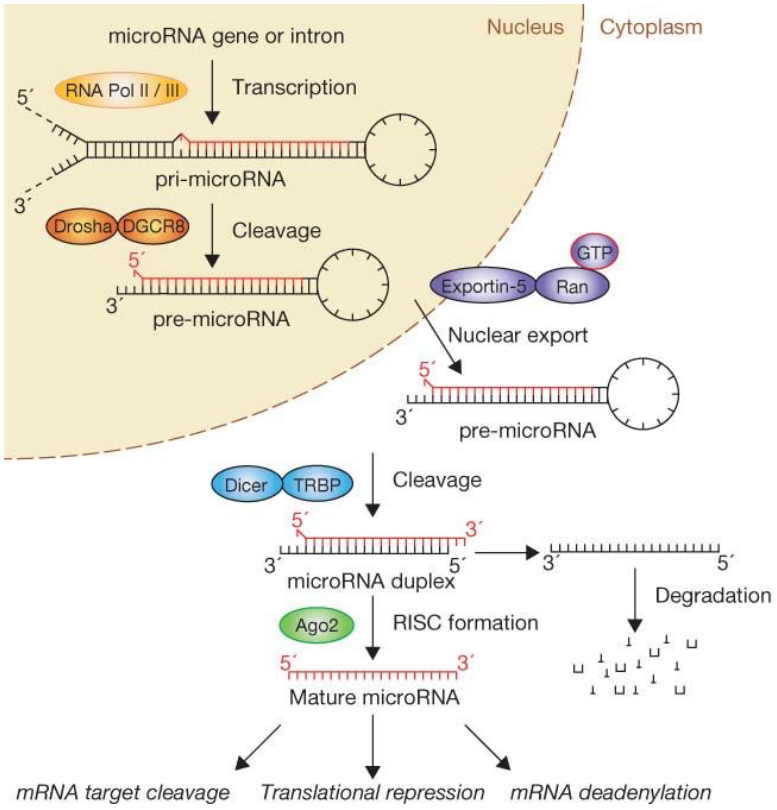


Figure 6. Schematic overview of miRNA processing. Maturation of miRNAs is initiated by the production of the primary miRNA transcript (pri-miRNA) by RNA polymerase II or III and cleavage of the pri-miRNA by Drosha–DGCR8 (Pasha) in the nucleus. The precursor hairpin structure, the so-called pre-miRNA, is then exported from the nucleus by Exportin-5–Ran-GTP. In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein TRBP cleaves the pre-miRNA hairpin to its mature length. Only one strand functions as the mature miRNA and is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage, translational repression or deadenylation, whereas the passenger strand (black) is degraded. (Winter et al. 2009).

1.2.2 MicroRNAs and Ageing

Interestingly those miRNAs having the largest effect on life span, namely *lin-4, mir-84 mir-241,* and *mir-71*, evidently regulate longevity by downregulating the insulin-signaling pathway (Boehm and Slack 2005) (Frost and Olson 2011) . Downregulation of the insulin/insulin-like growth factor 1 (IGF-1) signaling pathway is well known to promote longevity (C. J. Kenyon 2010) by activating the transcription factor *daf-16/FOXO.* In fact, several other longevity pathways, including the gonadal longevity pathway, mTOR, and intermittent fasting also impinge on daf-16/FOXO (Antebi 2007) (Yong et al. 2013) (Daitoku, Sakamaki, and Fukamizu 2011) (Becker et al. 2010) (Sengupta, Molkentin, and Yutzey 2009). Several studies in mammalians reported other miRNAs as participating in regulation of the IGF-1 pathway, for example *mir-1* (Elia et al. 2009). Although *mir-1* was not reported so far to have a

lifespan phenotype, *mir-1* targets IGF-1 itself in human cardiac and skeletal muscle in physiological as well as pathological conditions (Elia et al. 2009). Furthermore, the *mir-1* family member *mir-796* is reportedly upregulated with *C. elegans* ageing though longevity phenotypes remain uncharacterized (Kato et al. 2011).

1.2.3 mir-1

C. elegans mir-1 is a highly conserved miRNA (Tani et al. 2013) that shares complete homology with the human *mir-1* (Grad et al. 2003). Belonging to the class of so-called “myomirs”, *mir-1* is highly expressed in skeletal and heart-muscle and is of importance in muscle development (Xinran Xu 2014) and muscle structural organization (Mishima et al. 2009). Together with its family members *mir-133* and *mir-206*, *mir-1* is one of the most intensively studied myomirs, regulating fundamental processes of myogenesis, including myoblast or satellite cell proliferation and differentiation (Chen et al 2006). Accordingly, *mir-1* falls under the control of myogenic transcription factors, such as MEF-2 (Wang et al. 2018). Deregulation of *mir-1* is associated with many cardiovascular diseases ranging from arrhythmia to myocardial infarction and heart failure, and with certain types of cancer (J. Li et al. 2014). Cardiovascular diseases can be caused by decreased as well as increased levels of *mir-1*, supporting the thesis that a tight regulation of miRNA levels is crucial for normal physiological functions. Interestingly, *mir-1* is also reported to circulate after myocardial infarction, and is currently investigated to serve as biomarker of cardiovascular disease (C. Li et al. 2012), although it is still hotly debated whether circulating miRNAs are just biomarkers or substantial mediators of cardiovascular diseases (Fichtlscherer, Zeiher, and Dimmeler 2011). Identifying physiologically relevant circulating miRNAs could open a novel avenue of manipulating levels of these circulating molecules to treat disease progression. High circulating levels of *mir-1* are found not only in diseased states, but also after excessive exercise (Siracusa, Koulmann, and Banzet 2018). The proposed mechanism here is that passive muscle fiber damage releases *mir-1*. An unhealthy bout of exercise seems to dramatically increase *mir-1* levels in the muscle, which drop after 3h to 6h back to baseline levels. Interestingly, older men seem to have increased baseline *mir-1* levels in their muscles, which do not respond as rapidly to regulation by stimuli as exercise as in younger men (M. J. Drummond et al. 2008). The increased *mir-1* levels in older muscles and the general up regulation of *mir-1* after an unhealthy bout of exercise, strongly suggest that excess *mir-1* levels could have detrimental or pathological effects in adult individuals. However, as *mir-1* is crucial for heart muscle

development and *mir-1* knockout mice have severe cardiovascular abnormalities, it has proven challenging to decipher the optimal levels of this microRNA under stress, or to study the loss-of-function of *mir-1* in the ageing process. In contrast, *C. elegans*, *mir-1* knock-out worms do not show any developmental defects, making them an excellent model to study loss-of-function of *mir-1* in the adult organism.

1.3 Proteostasis

An organism's survival greatly depends on its ability to maintain a balance between the production of new and the degradation of old and potentially harmful proteins and cellular structures.

Protein homeostasis, or proteostasis, is therefore a crucial pathway that consists of pathways that control protein synthesis, folding, trafficking, aggregation, disaggregation, and degradation (Balch et al. 2009). Below I will discuss how these processes act in the maintenance of a healthy proteome.

1.3.1 Protein synthesis

Protein synthesis comprises the manufacture of polypeptide chains from amino acid building blocks on the ribosomes, and is one of the most energy intensive processes in the cell. It proceeds through three distinct phases, initiation, elongation and termination, which are tightly orchestrated through a plethora of highly coordinated factors. While mRNA translation is absolutely essential for survival under normal growth conditions and adaptation to stress, it was also shown that a modest decrease in translation rate extends lifespan in several model organisms, including yeast, Drosophila and nematodes (C. Kenyon et al. 2007) (Oakes et al. 2008) (Syntichaki, Troulinaki, and Tavernarakis 2007) (Katewa et al. 2009). Several theories have been proposed to explain these paradoxical findings. Rapid biosynthesis is accompanied by the production of non-functional proteins due to translational errors or mistakes in protein folding. Accumulation of damaged proteins is one of the main factors that causes cellular dysfunction and ageing. Therefore, by downregulating global protein biosynthesis, the load of misfolded proteins decreases which could lead to less toxic protein aggregates in the cell (Hipkiss 2007). In this context, the protein quality control mechanisms play a crucial role, which is known to decrease with age (López-Otín et al. 2013). Therefore, a decreased load of newly synthesized proteins would allow for improved protein quality control of existing proteins. Another possible theory is that protein synthesis is one of the most energy-consuming processes in the cell. Reduction of translation could therefore increase

overall energy availability. These resources could then be used for cellular maintenance and repair processes, promoting animal lifespan (C. Kenyon et al. 2007). Alternately, downregulation of translation can cause proteomic remodeling and selective expression of stress factors, which may help cells deal with multifactorial stress. Clearly these hypotheses are not mutually exclusive and could be at work simultaneously.

1.3.2 Protein degradation

Rapid protein synthesis can lead to misfolding of nascent proteins or introduce errors of translation that destabilize proteins (D. A. Drummond and Wilke 2010). Therefore, it is essential to have elaborate mechanisms that can re-fold or eliminate those proteins. Re-folding of proteins is assisted by chaperones. One well-studied example of ER resident chaperones involved in the correct folding of nascent peptides is the highly expressed heat-shock-protein 70 (HSP70) binding immunoglobulin protein (BiP/HSP-4) (Gething 1999). However, BiP is also a stress-induced master regulator of the unfolded stress response (UPR), a program designed to relieve ER stress by promoting the correct folding of peptides resident in the ER lumen (Y. Ma and Hendershot 2004).

However, when proteins become toxic aggregates, they need to be eliminated. The two major proteolytic pathways that degrade most cellular proteins in eukaryotic cells are: the ubiquitin–proteasome system (UPS) and autophagy.

1.3.2.1 Ubiquitin–proteasome system (UPS)

The UPS catalyzes the majority (around 80%) of protein degradation and is therefore absolutely required to assure a healthy proteome and cell survival (Collins and Goldberg 2017). To be destined for turnover by this pathway, proteins must be modified by polyubiquitin (polyUb) chains at lysine residues (Finley 2009). This polyubiquitylation of substrate proteins is mediated by an enzymatic cascade that involves the sequential actions of Ub-activating (E1), Ub-conjugating (E2), and Ub-protein ligase (E3) enzymes (Hershko 1998). The next steps in the degradation process involve the 26S proteasome, which consist of a barrel-shaped 20S core particle, capped on one or both ends by the 19S regulatory particle (Budenholzer et al. 2017) (Richly et al. 2005). The 26S proteasome is transcriptionally induced upon stress and its proteolytic activity and substrate specificity is highly regulated by post-translational modifications (Collins and Goldberg 2017) (Livneh et al. 2016).

In contrast to the former concept of separate proteolytic pathways there is growing evidence that there is crosstalk and interplay between UPS and autophagy (Korolchuk, Menzies, and Rubinsztein 2010) (Liebl and Hoppe 2016). Although it is widely accepted that UPS is the major degradation route for small and short-lived proteins (Schreiber and Peter 2014), whereas autophagy degrades mainly large proteins as well as aggregates (Ravikumar B., Duden R. 2002), the key regulatory factors that target selected substrates to one or the other proteolytic remain largely unknown. Ubiquitylation plays a major role in both degradation pathways and it has been reported that factors that direct a substrate toward a particular route of degradation might include ubiquitin chain length and linkage type (Clague and Urbé 2010). Interestingly, it has been shown that autophagy can even degrade proteasomes in response to starvation (Cuervo et al. 1995).

Although there is growing evidence that there is a crosstalk between both proteolytic pathways, I will concentrate on autophagy and especially the lysosome in the following sections and in my PhD work.

1.3.2.2 Autophagy

Christian de Duve was the first to discover lysosomes around 60 years ago. This discovery was, as many ground-breaking discoveries, an accidental but astonishing discovery while trying to purify his enzyme of interest. De Duve was interested in studying insulin signaling by investigating the in vitro function of hexose phosphatase. His enormous efforts of optimizing fractionation methods led him to the discovery that 95% of glucose-6-phosphatase was associated with the microsomal fraction. This finding led him to the assumption that enzymes have unique subcellular locations (Bowers 1998). More astonishing was the observation of another enzyme, again a serendipitous event. De Duve observed a low activity of acid phosphatase in all fractions he purified and placed it in the fridge for later experiments, only to observe a markedly increase in activity of the enzyme a few days later. Within a relatively short time, de Duve and his coworkers obtained solid evidence that the latency of acid phosphatases was due to a sequestration of the enzyme behind a membrane barrier which prevented access of the substrate. De Duve and his colleagues named these new particles lysosomes (de Duve et al. 1955).

However, autophagy did not get much attention as a cellular process for almost 30 years. A breakthrough in autophagy research came when Yoshinori Ohsumi conducted a laborious genetic screen in yeast to dissect the process (Tsukada 1993). He identified 15 autophagy-related proteins (ATG), which are essential to

protect yeast cell against nitrogen starvation and was awarded, as de Duve, a Nobel price.

In the early years of autophagy research, it was shown that autophagy is inhibited by insulin (Pfeifer 1977) and by amino acids (Mortimore, GE, Schworer 1977). These findings together with the groundbreaking observation that the drug rapamycin, inhibitor of TOR, induces autophagy (Blommaart, E.F., Luiken, J.J., Blommaart, P.J., van Woerkom, G.M. & Meijer 1995) confirmed our understanding of autophagy as a catabolic, energy-generating mechanism.

In the past years, intensive research in the field has uncovered the importance of autophagy in health and several diseases, including tumors, neurodegeneration and cardiomyopathies (Levine, Kroemer, and Roussy 2008). Autophagy is a vital process to maintain nutrient availability for the cell under stressful situations, like nutrient starvation. Initially, it was assumed that the cell would non-selectively 'self-eat' cellular components for survival. Only recently, has it become evident that autophagy can also selectively eliminate potentially harmful cytosolic materials, like misfolded proteins, and thereby acts as an important pathway to maintain proteostasis (Ravikumar et al. 2004) (Komatsu et al. 2007) (Levine, Kroemer, and Roussy 2008).

There are three distinct mechanisms of autophagy:

Macroautophagy (hereafter referred to as autophagy) is the process in which cytosolic components, big aggregates, parts of organelles or whole organelles are enclosed by membranes, the so called autophagsosome, and delivered to the lysosome for their degradation by lysosomal enzymes. Early studies already suggested that the lipid membrane used for autophagosome formation is taken from preexisting membranes as autophagosomes can even be formed when protein synthesis is almost completely inhibited (Hwang et al. 1974) (Locke and Sykes 1975). Recently, it has been established that ER, the Golgi apparatus, mitochondria and even the plasma membrane can serve as sources for the autophagosomal membrane (Locke and Sykes 1975) (Hayashi-Nishino et al. 2009) (Axe et al. 2008) (Hailey et al. 2010) (Ravikumar et al. 2010). The understanding of how various autophagy substrates are recruited or selectively sequestered by autophagosomes has developed rapidly (Stolz, Ernst, and Dikic 2014). Whereas the autophagy response to starvation is bulk degradation of cytosolic material, other types of stresses can trigger selective autophagy, whose selectivity is achieved trough autophagy receptors. These highly conserved receptors recognise on the one hand the cargo tagged with degradation signals and on the other hand the autophagosomal membrane through their LC3-interacting regions (LIR) (Rawet

Slobodkin and Elazar 2013) (Meijer et al. 2007) (Kirkin, Lamark, et al. 2009) (Behrends et al. 2010).

The most prevalent degradation signal in mammals is the modification of cargos with Ubiquitin (UB) (Kirkin, McEwan, et al. 2009). In agreement with Ubiquitin being important for cargo recognition in autophagy, it has been reported that most of the currently known autophagy receptors harbour UB-binding domains (Wild, McEwan, and Dikic 2014). Once the autophagosome is sealed, it can fuse with the lysosome to enable degradation of its cargo (Liou et al. 1997) (Berg et al. 1998).

The disintegrated cargo is then shuttled to the cytosol to be available as building blocks for nucleic acids, proteins and lipids (Yang, Z., Klionsky 2009).

The lysosome itself can also directly degrade cellular components. This process is called microautophagy and describes the engulfment of cytosolic components by invagination of the lysosomal membrane (De Duve 1966) (Kunz, Schwarz, and Mayer 2004). Besides macro- and microautophagy, there is also a third form of autophagy.

Chaperone-mediated autophagy (CMA) differs from the other forms of autophagy in both the way in which cargo proteins are recognized for lysosomal delivery and the way in which these proteins reach the lysosomal lumen. Cytosolic chaperones recognize a pentapeptide motif and unfold their substrates. Lysosomal chaperones are required to pull the unfolded protein inside the lumen of the lysosome, where it is degraded. A critical component for CMA is a receptor in the lysosomal membrane, the lysosome-associated membrane protein (LAMP) type 2A, to which the protein substrates bind (Majeski and Fred Dice 2004) (Dice 2007) (Cuervo and Wong et al. 2011).

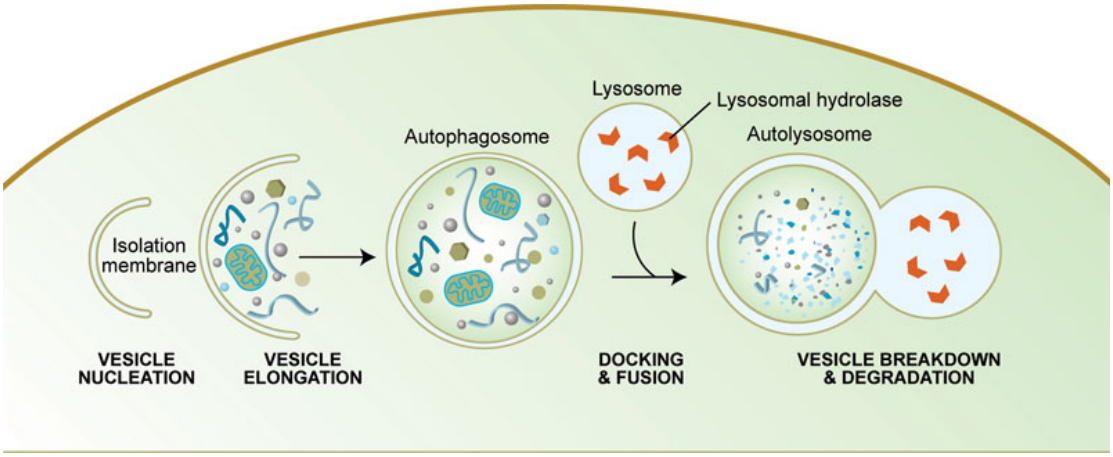


Figure 7. Schematic overview of the steps of autophagy. Autophagy is initiated by the formation of the phagophore (Vesicle nucleation). Expansion of the phagophore (Vesicle elongation) is a highly coordinated process which happens at the phagophore assembly site (PAS). The forming complex can engulf bulk cytoplasm nonspecifically, including

entire organelles, or target cargos specifically. Once the phagophore membrane is closed, it is called the autophagosome. When the outer membrane of the autophagosome fuses with an endosome (forming an amphisome before fusing with the lysosome) or directly with a lysosome (docking and fusion steps), it forms an autophagolysosome. Finally, the sequestered material is degraded inside the autophagolyosome (vesicle breakdown and degradation) and recycled. (wormbook.org)

1.3.2.3 The lysosome and v-ATPases

The key to substrate degradation in all three forms of autophagy is the acidic environment of the lysosome. The lysosome contains about 50 different degradative enzymes, all of them being acid hydrolases, that can hydrolyze proteins, DNA, RNA, polysaccharides and lipids (Pu et al. 2016). The acid hydrolases are inactive at a neutral pH (about 7.2) which is important to protect against uncontrolled digestion of the contents of the cytosol when the lysosomal membrane would break down (Alberts et al 2002). To maintain an acidic pH (about pH 5) for proper functioning of the acid hydrolases, lysosomes must actively concentrate H^+ ions (Huynh and Grinstein 2007). This is accomplished by a proton pump called the vacuolar ATPase, which hydrolyzes ATP to maintain the hundredfold higher H^+ concentration inside the lysosome (Mellman, I., Fuchs R. 1986).

V-ATPases are large multi-subunit complexes, composed of an ATP-hydrolytic domain (V_1) and a proton translocation domain (V_0), and operate by a rotary mechanism (Nishi and Forgac 2002). Electron microscopy image analysis has provided a general outline for the structural organization of the v-ATPase (Muench et al. 2009) (Wilkens, Zhang, and Zheng 2005) (Diepholz et al. 2008). The v-ATPase consists in total of 14 subunits. V_1 is composed of subunits A-H and is responsible for ATP hydrolysis. The V_0 domain is composed of subunits a, d, e, c, and c´ and carries out the proton transport (Cotter et al. 2015).

However, the v-ATPase is far more than just a proton pump and is involved in the regulation of numerous pathways.

Besides its function in protein degradation, lysosomes are also important for receptor recycling and endosomal trafficking (Scott et al 2010) (Huotari J. 2011). It has been shown that the acidic pH of the lysosome is important for processing of pro-hormones, like insulin, gastrin and thyroxine, to their biologically active form (Fisher and Scheller 1988). In specialized cells, the v-ATPase can confer certain important features of the cells, like maintaining a neutral cytoplasmic pH in neutrophils and macrophages (Brisseau et al. 1996) or neurotransmitter release into the synaptic space in neurons (Moriyama, Maeda, and Futai 1992).

Furthermore, the v-ATPase is more and more considered to serve as important metabolic sensor as it is shown that mTOR, which is activated on the lysosomal membrane, needs the activity of the v-ATPase for its activation (Zoncu et al. 2011). Furthermore, it has been shown that the v-ATPase can sense amino acid availability (Zoncu et al. 2011). In addition, the a subunit of V-ATPase also interacts with 1-phosphofructokinase, an enzyme that catalyzes the rate-limiting step in glycolysis (Su et al. 2003) (Su et al. 2008)

In agreement with its important role in multiple, essential processes, the v-ATPase is also reported to impact lifespan. Lysosomal pH decreases with age and this decrease was directly associated with mitochondrial dysfunction and shortened lifespan in yeast (Hughes and Gottschling 2012). Moreover, it was shown that lysosomes and mitochondria physically interact with other, possibly to exchange lipids and other nutrients (Elbaz-Alon et al. 2014) (Hönscher et al. 2014).

In *C. elegans*, one regulatory subunit of the v-ATPase, *vha-13*, was described to play an important role in germ line rejuvenation (Adam Bohnert and Kenyon 2017). Furthermore, the lysosomal acid lipase LIPL-4 is highly expressed under conditions associated to extended lifespan in *C .elegans* (Lapierre et al. 2011), and overexpression of LIPL-4 itself is sufficient to extend lifespan via the generation of the fatty acid oleoylethanolamide (OEA), which shuttles to the nucleus triggering the transcriptional activation of target genes (Folick et al. 2015).

Due to the importance in regulating multiple essential cellular programs and its implication in lifespan regulation, it is of great interest to understand the impact of the v-ATPase and lysosomes in the process of ageing.

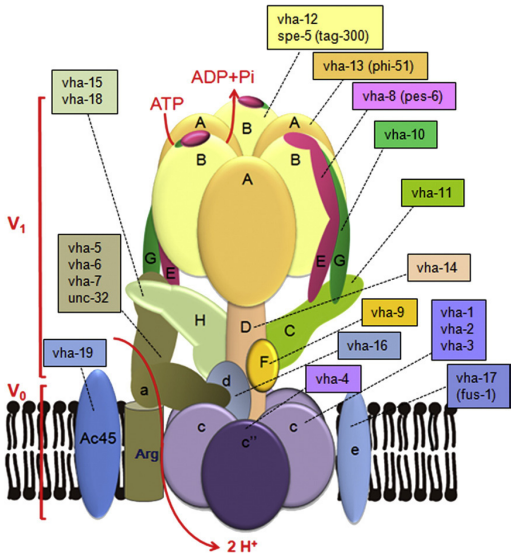


Figure 8. Schematic overview of V-ATPase complex

V-ATPase is a highly conserved complex which consists of a peripheral V1 domain that hydrolyzes ATP and an integral Vo domain that translocates protons across the membrane. The A and B subunits, which are the core subunits of the V1 domain, form ATP binding and hydrolysis pockets. In the Vo domain, the a and e subunits are adjacent to a proteolipid ring, which is composed of the c, c′, and c″ subunits. The V1 and Vo domains are connected by a central stalk composed of the D, F, and d subunits and surrounded by a peripheral stalk composed of the C, E, F, G, and H subunits of the V1 domain. Protons are translocated from the cytoplasm to the lumen with a force driven by ATP hydrolysis.

1.3.2.4 Regulation of the autophagic process

Lysosomes are not only the terminal degradation compartments, but are also connected to the autophagic process at the signaling level. For example, they represent a molecular hub controlling the activity of the mammalian target of rapamycin complex 1 (mTORC1) complex, which negatively regulates autophagy. Upon nutrient availability, mTORC1 inhibits autophagy by directly phosphorylating and thereby suppressing the activity of the autophagy initiator kinase complex (Jiang et al. 2009). Interestingly, mTORC1 exerts its function on the lysosomal surface and its docking can directly be influenced by the lysosome. Amino acid abundance in the lysosomal lumen seem to enable the docking of mTORC1 to the lysosomal surface. More precisely, ATP hydrolysis by the v-ATPase was necessary for amino acids to regulate the translocation of mTORC1 (Zoncu, Liron Bar-Peled, Alejo Efeyan, Shuyu Wang, Yasemin, Sancak 2011). Thus, there seems to be a tight link of regulation between regulators of autophagy and the autophagic process itself. Another important regulator of autophagy is the transcription factor EB (TFEB), which is involved in the expression of lysosomal biogenesis by directly regulating lysosomal genes (Di Malta et al. 2011). One major regulator of TFEB is –among others- mTORC1, which can phosphorylate TFEB and thereby prevent its nuclear translocation (Y. Chen et al. 2012).

Yet another interesting regulator of autophagy is the Forkhead Transcription factor FOXO, especially in muscle cells. Christina Mammucari et al showed that FOXO3 regulates autophagy in skeletal muscle atrophy independent of mTOR. Overexpression of FOXO3 significantly increased the level of lipidated LC3, which was found to be due to direct regulation of LC3 transcript by FOXO (Mammucari et al. 2007). Although it was reported that FOXO increases autophagy during muscle atrophy and is therefore contributing to muscle wasting in the young (Mammucari et al. 2007) (Brault et al. 2007). As myofiber atrophy typically results from decreased protein synthesis and increased protein degradation, it was thought that also in ageing, increased autophagy would lead to sarcopenia. However, it is well known that many age-related diseases arise from accumulation of misfolded proteins and

dysfunctional organelles and that autophagy can ameliorate proteostasis in such conditions (Douglas and Dillin 2010). The muscle is a metabolically active tissue and is challenged with constant oxidative stress, for example during exercise. Therefore, it requires a well-functioning proteostasis-network, which declines with ageing (López-Otín et al. 2013). Autophagy genes (Micah J. Drummond et al. 2014) as well as proteins (Russ et al. 2015) are shown to be decreased in aged muscles, which could contribute to the decline in autophagic function. Furthermore, muscle-specific deletion of a crucial autophagy gene, Atg7, resulted in profound muscle atrophy and an age-dependent decrease in force (Masiero et al. 2009). Thus, autophagy is required to maintain muscle mass and FOXO seems to play an important role in autophagy regulation.

1.3.2.5 Autophagy and Ageing

Accumulating evidence is revealing autophagy as important regulator of organismal lifespan (Rajawat, Hilioti, and Bossis 2009) (Martinez-lopez et al. 2015). Decreased macroautophagy with age has been reported extensively in a variety of different model organisms (Del Roso et al. 2003) (Rubinsztein, Mariño, and Kroemer 2011). In 2010, an unbiased large-scale genetic screen for yeast chronological ageing factors found that the confirmed short-lived mutants were those defective for autophagy, indicating a key requirement for the recycling of cellular organelles in longevity (Smith et al. 2010). Also in Drosophila, a mutation in the *atg8* gene reduced lifespan, whereas overexpression of *atg8* in older flies extended their lifespan (Simonsen et al. 2008). In *C. elegans,* loss-of-function mutations in several autophagy genes, like *atg9, atg-18, bec-1, lgg-1* and *unc-5,* led to a decrease in lifespan. Furthermore, Márton L. Tóth et al showed that autophagy genes are required for life-extension in various long-lived mutant strains, implying that the effects of mutations affecting distinct longevity pathways converge on common downstream processes that involve autophagy (Takács-Vellai et al. 2014). The knockdown of autophagy leads not only to shortened lifespan, but also increased age-associated pathologies like triglyceride accumulation, mitochondrial dysfunction and muscle degeneration (Karin et al. 2010). In mice, knockdown of autophagy genes is lethal during the early postnatal period, but tissue-specific knockout of *Atg* genes have a less dramatic phenotype and instead also manifest multiple age-associated pathologies like neurodegeneration (Murata et al. 2006) or accumulation of Amyloid-beta (Aß) in the mouse brain (Pickford et al. 2008). Zhan and Cuervo noted that the expression of Lamp2a, a lysosomal protein, is decreased with age and

that reinstatement of normal LAMP2a levels averts the aging-associated defect in chaperone-mediated autophagy (CMA) and macroautophagy (C. Zhang and Cuervo 2008). Lysosomal biogenesis and microautophagy could therefore also play a crucial role in the ageing process. In line with that, Bohnert and Kenyon reported that a lysosomal switch triggered proteostasis renewal in *C. elegans* germ lineage to „rejuvenate“ the oocyte (Adam Bohnert and Kenyon 2017). Sperm stimulates v-ATPase activity in oocytes which in turn activates lysosomes and promote aggregate clearance to reset proteostasis. So far, this was only shown in the immortal germ line, but it would be of particular interest to test whether lysosomal proteostasis can be induced in the ageing soma to reverse age-related phenotypes.

The question how autophagy decreases with age remains unclear. Given the complexities in the orchestration of autophagy, it is likely that the mechanisms contributing to inhibition of autophagy are complex and multifactorial.

1.4 Link between microRNAs and proteostasis

As miRNAs are regulating multiple cellular processes, it is unsurprising that they play a part in complex, multifactorial cellular processes such as proteostasis and therefore influence human disease and cellular and organismal ageing. MiRNAs are implicated to regulate many of the hallmarks of ageing (Harries 2014).

As miRNAs have hundreds of targets and modulate gene expression by fine-tuning mRNA levels, it is challenging to identify their involvement in one particular pathway. In this chapter, I want to highlight studies that implicated a direct link between miRNAs and the regulation of the proteostasis network.

One recent study could show that a miRNA, which is only expressed in the nervous system, can regulate proteostasis in the intestine of *C. elegans*. Finger et al. showed that *mir-71* promotes ubiquitin-dependent protein turnover, particularly in the intestine. Interestingly, this process is regulated by olfaction and *mir-71* triggers this cell-nonautonomous communication from the olfactory neurons to the intestine by directly regulating *tir-1*, a Toll-receptor-domain adaptor protein in AWC neurons (Finger et al. 2018). Until now, it was largely unknown whether miRNAs are able to respond to metabolic clues and attempt to regulate homeostasis. This work contributed largely to the understanding of the relation between metabolism and proteostasis and showed that miRNAs can greatly influence the communication between both pathways.

Furthermore, Zhang et al recently identified miRNAs, especially *mir-122*, as critical mediators of caloric restrichtion (CR) in inducing mitochondrial UPR (UPR^{mt}) to improve mitochondrial proteostasis (Zhang et al. 2019).

In addition, knockdown of a single miRNA, *mir-378*, causes muscle atrophy in mice. The miRNA was previously established as a critical regulator of hepatic insulin signaling during fasting (Liu et al. 2014). The muscle atrophy could be explained by the regulation of autophagy by *mir-378* (Y. Li et al. 2018). *PDK-1*, being a direct target of *mir-387*, mediates the effect of the miRNA on autophagy in skeletal muscle. These findings again show that miRNAs are powerful regulators of cellular and organismal processes, by acting autonomously and non-autonomously.

MiRNAs are also being implicated in regulating proteins of the autophagic process directly (Jing et al. 2015). This direct regulation of autophagic components could potentially be interesting for treatment of cancer. Treatment of tumor cells with the *miR-30a* mimic decreased, and with the antagomir increased, the expression of *beclin-1* mRNA and protein (Yang et al. 2014). Also *mir-1* was recently shown to regulate the autophagic process in a drug resistant non-small cell lung cancer (NSCLS) cell line (Hua, Zhu, and Wei 2018). Overexpression of *mir-1* reduced GFP-LC3 positive cell percentage by directly inhibiting *ATG3*. There is still debate in the field whether induction or inhibition of autophagy is beneficial in cancer treatment, but miRNAs, as druggable targets, could play an important role in this regulation.

Although, intensive effort is made, the interaction between autophagy and miRNA is extremely complex and still poorly understood.

1.5 Role of non-autonomous regulation of proteostasis

All biological systems are constantly exposed to a diversity of physiological and environmental stimuli, ranging from acute to chronic, leading to an accumulation of age-associated damage and dysfunction. Horvath postulated that tissues age at different rates (Horvath 2013). He based his finding on methylation profile as indication of ageing status of the different tissues. This finding raises the question whether tissue ageing is an isolated, autonomous process or if tissues communicate with each other to inform neighboring or distant tissues about their state of ageing. In *C. elegans,* reducing insulin/IGF-1-receptor DAF-2 doubles the lifespan of the animals. Throughout their lives, they closely resemble younger wild-type animals. Thus, the ageing in all tissues seem to happen normally, just at a slower pace, suggesting an equal cell-autonomous reduction of insulin/IGF-1

action in all tissues. Surprisingly, a genetic mosaic analysis indicates that signaling between different tissues plays an important role in *daf-2* longevity (Apfeld and Kenyon 1998). Apfeld and Kenyon removed *daf-2* from early blastomeres, so that only some tissues have reduced *daf-2* expression. Although they observed some variability in the phenotype they could show that mosaic animals went into Dauer and lived longer than wild-type controls. Their results indicate that cells that lack *daf-2* activity not only change their own rates of ageing, but also signal to wild-type cells to change their rates of ageing. In a later study, Coleen T. Murphy, Seung-Jae Lee, and Cynthia Kenyon could establish the intestine, which is the animals entire endoderm, as an important non-autonomous insulin-signaling center (Murphy, Lee, and Kenyon 2007).

The proteostasis network serves in all cells to maintain proteostasis and prevent protein misfolding in the face of development and aging. Small heat-shock proteins that help refolding misfolded proteins are a crucial facet in the maintenance of cellular proteostasis (Morimoto 2008). Even though the proteomes expressed for example in muscle or neuronal cells are highly distinct according to their specialized function, the highly conserved cellular stress responses are functioning in all tissues (Balch et al. 2009). It might seem counterintuitive to communicate the protein folding defects that occur in a single tissue to elicit the same stress response in other non-affected tissues or even throughout the entire organism. Therefore, proteostasis regulation had been considered to be a cell-autonomous process. However, in a multicellular organism, intercellular communication to ensure that tissue-level proteostasis is balanced across the organism throughout development, acute and chronic stimuli and the ageing process seems useful.

1.5.1 Neuronal control of organismal proteostasis

An important organ to coordinate stress responses in the organism is the brain. As for many groundbreaking discoveries, *C. elegans* was the model organism in which Prahlad et al showed in 2008 that the AFD neuron is required to induce heat-shock-factor-1 (HSF-1)-dependent heat-shock protein 70 (*hsp70)* induction in multiple tissues (Prahlad, Cornelius, and Morimoto 2008). This study demonstrated that the organismal heat-shock response can be regulated in a non-autonomous manner. Prahlad used a temperature-stress and found that *gcy-8*, a guanylyl cyclase that is solely expressed in the two AFD neurons, is necessary for the induction of the stress responses in other tissues. But what about a stress that only one single tissue experiences? Jenni Durieux et al. used tissue-specific knock-down of *cco-1*, an

essential component of the electron transport chain (ETC), to induce mitochondrial dysfunction in a tissue-specific manner. Strikingly, they found that this perturbation is received and acted on in other tissues than that tissue experiencing the stress (Durieux, Wolff, and Dillin 2011). Furthermore, they could show that reducing mitochondrial ETC in only one tissue can confer longevity, probably trough the regulation of global stress responses. Especially the neuronal control, to communicate the stress, seems to be important. After further investigations of the non-autonomous stress regulation pathway, Rebecca C. Taylor and Andrew Dillin reported XBP-1 as cell non-autonomous regulator of stress resistance and longevity (Taylor and Dillin 2013). Global stress responses like the endoplasmic reticulum unfolded protein response (UPR^{ER}) are not only declining with the aging process, their induction is also less efficient in aged worms. This age-related loss of ER proteostasis could be reversed by expressing constitutively active XBP-1 s, thereby prolonging lifespan. Interestingly, expressing the constitutively active form of XBP-1s solely in the neurons, was sufficient to rescue the age-related loss of proteostasis in distal tissues and confer longevity. Furthermore, they could show that small neurosecretory vesicles confer the systemic effect as *unc-13* mutants failed to communicate the neuron-specific XBP-1s stress response to distal tissues. This finding suggests that neurotransmitter could act as messenger molecules in this non-autonomous signaling pathway.

1.5.2 Non- neuronal tissues as non-autonomous regulators

Neurons provide rapid systemic communication across tissues as they sense and transmit physiological and environmental signals to coordinate and integrate cellular and tissue responses. Interestingly, observations in *C. elegans* revealed that also nonneuronal communication is essential for organismal proteostasis and stress responses. The imbalance of proteostasis through the expression of a metastable myosin increases heat-shock protein 90 (HSP90*)* not only in muscle cells but also in different cell-types, such as the intestine (Van Oosten-Hawle, Porter, and Morimoto 2013). Also neuronal HSP90 expression was regulated through muscular proteostatic stress, indicating that neurons respond to signals coming from peripheral tissues. In *D. melanogaster* it was further demonstrated that dFOXO regulates ageing when activated only in pericerebral fat body and that this fat-specific increase in dFOXO decreased insulin signaling in the brain as measured by RT-PCR of *dilp-2* (insulin-like-peptide) (Hwangbo et al. 2004). Among all age-related pathological

conditions, the gradual decay in muscle strength is one of the first hallmarks of ageing in many organisms, including Drosophila, *C. elegans*, mice and humans. FOXO regulates the expression of a series of target genes involved in stress responses and has been reported to prevent the pathogenesis of some age-related diseases. For example, FOXO reduces the toxicity associated with aggregation-prone human mutant Alzheimer´s and Huntington´s disease proteins in *C. elegans* (Hsu, Murphy, and Kenyon 2003) (Cohen et al. 2006). In 2010, Demontis and Perrimon showed that FOXO/4E-BP signaling regulates proteostasis via an autophagy/lysosome system in the muscle of Drosophila. This prevention of muscle ageing by FOXO and 4E-BP was sufficient to extend lifespan. Furthermore, they demonstrated that muscle-specific FOXO/4E-BP signaling regulates proteostasis in other ageing tissues (Demontis and Perrimon 2010). This non-autonomous effect was due to a decreased feeding behavior of the flies and can therefore be attributed to dietary restriction rather than a direct effect of FOXO signaling to other tissues. This study supports again the common belief that preserving muscle function is beneficial for overall ageing and that the muscle can serve as central tissue to coordinate organism-wide processes in a non-autonomous manner. For this particular study, as well as many others, it is difficult to find the mediator that communicates the non-autonomous signal to other cells or tissues. Two distinct scenarios could be conceivable:

1. **direct communication**: The responsible molecule of the „sender“ tissue itself is directly being transported to the „receiver“ tissue to induce the same response
2. **indirect communication**: The „sender“ releases a distinct messenger molecule that can communicate the state of the „sender“ and is able to evoke a response in the „receiver“ tissue.

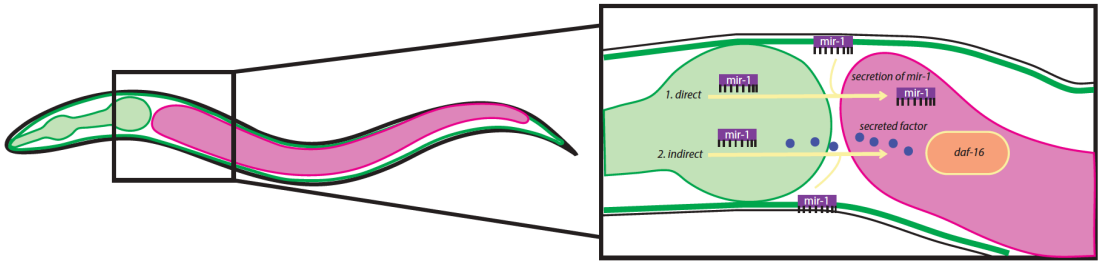


Figure 9. Schematic overview of possible mechanism for tissue non-autonomous signaling. 1. Direct signaling though a direct release of mir-1 by the muscle to the

"receiver" tissue. 2. Indirect signaling through activation of mechanism in the muscle by mir-1, which passively evoke a response in the "receiver tissue".

1.5.3 MiRNAs as non-autonomous regulators

Secreted miRNAs represent a newly discovered mechanism by which donor cells can influence the gene expression of recipient cells. Before, it was assumed that extracellular miRNAs originate from cell lysis or are released in vesicles as „waste products" of the cells. However, evidence suggests that miRNA packaging occurs non-randomly and that specific miRNAs are preferentially sorted into microvesicles (Y. Zhang et al. 2010) (Mittelbrunn et al. 2011). Neutral sphingomyelinase 2 (nSMase2) was reported to regulate the secretion of exosomal miRNAs, suggesting exosomes as essential transport vehicle for miRNAs (Kosaka et al. 2010). The nomenclature of small subcellular vesicles is still controversial; however, exosomes can be classified as small secreted vesicles with a diameter between 30 and 100 nm, containing a variable spectrum of molecules, including RNA, proteins and lipids. As it was shown that the endosomal sorting complex required for transport (ESCRT) system was not necessary for the release of miRNAs (Kosaka et al. 2010), it is still not fully understood how miRNAs are recognized and sorted into excretory vesicles. However, evidence has been provided that the loading of miRNAs into exosomes might be controlled by specific proteins of the RNA-induced silencing complex (RISC). This evidence comes from studies that showed GW182 and Ago2, which are parts of the RISC complex, to be enriched in exosomes (Gibbings et al. 2009) (Y. Zhang et al. 2010). One other poorly understood issue is how secreted miRNAs are taken up into recipient cells and whether specific cells can be targeted for uptake. Microvesicle-enclosed secreted miRNAs are postulated to be taken up when they are internalized by endocytosis, phagocytosis or direct fusion with the plasma membrane. Whether there are also cell recognition molecules on the surface of microvesicles that could allow for specificity in uptake is still under investigation and could provide interesting possibilities for therapeutic approaches (X. Chen et al. 2012). It has also been demonstrated that high-density lipoproteins (HDL) can serve as transport vehicle for miRNAs and can deliver endogenous miRNAs to recipient cells (Vickers et al. 2011). MiRNAs were once thought to be unstable molecules, but were recently demonstrated to circulate in a highly stable, cell-free form in body fluids (Mitchell et al. 2008). As there is accumulating evidence of a targeted release of miRNAs, the burning question whether these miRNAs are entering and controlling gene expression in recipient cells is debated.

1.5.4 Can secreted miRNAs affect gene expression in recipient cells?

Kickers et al tested whether HDL has the capacity to deliver small RNAs to recipient cells. They incorporated HDL with exogenous miRNAs and induced those to cultured hepatocytes. In cells treated with HDL-*mir-375* complexes, not only the intracellular levels of *mir-375* increased, but also target mRNA of *mir-375* was decreased (Vickers et al. 2011). This finding provides evidence that miRNAs enter and also influence gene expression of recipient cells. Evidence for non-autonomous gene regulation by miRNA is not only coming from in vitro studies but there are also a limited number of in vivo studies. The group of Jing Ai observed that cardiac overexpression of *mir-1* caused cognitive impairment in mice (J. C. Ma et al. 2015).

Although *mir-1* was expressed under a muscle-specific promotor, *mir-1* levels were significantly increased in the hippocampus of the mice. RT-PCR analysis revealed that *pri-mir-1* and *pre-mir-1*, the precursor forms of *mir-1*, were not elevated in the hippocampus. This suggests that the increased *mir-1* levels in the hippocampus are due to an uptake of the mature form of *mir-1*. Blood *mir-1* levels were also increased, further indicating that *mir-1* was secreted. Furthermore, it was shown that brain-derived neurotrophic factor (*BDNF*), a direct target of *mir-1*, was down regulated in the hippocampus of the *mir-1* transgenic mice. This down regulation could be reversed by silencing *mir-1* using anti-*miR-1* oligonucleotide fragments, carried by a lentivirus vector that was injected directly into the hippocampus. The same group recently published a follow-up paper, showing that GW4869, an exosome inhibitor, was able to block the elevated levels in the hippocampus of the *mir-1* Tg mice (Duan et al. 2018). Interestingly, this finding could even be conferred to humans as it was shown that *mir-206*, belonging to the *mir-1* family, was up regulated in the brain of AD patients and suppressed the expression of *BDNF* (Moon et al. 2016) (Soon-Tae et al. 2012). *Mir-206* expression was also increased in the group of mild cognitive impaired patients and further increased with AD disease progression. The group of Kon Chu showed that *mir-206* was also elevated in olfactory mucosal cells of AD patients, which can be easily isolated and can be utilized as an excellent biomarker for the diagnosis of early AD (Moon et al. 2016). Biomarkers for diseases are urgently needed in the clinics and circulating miRNAs are very interesting candidates in this field of research. Exosomes isolated from the CSF of Alzheimer´s and Parkinson´s disease patients identified a variety of deregulated miRNAs compared to healthy age-matched controls. *Mir-1* was among the most down regulated miRNAs in exosomes isolated from disease patients (Gui et al. 2015) and correlated with a strong ´degenerative´ pathology. In a different context, *mir-1* was suggested as a

promising biomarker for measuring exercise intensity. Circulating levels of the *mir-1* family, *mir-1*, *mir-133a*, and *mir-206* are increased after a half-marathon race or marathon race (Gomes et al. 2014) (Clauss et al. 2016), as well as after resistance training (Cui et al. 2017). Plasma *mir-133a* level correlated with increased cortisol levels, suggesting *mir-133a* as a potentially useful biomarker indicating stressful training (Cui et al. 2017).

Taken together, accumulating evidence suggests *mir-1* could not only serve as promising biomarker for AD progression or stressful exercise, but there might also be a link between elevated *mir-1* level and pathologies in humans. Although *mir-1* is absolutely essential for early muscle development, it becomes more and more evident that increased *mir-1* levels later in life might be associated with muscle malfunctioning. Muscle tissue makes up about 40% of body mass and due to its high metabolic activity, it is extremely important to maintain cell maintenance pathways like proteostasis throughout the ageing process. However, as proteostasis declines with age, muscle tissue accumulates misfolded proteins which causes a gradual decline of muscle function and sarcopenia. Furthermore, despite intensive research, secretory mechanism as well as the biological pathways regulated by circulating miRNAs in non-autonomous regulation is still mainly unclear. *Mir-1* is found in the circulation, especially in pathologic disease background and could have a potential role in clinical applications. In summary, it is of crucial importance to understand the underlying mechanism of muscle ageing, especially muscle specific decline of repair mechanisms and the potential impact of myomirs like *mir-1*, as they could serve as therapeutic targets. Furthermore, non-autonomous regulation and action of miRNAs in disease pathologies is greatly unknown and *mir-1*, being specifically expressed in the muscle, could be an interesting candidate to investigate potential non-autonomous effects of miRNAs as circulating levels of *mir-1* are already described.

1.6 Aims of this study

Healthy, functional skeletal and heart muscle throughout the life is important to maintain physical activity which is crucially important for overall health. Correlation studies suggest a potential detrimental effect for elevated *mir-1* level in aged mammals. Furthermore, *mir-1* was found in the circulation and elevated *mir-1* levels were associated with other pathologies like AD, suggesting a potential cell non-autonomous regulation by *mir-1*. As knock-out of *mir-1* in higher organism is embryonically lethal, it is difficult to address the effect of *mir-1* in aged animals. Interestingly, *mir-1 C. elegans* mutants are viable and do not show any developmental abnormalities. Furthermore, levels of *mir-1* family members increase in aged worms (Lucanic et al. 2013) (Kato et al. 2011), and components of the proteostasis network (v-ATPases) are predicted by bioinformatical methods to be potentially regulated by the *mir-1* family in *C. elegans* (Kato et al. 2011). However, no studies investigated the link between *mir-1* and proteostasis so far.
Taken together, studying the effect of *mir-1* deletion on ageing in general and on muscle proteostasis in particular, could uncover a novel player in muscle ageing which could serve as interesting target for drug development.

Aim 1: Does *mir-1* affect lifespan and muscle proteostasis?

Aim 2: What are the downstream mediators of *mir-1,* responsible for improvement of protein quality control (PQC)?

Aim 3: Does *mir-1* act non-autonomously?

2 Results

Ageing is the cause of progressive decline in all organ systems throughout the body. This is particularly evident in the musculature and often manifests as sarcopenia, the loss of muscle mass and strength. In fact, muscle frailty is a hallmark of tissue ageing seen in species as diverse as worms, flies, mice and humans (Herndon et al. 2002) (Miller et al. 2008) (Martinez et al. 2007) (Demontis et al. 2013) (Cruz-Jentoft et al. 2010) (Nair 2005). At the molecular level, frailty is often accompanied by a decline in muscle structure and function, as well as alterations in muscle proteostasis and metabolism. Nevertheless, muscle can often respond positively to exercise and stress and rejuvenate even into older age, showing remarkable plasticity (Pollock et al. 2018) (Cartee et al. 2016) (Distefano and Goodpaster 2018). Thus, a molecular study of muscle ageing and plasticity in a genetically tractable model could shed light on fundamental aspects of tissue ageing and regeneration.

microRNAs are small 22-26 nucleotide RNAs that bind with complementarity through their seed sequence to target mRNAs to downregulate gene expression (Gu and Kay 2010). They can work as molecular switches or fine tune gene regulation through feedback, and typically have multiple targets, thereby coordinating cellular programs. Many microRNAs are expressed in a tissue specific manner and regulate programs intrinsically, and could therefore potential serve as tissue specific therapeutic targets (Guo et al. 2014) (Panwar, Omenn, and Guan 2017). In addition, some microRNAs are also secreted and found in serum, and other body fluids (Weber et al. 2010) (X. Chen et al. 2008) , raising the possibility that they can act extrinsically as well.

Mir-1 is a muscle enriched microRNA highly conserved in evolution and is essential to mammalian muscle and heart function. *Mir-1* mouse knockouts, however, are lethal and therefore difficult to study (Zhao et al. 2007). By contrast, *C. elegans mir-1* deletion mutants are viable, and exhibit modest changes in the behavior of the neuromuscular junction. Like its homologs, *mir-1* is expressed in body wall (skeletal) and pharyngeal (cardiac) muscle (Simon et al. 2008). Furthermore, the *mir-1* family member *mir-796* is reportedly upregulated with *C. elegans* ageing though longevity phenotypes remain uncharacterized (Kato et al. 2011).

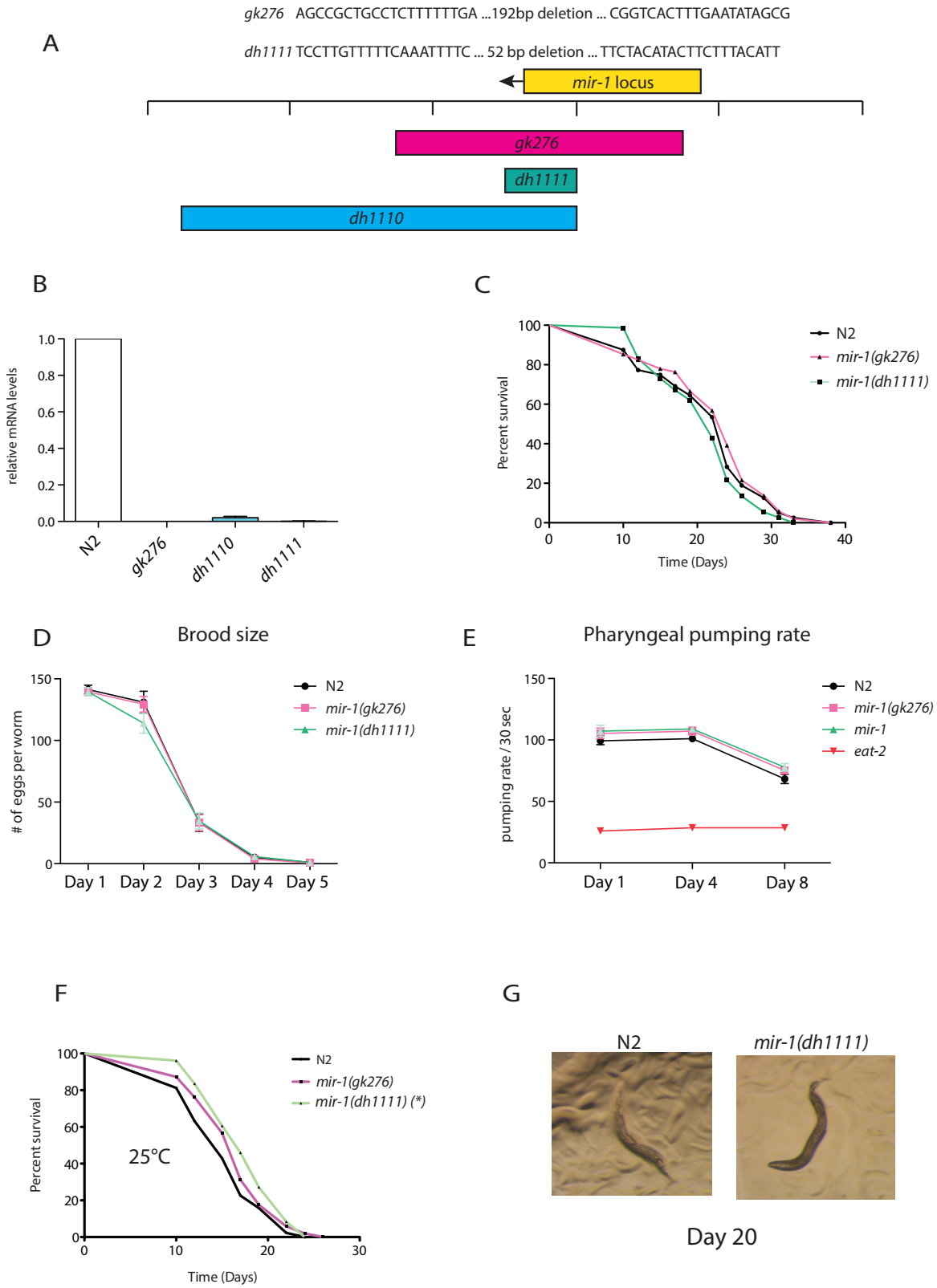
In this work I focused on the potential role of the *mir-1* in regulating muscle function, proteostasis and organismal ageing. I found that *mir-1* null mutations result in increased motility and reduced aggregate formation under proteotoxic challenge during ageing, and show enhanced autophagy and lysosomal function. These studies reveal that muscle expressed mir-1 impacts organismal function, and imply both cell autonomous and non-autonomous control.

2.1 *mir-1* mutants have improved muscle PQC

To unravel *mir-1* physiologic functions, I first characterized the nature of *mir-1* mutations. *gk276* is the canonical allele and consists of a large 192 base pair deletion that removes the *mir-1* coding region as well as part of the downstream region which does not contain any coding sequence. I also created two independent *mir-1* alleles by CRISPR genome engineering (Figure 10a): *dh1111* yielded a 52 base pair deletion within the *mir-1* locus, while *dh1110* yielded a 267 base pair deletion. All three alleles failed to express the coding miRNA, and are thus *mir-1* null mutants (Figure 10b). I predominately used *gk276* and *dh1111* for further analysis.

To investigate whether *mir-1* impacts ageing, I performed demography experiments at various temperatures with *mir-1* mutants. At 20°C, *mir-1* mutants developed normally and exhibited no effect on lifespan (Figure 10c). They also showed nearly normal brood size and pumping rate (Figure 10d+e). Surprisingly, at 25°C, however, *mir-1* mutants showed a moderate extension of lifespan (Figure 10f). In particular, the median lifespan of *dh1111* was significantly extended (cf. 15 versus 17 days), but the maximum lifespan was unchanged, suggesting an effect on healthspan. In support of this idea, *mir-1* mutants adopted a more youthful posture and remained mobile at Day 20 of adulthood compared to wild-type controls (Figure 10g). Interestingly, *mir-1* mutants were more resistant to heat shock at 35°C (Figure 1h) but were not more resistant to oxidative challenge (Figure 10i).

For *C. elegans,* the temperature of 25°C represents a condition of moderate thermal stress, which can induce protein misfolding and modest heat shock response (Gomez-Orte et al. 2018). Preliminary data suggested that *mir-1* mutants showed elevated expression of ER UPR heat shock protein *hsp-4* and mitochondrial UPR *hsp-6* compared to wild type under basal conditions (Figure 10j+k).


A
gk276 AGCCGCTGCCTCTTTTTTGA ...192bp deletion ... CGGTCACTTTGAATATAGCG
dh1111 TCCTTGTTTTTCAAATTTTC ... 52 bp deletion ... TTCTACATACTTCTTTACATT
mir-1 locus
gk276
dh1111
dh1110
B
relative mRNA levels
1.0
0.8
0.6
0.4
0.2
0.0
N2
gk276
dh1110
dh1111
C
Percent survival
100
80
60
40
20
0
0
10
20
30
40
Time (Days)
N2
mir-1(gk276)
mir-1(dh1111)
D
Brood size
# of eggs per worm
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Day 5
N2
mir-1(gk276)
mir-1(dh1111)
E
Pharyngeal pumping rate
pumping rate / 30 sec
150
100
50
0
Day 1
Day 4
Day 8
N2
mir-1(gk276)
mir-1
eat-2
F
Percent survival
100
80
60
40
20
0
25°C
0
10
20
30
Time (Days)
N2
mir-1(gk276)
mir-1(dh1111) (*)
G
N2
mir-1(dh1111)
Day 20

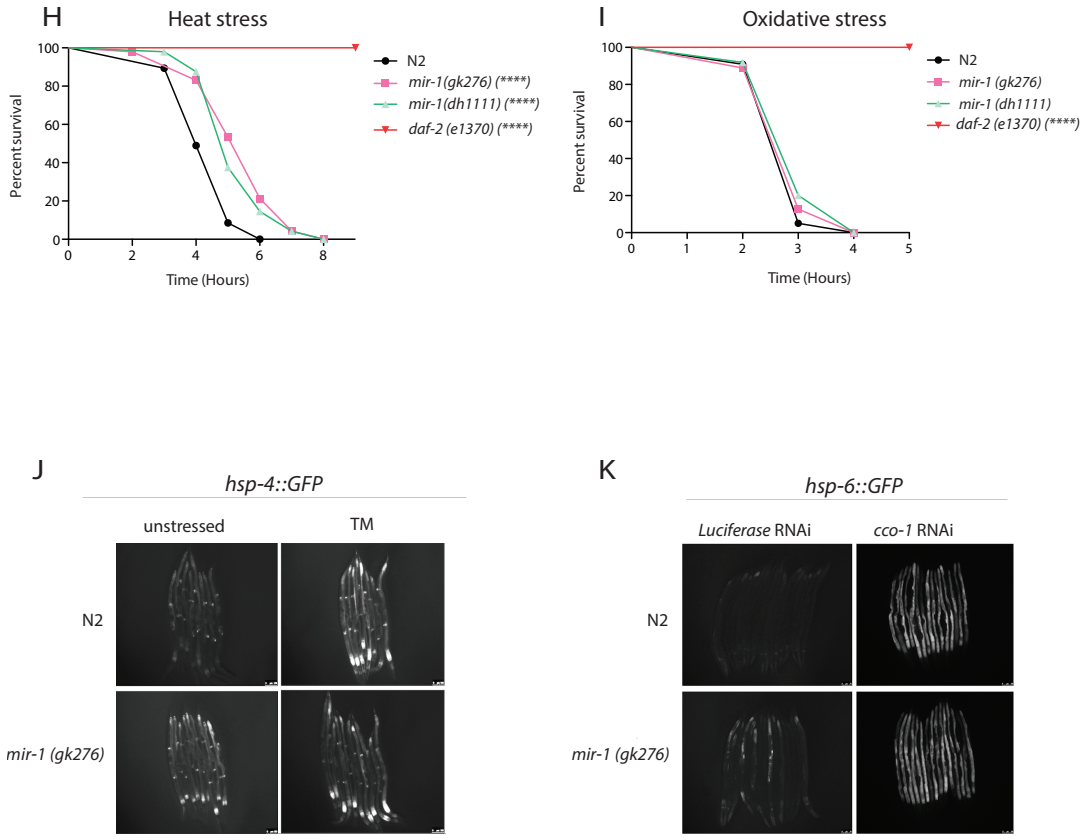


Figure 10. *mir-1* mutants develop normally and are more heat stress resistant and modestly long-lived at 25°C compared to wild-type.
(A) Schematic showing *mir-1* locus and the three *mir-1* deletion alleles. *mir-1(gk276),* obtained from the million mutant project, is mainly used for this thesis. *mir-1 (dh1111)* and *mir-1 (dh1110)* CRISPR deletion allele is made using the sgRNA Guide described in Material and Methods. **(B)** TaqMan qPCR measuring mature *mir-1* levels confirm that all alleles are null, N=2. **(C)** Lifespan analysis performed at 20°C for two independent *mir-1* mutant alleles compared to wild-type. 75 worms/strain, N=3, Log-rank Test, ns. **(D)** Brood size measurement of *mir-1 (gk276)* and *mir-1 (dh1111)* compared to wild-type, N=1. **(E)** Pharyngeal pumping rate measured on day 1, day 4 and day 8 in two *mir-1* alleles compared to wild-type, N=1. **(F)** Lifespan analysis performed at 25°C of two *mir-1* mutant alleles compared to wild-type. 75 worms/strain, N=3, Log-rank Test, *, p<0.05. **(G)** Representative picture of worm posture of N2 and *mir-1 (dh1111)* mutant on Day 20 of adulthood (right). **(H)** Heat stress resistance measured by exposing *mir-1* mutants and wild-type worms to 35°C for 8h, N=1, Log-rank, ****, p<0.0001. (I) Oxidative stress resistance measured by exposing worms to 10mM H_2O_2 for 5h, N=1, ns. **(J)** Images of the ER stress reporter *hsp-4p::hsp-4::GFP* in *mir-1* and wild-type Day 1 adults under basal and induced (4h 10uM Tunicamycin (TM)) condition, N=1. **(K)** Images of the mito-UPR stress reporter *hsp-6p::hsp-6::GFP* in *mir-1* and wild-type Day 1 adults under basal and induced (egg-on cco-1 RNAi) condition.

I therefore surmised that the enhanced health of *mir-1* mutants at 25°C might reflect differences in protein folding or quality control (PQC). To assess the ability of *mir-1* to withstand proteotoxic challenge, I used strains expressing various lengths of polyglutamine tracts fused to yellow fluorescent protein (YFP) under the control of the muscle myosin specific *unc-54* promoter, which have been used previously as models of proteotoxicity and Huntingtin's disease (Morley et al. 2002) (Brignull et al

2006). In these strains, initially soluble proteins become sequestered into insoluble aggregates, visible as foci in the muscle of the worm, with longer polyQ stretches developing more aggressive aggregation. Indeed, I observed that wild type worms harboring polyQ40 stretches already expressed the maximum number of aggregates by the L4 stage. Interestingly, *mir-1* deletion strongly reduced the amount of aggregates formed (Figure 11a+b). Worms expressing the milder polyQ35 stretches showed a more progressive age-dependent protein aggregation, which typically appeared by Day 4 of adulthood. In this case, too, *mir-1* mutants displayed significantly less aggregates (ca. 32) compared to age-matched wild-type controls (ca 42) (Figure 11c+d). Furthermore, *mir-1* mutants not only decreased aggregate number, but also improved the solubility of polyglutamine (Figure 11e). The effect of proteotoxicity was further investigated by measuring organismal motility, counting body bends in liquid culture on day 8 of adulthood. Though the degree of paralysis was quite variable between replicates, *mir-1* mutants were significantly more mobile in all replicates compared to age-matched wild-type controls (Figure 11f+g). Allele *dh1111* showed the same, albeit slightly weaker phenotype on Q35 motility as the canonical *gk276* deletion (Figure 11h). The decrease in muscle aggregates was not due to a general decrease in transgene expression as the total amount of polyglutamine::YFP protein was similar in wild-type worms and *mir-1* mutants, as measured by Western blot (Figure 11i).The effect was specific for the muscle, as *mir-1* mutants did not show improvements in motility when polyglutamine stretches were expressed under a neuronal- promotor (Figure 11j). Importantly, both the decreased aggregates and the increased motility could be rescued by re-introducing a wild type *mir-1* transgene (Figure 11c,d+g). The similarity of behavior among the different alleles and the rescue of these phenotypes by the wild type transgene demonstrate that *mir-1* is causal for removing muscle aggregates and improving mid-life motility.

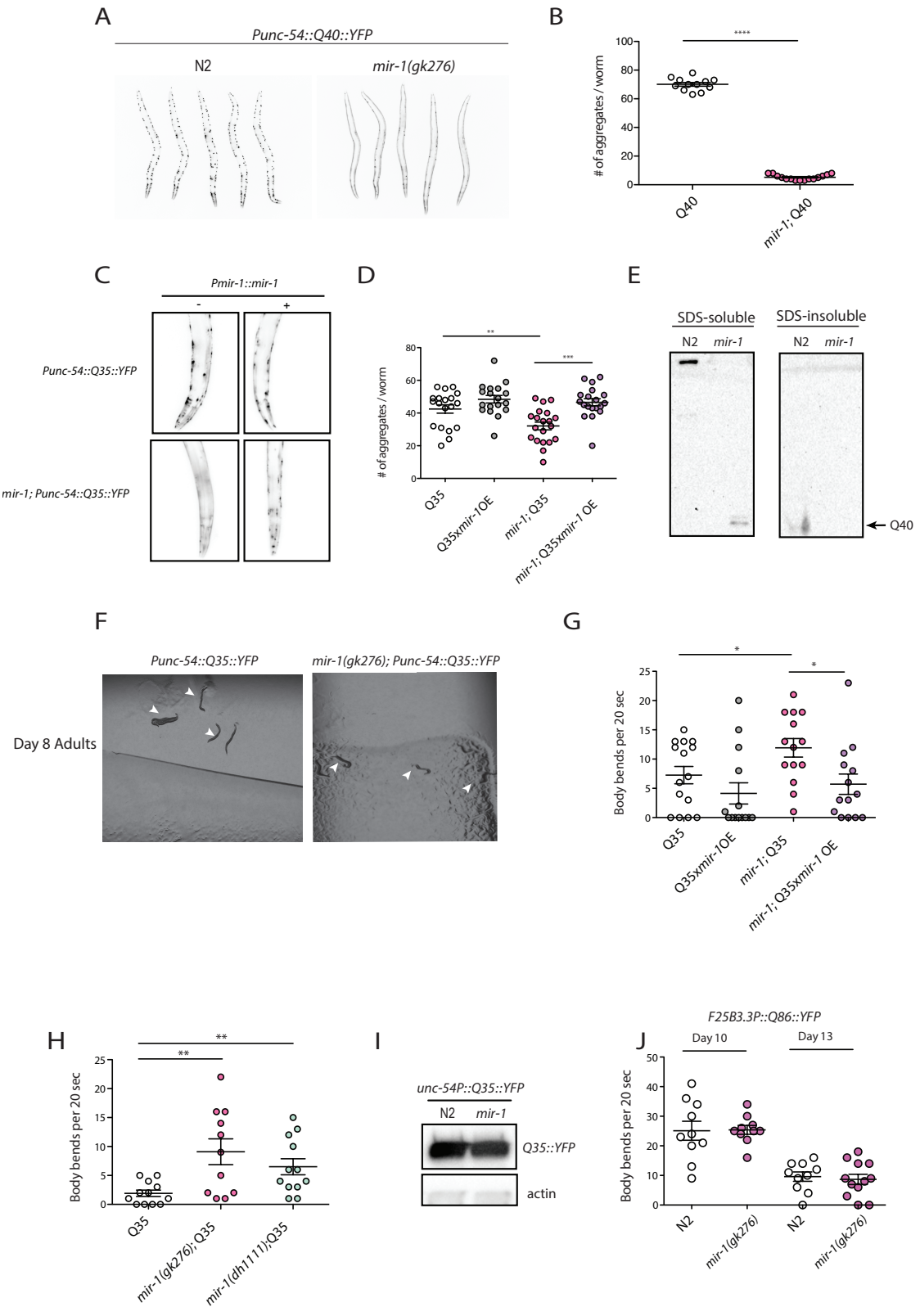


Figure 11: *mir-1* mutants have improved muscle PQC
(A) Representative images of wild-type and *mir-1* mutant animals expressing *unc-54P::Q40::YFP* at day 1 of adulthood. **(B)** Quantification of *Q40::YFP* aggregates using Image J (like in A.), each dot represents one animal, >10 worms per condition, line and error bar indicate mean ± SD of one representative experiment, N=3, t-test, ****, p<0.0001. **(C)** Representative images of wild-type and *mir-1* mutant animals expressing *unc-54P::Q35::YFP* in the presence or absence of *mir-1P::mir-1* at Day 4 of adulthood. **(D)** Quantification of *Q35::YFP* aggregates using Image J (like in C.), each dot represents one animal, >15 worms

per condition, line and error bar indicate mean ± SD of one representative experiment, N=3, 1way ANOVA, Tukey´s multiple comparisons test, **, p<0.01, ***, p<0.001.
(E) Representative Western blot of SDS-soluble and SDS-insoluble Q40::YFP fractions of day 1 adult unc-54P::Q40::YFP animals in *mir-1* mutant and wild-type background, N=1.
(F) Representative pictures of wild-type and *mir-1* mutants worms expressing *Q35::YFP* at day8 of adulthood. Arrowheads indicating position of the worms. Wild-type worms failed to reach bacterial lawn due to paralysis. **(G)** Motility of wild-type and *mir-1(gk276)* mutant animals expressing *unc-54P::Q35::YFP* in presence or absence of *mir-1* OE at day 8 of adulthood, measured by swimming assay. 15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SD of one representative experiment, N=3, 1way ANOVA, Tukey´s multiple comparisons test, *, p<0.05. **(H)** Motility of two independent *mir-1* alleles *(gk276)* and *(dh1111)* and wild-type animals expressing *unc-54P::Q35::YFP*, measured by swimming assay. 15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SD of one representative experiment, N=3, 1way ANOVA, Tukey´s multiple comparisons test, **, p<0.01. **(I)** Representative Western blot of total *Q35::YFP* in day 1 adults, N=2. **(J)** Motility as measured by swimming assay of wild-type and *mir-1* mutant worms expressing *Q86::YFP* under a pan-neuronal promotor, N=1.

I next sought to investigate whether *mir-1* exerts a general effect on proteotoxicity or if it might be specific to the polyQ model. To do so, I examined the effect of *mir-1* on a model of amyloid toxicity, the human *A-beta peptide 1-42* minigene, which is expressed in the muscle of the worm and gives rise to progressive paralysis during adulthood (Link 1995). *mir-1* mutants also rescued the gradual decline in motility caused in this model, suggesting a general improvement in muscle proteostasis (Figure 12a,b).

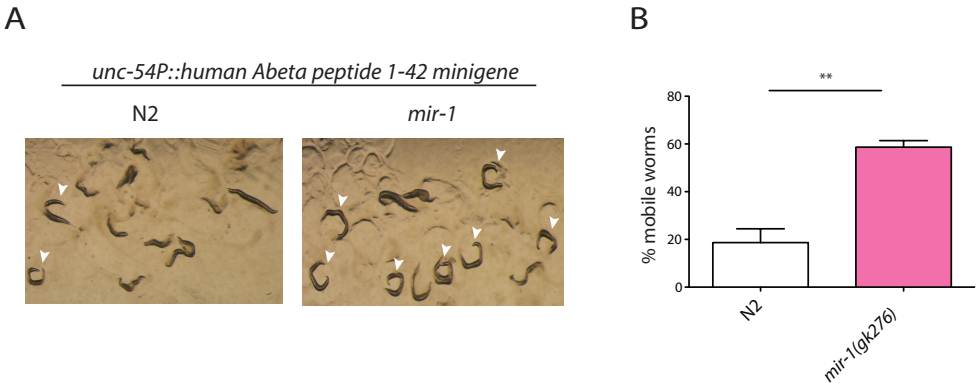


Figure 12. *mir-1* deletion also protects against A-beta toxicity.
(A) Representative pictures of paralyzed vs rolling phenotype in wild-type and *mir-1* mutant worms expressing *unc-54P::human A-beta peptide 1-42 minigene* at day 8 of adulthood. Arrowheads indicating worms which were able to roll at day 8.
(B) Quantification of motility (like in A.) measured by percentage mobile worms, >15 worms per genotype, N=3, shown mean +SEM, t-test, **, p<0.01.

2.2 Informatic screen for downstream mediators of *mir-1* induced PQC improvement

To identify downstream mediators of *mir-1* PQC improvement, I looked for potential *mir-1* regulatory targets. I first took an informatic approach, and used publicly available prediction tools to select potential genes harboring *mir-1* seed binding sites in their 3'UTR, namely microRNA.org, TargetScanWorm, and PicTar (Betel et al. 2008) (Calvin et al. 2011) (Krek et al. 2005). These different prediction tools use distinct algorithms that yield different sets of candidates and these lists are usually quite extensive. Therefore, I restricted my candidates based on the prerequisite that the target was predicted by all three target prediction tools. This yielded 60 candidates, 50 of which had available RNAi clones.

To carry out the primary screen, I looked for clones that abrogated the improved motility of *mir-1;Q35* worms. Specifically, synchronized L4 larvae were transferred to RNAi FUDR plates and transferred twice to fresh plates until day 8 when motility was scored using the circle test (Figure 13a). This test measures the ability of strains to migrate out of a circle within a limited amount of time. String analysis of all predicted targets showed a strong enrichment for v-ATPase subunits (Figure 13b). Top candidates were selected based on the criteria to fully suppress the motility phenotype and were then validated in a secondary screen using the more laborious body bend motility assay (Figure 13c). Confirmed candidates (Figure 13d) were then counter-screened in wild-type worms expressing Q35 to investigate whether the RNAi clones caused general loss of motility in WT or whether they specifically reduced *mir-1* motility (Figure 13e). Some candidates completely abolished *mir-1* motility but barely reduced wild-type motility. Strikingly, among them I obtained vacuolar-ATPase subunits, as well as the stress response transcription factor *daf-16*/FOXO, a known transcriptional regulator of many v-ATPase subunits (Baxi et al. 2017). These candidates contained one conserved *mir-1* binding site. One caveat of this approach, however, was that wild-type and *mir-1* motility assays had to be scored on different days (wildtype: day 5, *mir-1*: day8) as wild-type worms were already paralyzed at a timepoint where *mir-1* motility was still too strong to observe an RNAi phenotype. Therefore, to obtain a more comparable result, I scored motility on the day of adulthood where the same percentage of immobile worms was observed between the genotypes on luciferase control RNAi (i.e., wild-type = Day 5; *mir-1* = Day 8.

To measure the impact of candidates on proteostasis, I measured the effect of their cognate RNAi on Q35 aggregation. In pilot experiments using *vha-13* and *daf-16* RNAi, however, I failed to observe changes in the aggregate phenotype of *mir-1*, when animals were fed RNAi from L4 onwards. (Figure 13f), while earlier *vha-13* RNAi exposure (egg on) led to lethality. Conceivably, the L4 exposure to RNAi was insufficient to affect Day 3 adult aggregate formation. In line with this hypothesis, I observed that *mir-1;daf-16* double mutants suppressed the aggregate phenotype of *mir-1* single mutants (Figure 13g). Because *vha-13* mutants are embryonic lethal, I was unable to assess its impact on aggregate formation. Therefore, I focused on the motility phenotype.

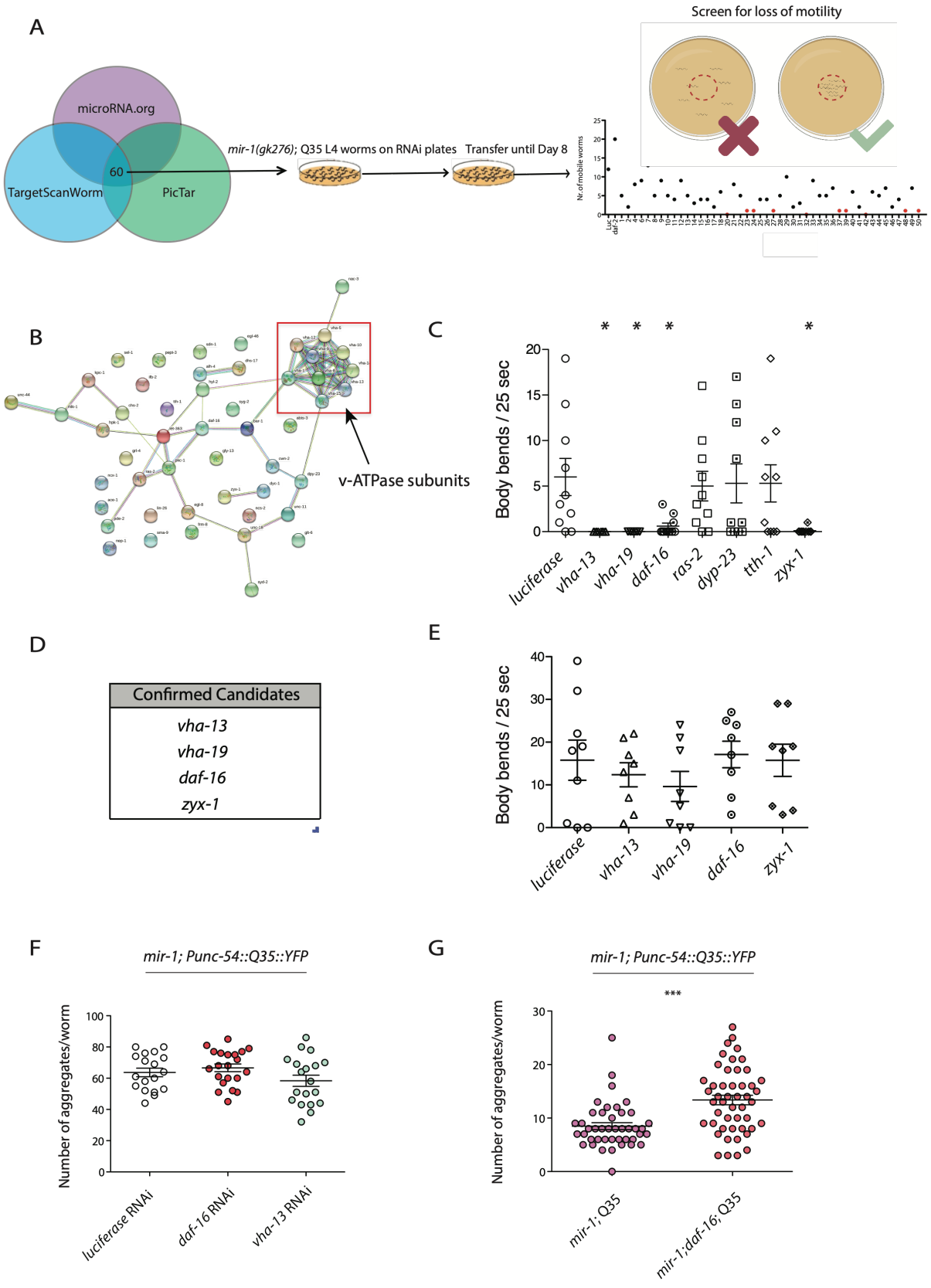


Figure 13. RNAi screen for predicted *mir-1* targets identifies *daf-16* and *vha-13* as strong candidates for *mir-1* mediated regulation of polyQ toxicity.
(A) RNAi screen workflow with a total of 50 RNAi clones, readout motility was tested using the circle test on day 8 of adulthood. **(B)** String analysis of predicted targets shows strong clustering of v-ATPase subunits **(C)** Validation of candidates of initial screen by swimming assay at day 8 of adulthood, 15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SD of one representative experiment, N=1, 1way ANOVA,

Tukey´s multiple comparisons test, *, p<0.05. **(D)** List of confirmed candidates. **(E)** Counter screen testing motility effect of RNAi on *N2;Q35* worms. Motility was performed using swimming assay at day 5 of adulthood, N=1, 15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SD of one representative experiment, N=1, 1way ANOVA, Tukey´s multiple comparisons test, ns. **(F)** Quantification of aggregates of mir-1;Q35 worms on RNAi against top candidates *daf-16* and *vha-13*. *Luciferase* was used as control RNAi. Each dot represents one animal,error bars indicate mean ± SD, 1way ANOVA, Tukey´s multiple comparisons test, N=1, ns. **(G)** Quantification of aggregates of *mir-1(gk276); daf-16(mu86)*;Q35 mutants compared to mir-1;Q35 mutants, N=3, each dot represents one animal,error bars indicate mean ± SD, t-test, *** p<0.0001.

2.3 *Mir-1* proteomics screen for downstream mediators of *mir-1* induced PQC improvement

As a second approach to identify *mir-1* regulatory targets in an unbiased manner, I performed TMT shotgun proteomics analysis comparing *mir-1* mutants to wild-type worms on day 1 of adulthood. (Previous attempts to use SILAC labeling of the worms did not yield any useful data). Approximately 2000 proteins were identified in the various replicates. With TMT labeling, PCA analysis did not show a separation of WT and *mir-1* genotypes, perhaps reflecting the limited measurable changes induced by *mir-1* mutation. Using a p-value of 0.05 as cutoff yielded 56 proteins as significantly upregulated in *mir-1* mutants compared to wild-type (Figure 14a). GO term analysis did not yield any commonly regulated pathway, probably due to the small sample size (Figure 14b). Interestingly, though two of the significantly upregulated proteins were v-ATPase subunits (*vha-10* and *vha-19*), one of which (*vha-19)* was also found in the informatic screen. Seven other *vha* subunits were also detected including *vha-13*, but expression levels were unchanged (Figure 14c).

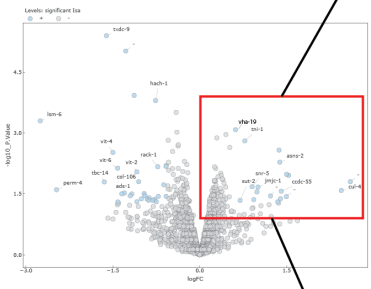
I performed another motility screen using RNAi against the significantly upregulated candidates from the proteomics analysis (Figure 14d). Using the circle test, I initially identified 6 candidates that reversed *mir-1* motility and contained a *mir-1* binding site (Figure 14e). These included *vha-19*, *ccdc-55*/NRSP1, *nrfl-1*/SLC9A3R1, *apl-1/*amyloid precursor protein, *dtmk-1*/deoxythymidylate kinase, and *hsp-17*.

In the secondary screen that was carried out using the more laborious body bend motility assay, *ccdc-55* and *vha-19* could be further confirmed as top candidates (Figure 14f).

Detecting two v-ATPase subunits and identifying *vha-19* in both screens as top candidates strengthened the evidence of the v-ATPase playing an important role in *mir-1* dependent regulation of proteostasis. Moreover, *daf-16* is proposed as a regulator of v-ATPAse expression (Baxi et al. 2017). Therefore, we focused further

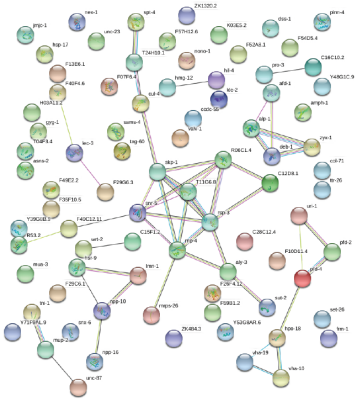
on the investigation of this pathway and used the services of sunybiotech to endogenously tag *daf-16*, *vha-13* and *vha-19*. As endogenously tagging of *vha-19* led to a lethal phenotype, I focused on *vha-13* and *daf-16* for further analysis.

A



Gene names	logFC
cul-4	2,44
ccdc-55	1,41
spt-4	1,39
asns-2	1,38
vha-19	1,36
jmjc-1	1,00
uri-1	0,93
snr-5	0,90
tni-1	0,78
sut-2	0,70
gyg-1	0,52
ttr-26	0,45
pinn-4	0,45
hpo-18	0,42
valv-1	0,42
zyx-1	0,40
sams-4	0,39
dtmk-1	0,37
dss-1	0,37
alp-1	0,36
wrt-2	0,35
aly-3	0,32
unc-23	0,32
klc-2	0,31
npp-16	0,30
mup-2	0,29
set-26	0,29
rnp-4	0,28
hpo-34	0,28
nrfl-1	0,28
pfd-4	0,27
hsp-17	0,27
vha-10	0,27
hil-4	0,27
snx-6	0,26
deb-1	0,25
hsr-9	0,25
nex-1	0,25
lmn-1	0,25
mrps-26	0,25
unc-87	0,25
pro-3	0,25
irg-7	0,25
afd-1	0,24
col-71	0,24
fubl-1	0,23
frm-1	0,22
amph-1	0,21
hmg-12	0,21
lec-3	0,21
rbm-22	0,20
nono-1	0,20
pfd-2	0,20
mua-3	0,20
rsp-3	0,17
npp-10	0,17

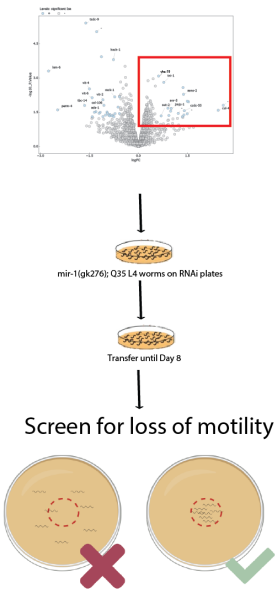
B



C

Gene names	P.Value	logFC
vha-8	0,12	0,12
vha-14	0,17	0,16
vha-13	0,20	-0,28
vha-11	0,25	-0,29
vha-15	0,25	0,12
vha-9	0,49	-0,17
vha-12	0,61	-0,05

D



E

candidate list
ccdc-55
nrfl-1
apl-1
dtmk-1
vha-19
hsp-17
tni-1
mua-3
afd-1
spt-4
uri-1
lec-3
snr-5
cul-4
col-71
rsp-3

F

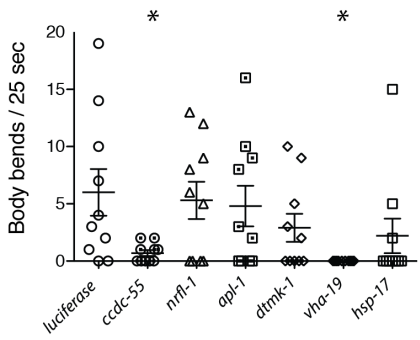


Figure 14. RNAi screen for upregulated proteins in *mir-1* mutants compared to wild-type identifies *vha-19* as candidate for *mir-1* mediated regulation of polyQ toxicity.
(A) List of proteomics results showing gene name and logFC of upregulated candidates with p-value>0.05 in 5 BR of *mir-1* vs N2. **(B)** String analysis of candidates listed in (A). **(C)** v-ATPase subunits detected in proteomics including the respective p-value and logFC comparing *mir-1* mutants to wild-type. **(D)** RNAi screen workflow using proteomics results (upregulated with p-value>0.05 in 5 BR of *mir-1* vs N2 5 BR) with a total of 59 RNAi clones,

readout motility was tested using the circle test. Motility scored on day 8 of adulthood. **(E)** Candidates of screen; red indicates candidates containing *mir-1* binding site. In total 6/16 top hits contained *mir-1* binding site. **(F)** Validation of candidates of initial screen by swimming assay at day 8 of adulthood, 15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SD of one representative experiment, N=1, 1way ANOVA, Tukey´s multiple comparisons test, *, $p<0.05$.

2.4 Requirement of *vha-13* and *daf-16* for *mir-1* induced PQC improvement

Both *daf-16* and *vha-13* (kd) completely reversed the motility of *mir-1;Q35* mutants (Figure 15a) and are therefore most likely be involved in *mir-1* dependent proteostasis regulation. Interestingly, overexpression of *vha-13* under a muscle-specific promoter in Q35 worms, recapitulated the improved motility seen in *mir-1;Q35* worms (Figure 15b). However, overexpression of *vha-13* in the muscle of Q35 worms did not affect aggregate number (Figure 15c), consistent with previous observations that the aggregate phenotype is difficult to modulate.

2.5 Vha-13 3'UTR is regulated by *mir-1* in cell culture

These findings raise the question whether the regulation by *mir-1* is direct or indirect. To address this, I turned to in vitro cell culture studies. I cloned the *C. elegans vha-13* 3´UTR into PmirGLO, a vector used to study regulation of 3´UTRs by miRNAs in a cell culture system. *Mir-1* overexpression was achieved by a *mir-1* overexpression construct, which increased the mature form of *mir-1* by 1000-fold as measured by RT-qPCR (Figure 15d). PmirGLO construct and either empty vector control or the vector expressing *mir-1* was co-transfected and firefly and renilla (internal control) expression was measured after 24 hours on a luminescence spectrometer. Overexpression of *mir-1* suppressed the expression of firefly luciferase compared to renilla luciferase, suggesting that *mir-1* directly regulates *vha-13* 3´UTR (Figure 15e).

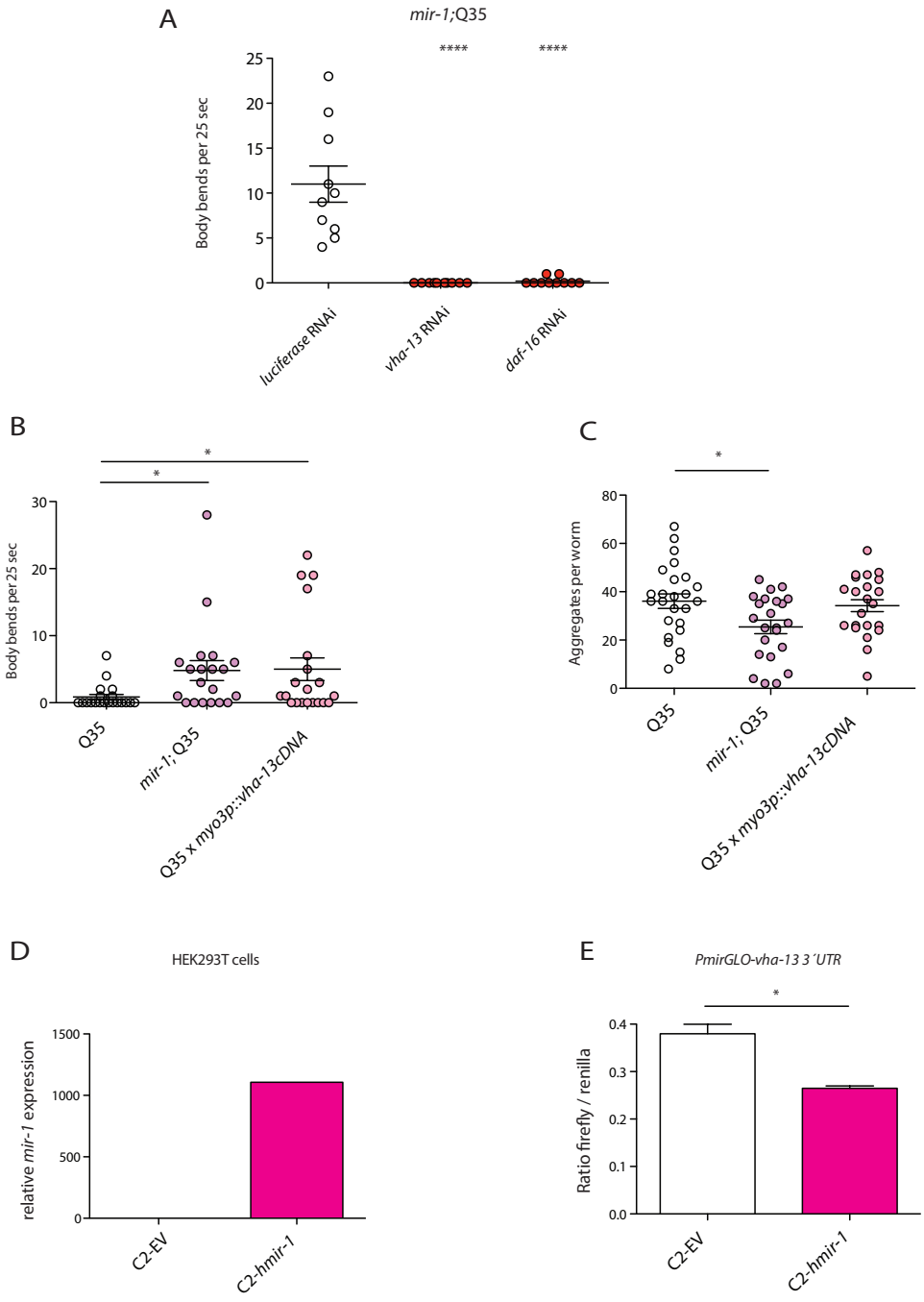


Figure 15. *Vha-13* might be directly regulated by *mir-1* and muscle-specific overexpression of *vha-13* improves motility of Q35 worms, but does not affect aggregate formation. *Daf-16* knockdown reverses motility as well as aggregate phenotype of *mir-1* mutants.

(A) Motility of *mir-1;Q35* transgenic worms grown on RNAi against *vha-13*, *daf-16* and *luciferase* L4 on, motility scored at day 8 of adulthood using swimming assay, each dot represents one animal, >15 worms per condition, line and error bar indicate mean ± SD of one representative experiment, N=3, individual t-tests, ****, p<0.0001. **(B)** Motility of wild-type and *mir-1* mutant animals expressing *Q35::YFP* and wild-type animals expressing *Q35::YFP* and *myo3p::flag::mcherry::vha-13cDNA* at day 8 of adulthood, measured by swimming assay. >15 worms per condition, each dot represents one animal, line and error bars indicate mean ± SEM of one representative experiment, N=3, 1-way ANOVA, Tukey´s multiple comparisons test, *, p<0.05. **(C)** Quantification of *Q35::YFP* aggregates using Image J at day 4 of adulthood, each dot represents one animal, >15 worms per condition, line and error bar indicate mean ± SEM of one representative experiment, N=3, 1way ANOVA, Tukey´s multiple comparisons test, *, p<0.05. **D)** qRT-PCR levels showing relative *mir-1* levels normalized to *U6* in HEK293T cells transfected with C2 plasmid expressing *mir-1* or empty vector (EV)

control. **(E)** Quantification of luciferase assay showing firefly luciferase levels under the control of *vha-13* 3´UTR normalized to renilla luciferase in HEK293T cells co-transfected with C2 plasmid expressing *mir-1* or empty vector (EV) control, shown mean +SEM, N=2, t-test, *, p<0.05.

2.6 *Mir-1* mutation derepresses mRNA levels of v-ATPase subunits during ageing

MicroRNAs can regulate both mRNA and protein levels of their targets. If *mir-1* regulates v-ATPase subunits, then *mir-1* loss would be predicted to derepress v-ATPase mRNA expression. Because v-ATPase activity is suggested to decline with age (Hughes and Gottschling 2012), I first examined the expression of subunits during the ageing process. RT-qPCR analysis confirmed a decline in mRNA levels of v-ATPase subunits already at day 4 of adulthood in wild-type worms (Figure 16a). In contrast, *mir-1* mutants maintained mRNA levels of v-ATPase subunits between Day 1 and Day 4 of adulthood (Figure 16b). Although a modest decrease could also be observed in v-ATPase mRNA in *mir-1* mutants, levels of the *vha-13* subunit seem to be especially stable between day 1 and day 4 of adulthood (Figure 16b). In summary, at the onset of visible Q35 protein aggregates (day 4), the mRNA of v-ATPase subunits was 2-fold increased in *mir-1* mutants compared to wild-type worms (Figure 16c). To investigate whether *mir-1* regulates our candidates on the protein level, we endogenously tagged *daf-16*, *vha-13* and *vha-19* by CRISPR/Cas9 with either N-terminal 3xflag-tag (*daf-16u*) or 3xflag-neongreen-tag (*vha-13u* and *vha-19u*) (courtesy of Sunnybiotech). Tagging of *vha-19* caused lethality, so I focused on *daf-16* and *vha-13*. In addition, we mutated putative *mir-1* binding sites in the 3´UTRs of *daf-16* and *vha-13* (*daf-16um* and *vha-13 um*) to see if this would affect expression levels (Figure 17a-c). Interestingly, I found that protein levels of *vha-13* behaved similar to the mRNA, and were increased at day 4 of adulthood in *mir-1* mutants relative to wild type. Notably Western blot analysis revealed that VHA-13 protein levels were slightly increased in all 4 replicates in *mir-1* mutants, though not reaching significance (Figure 16d).

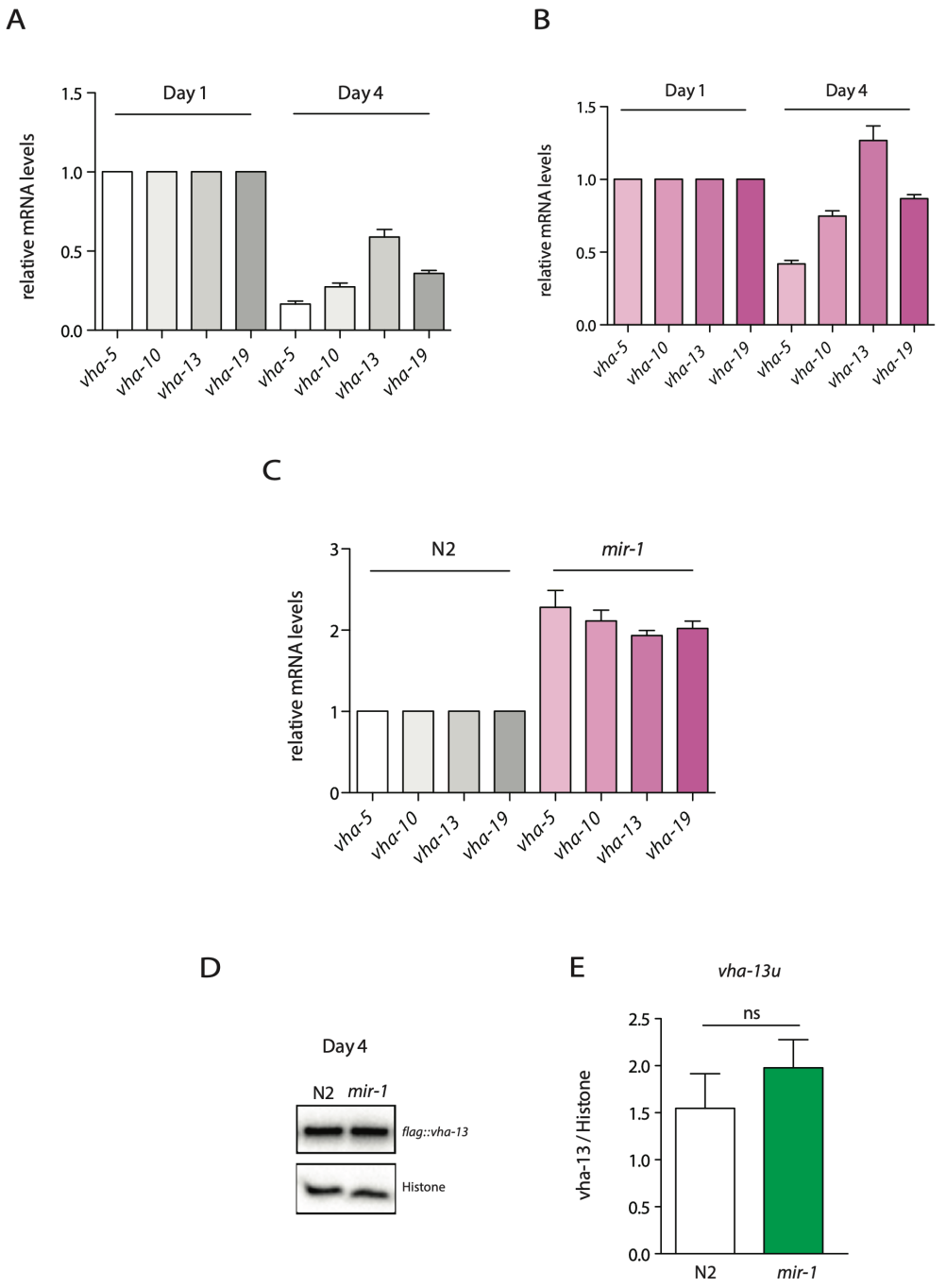


Figure 16. v-ATPase subunits are downregulated with age in wild-type, but not *mir-1* mutants. (A) qRT-PCR showing levels of different v-ATPase subunits at day 4 normalized relative to day 1 in wild-type worms. V-ATPase subunits expression levels normalized to *cdc-42*, shown mean +SEM, N=3. **(B)** qRT-PCR showing levels of different v-ATPase subunits at day 4 relative to day 1 in *mir-1* mutant worms. V-ATPase subunits expression levels normalized to *cdc-42*, shown mean +SEM, N=3. **(C)** qRT-PCR showing levels of different v-ATPase subunits in *mir-1* mutants relative to wild-type at day 4 of adulthood. **(D)** Representative western blot showing *vha-13u expression* in *mir-1* mutants and wild-type controls at day 4 of adulthood **(E)** Quantification of Western Blot analysis comparing protein levels of *vha-13u* in *mir-1* mutants compared to wild-type controls at day 4 of adulthood, shown mean +SEM, N=4, individual t-tests, ns.

On the other hand, I saw no difference in expression of various *daf-16* and *vha-13* constructs by Western blot in WT and *mir-1* backgrounds in day 1 adults, either at 20°C or 25°C (Figure 17 d-g), Nor did I observe regulation by *mir-1* OE on the *vha-13* reporter (Figure 17h). These findings suggest that there might be an age-dependent regulation.

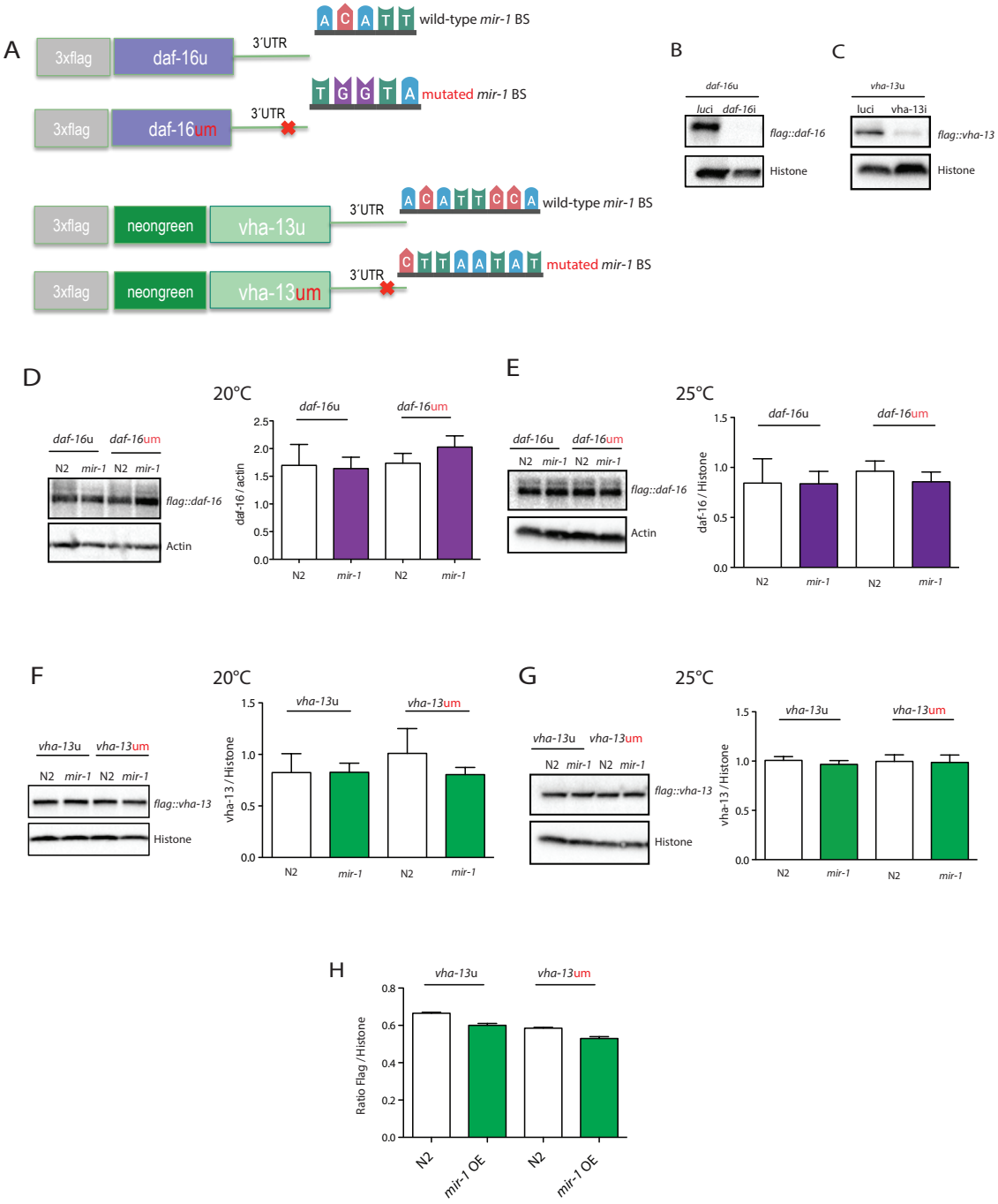


Figure 17. Endogenously-tagged *daf-16* and *vha-13* protein level show no regulation by *mir-1* on Day 1 of adult.

(A) Schematic overview of strains made by sunybiotech. Endogenously N-terminally tagged *daf-16* and *vha-13* with wild-type (*daf-16u/ vha-13u*) or mutated (*daf-16um/ vha-13um*) *mir-1* binding site. **(B)** Western blot of total protein extracts of synchronized day 1 *daf-16u* transgenic worms incubated with anti-flag antibody grown on *daf-16* or *luciferase* RNAi egg-on. **(C)** Western blot of total protein extracts of synchronized day 1 *vha-13u* transgenic worms incubated with anti-flag antibody grown on *vha-13* or *luciferase* RNAi L4 on. **(D)** Representative Western blot of total protein extracts of synchronized day 1 *daf-16u* and *daf-16um* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody, grown at 20°C egg-on (left). Quantification of Western Blot analysis showing *flag::daf-16* levels normalized to actin, shown mean +SEM, N=3, t-test, ns (right).
(E) Representative Western blot of total protein extracts of synchronized day 1 *daf-16u* and *daf-16um* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody, grown at 25°C egg-on (left). Quantification of Western Blot analysis showing *flag::daf-16* levels normalized to actin, shown mean +SEM, N=3, t-test, ns (right). **(F)** Representative Western blot of total protein extracts of synchronized day 1 *vha-13u* and *vha-13um* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody, grown at 20°C egg-on (left). Quantification of Western Blot analysis showing *flag::vha-13* levels normalized to histone, shown mean +SEM, N=3, t-test, ns. **(G)** Representative Western blot of total protein extracts of synchronized day 1 *vha-13u* and *vha-13um* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody, grown at 25°C egg-on (left). Quantification of Western Blot analysis showing *flag::vha-13* levels normalized to histone, shown mean +SEM, N=3, t-test, ns.
(H) Quantification of Western Blot of *vha-13u vha-13um* transgenic worms in the presence or absence of the *mir-1* OE construct *mir-1p::mir-1* on day 1 of adulthood grown at 20°C, normalized to histone, shown mean +SEM, N=3, t-test, ns.

2.7 Tissue specific regulation

The challenge to detect regulation of *vha-13* by *mir-1* using western blot is the ubiquitous expression of *vha-13*, while *mir-1* most probably works in a muscle-autonomous fashion. VHA-13 levels can be strongest observed in hypodermis, excretory cell, canal and gut (Figure 18a). *vha-13* protein levels can also be measured in the pharyngeal isthmus, which is muscular tissue (Figure 18b). Interestingly, I observed that *mir-1* mutants have increased VHA-13 protein level in the isthmus (Figure 18c+d), suggesting potential regulation. In line with regulation of VHA-13 by *mir-1*, overexpressing *mir-1* decreased VHA-13 expression in the isthmus (Figure 18e). However, this regulation appeared to be independent of the *mir-1* binding site. It is possible that similar regulation might also take place in body wall muscle, but the expression levels were too difficult to determine because background from other tissues, especially hypodermis, masked the low expression in body wall muscle. Both the tissue-specificity of *mir-1* expression and the high expression of *vha-13* outside of *mir-1* expressing tissues explain why it is challenging to measure target regulation both by immunofluorescence and Western blot.

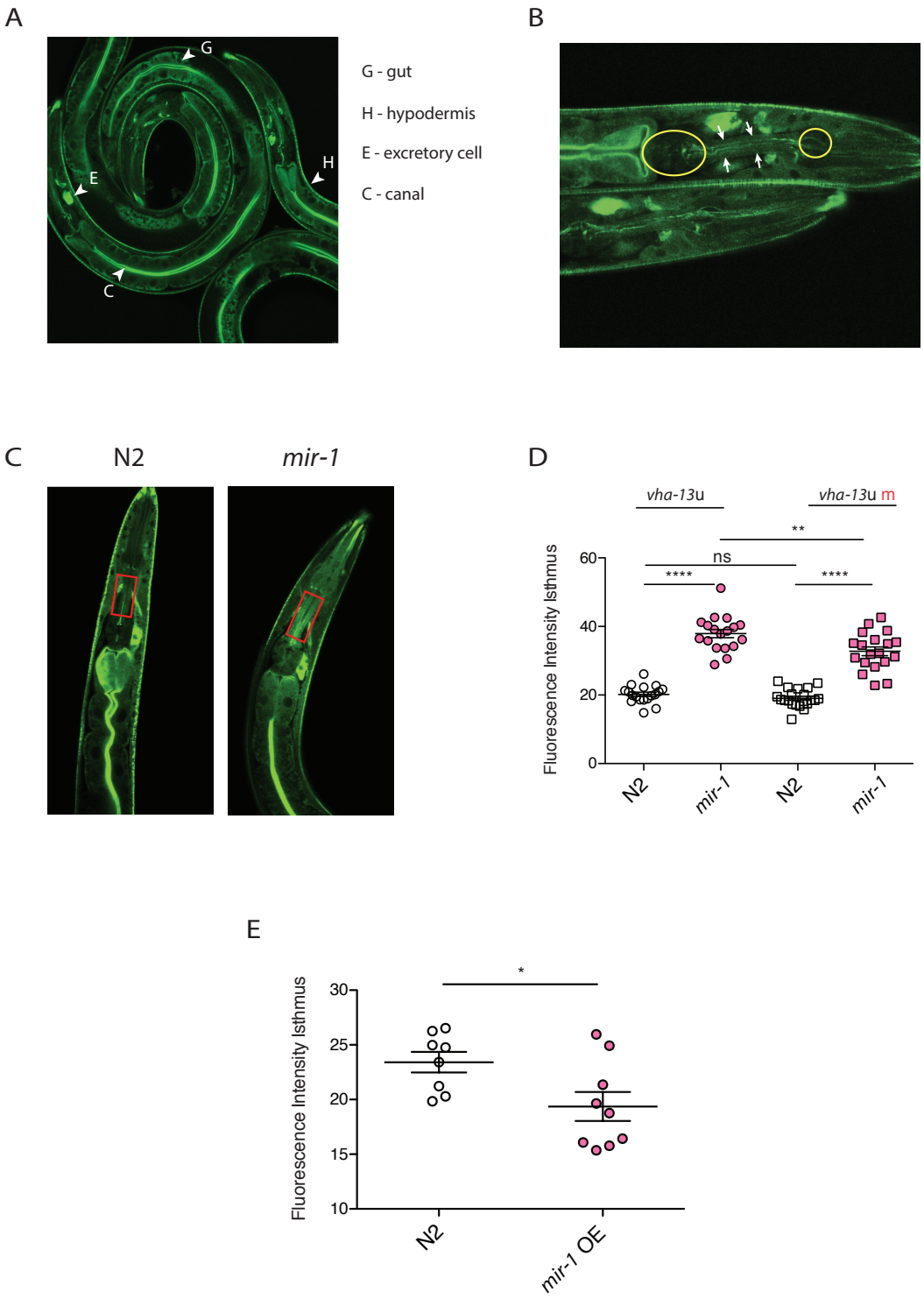


Figure 18. *Mir-1* regulates *vha-13* protein level in the isthmus
(A) Representative confocal image showing expression pattern of *vha-13u*. Arrow indicate tissues that strongly express *vha-13*. G - Gut, H - Hypodermis, E - excretory cell, C - canal. **(B)** Close-up on pharynx of vha-13u, yellow circles mark the metacorpus (small circle) and the terminal bulb (large circle), where expression levels of vha-13 are low. White arrowheads indicate the isthmus, where expression of vha-13 is observed. **(C)** Representative picture of pharyngeal expression of vha-13 in wild-type and *mir-1* mutant worms. Red box marks the isthmus. **(D)** Quantification of fluorescent intensity of the isthmus as in **(C)**, each dot represents one animal, >15 worms per condition, line and error bar indicate mean ± SD of one representative experiment, N=3, t-test, **, p<0.01, ****, p<0.0001. **(E)** Quantification of fluorescent intensity of VHA-13::neongreen expression in the isthmus in wild-type and *mir-1*

OE worms, line and error bar indicate mean ± SD of one representative experiment, N=3, t-test, *, p<0.05

To overcome the problem of high expression in tissues other than the muscle, I sought to investigate *vha-13* and *daf-16* regulation in the muscle using tissue specific promoters. To do so, I first used a myo-3 driven *daf-16* reporter strain containing the endogenous *daf-16* 3´UTR. Notably, DAF-16 protein levels were highly upregulated in *mir-1* mutants compared to wild-type controls as measured by Western Blot analysis and microscopic imaging (Figure 19a). The upregulation was not due to a general increase in *myo-3* transcription in *mir-1* mutants as measured by RT-qPCR (Figure 19b). To further investigate whether this effect is due to a direct regulation by *mir-1*, I am currently mutating the binding site for *mir-1* in the muscle-specific *daf-16* expressing plasmid. To further exclude a regulation of the extrachromosomal plasmid itself by *mir-1*, I performed Western Blot analysis using a strain expressing the *myo-3* promoter plasmid only driving a GFP. No regulation was observed when comparing *mir-1* mutants to wild-type controls (Figure 19c). To investigate muscle-specific *vha-13* levels, I cloned *vha-13* cDNA and its endogenous 3´UTR into a *myo-3p::mcherry::flag::HA* expression vector and created an extrachromosomal line by microinjection. Using Western Blot technique, VHA-13 was observed at the estimated size and the band disappeared upon *vha-13* RNAi (data not shown), confirming that *vha-13* was expressed. When I compared muscle-specific *vha-13* expression in *mir-1* mutants compared to wild-type controls, the construct was upregulated in *mir-1* mutants. (Figure 19d+e). Interestingly, when I swapped the 3´UTR of the construct to *unc-54* 3´UTR, which lacks the *mir-1* binding sites, the construct was no longer regulated in *mir-1* mutants (Figure 19f+g). This suggests that *mir-1* directly regulates *vha-13* by binding to its 3´UTR. I also investigated the possible regulation of other predicted targets, like *zyx-1*, but could not observe any regulation of a *zyx-1P::zyx-1::GFP* reporter, further narrowing the pool of true targets that mediate the *mir-1* dependent effect on PQC to *vha-13* and *daf-16* (Figure 19h).

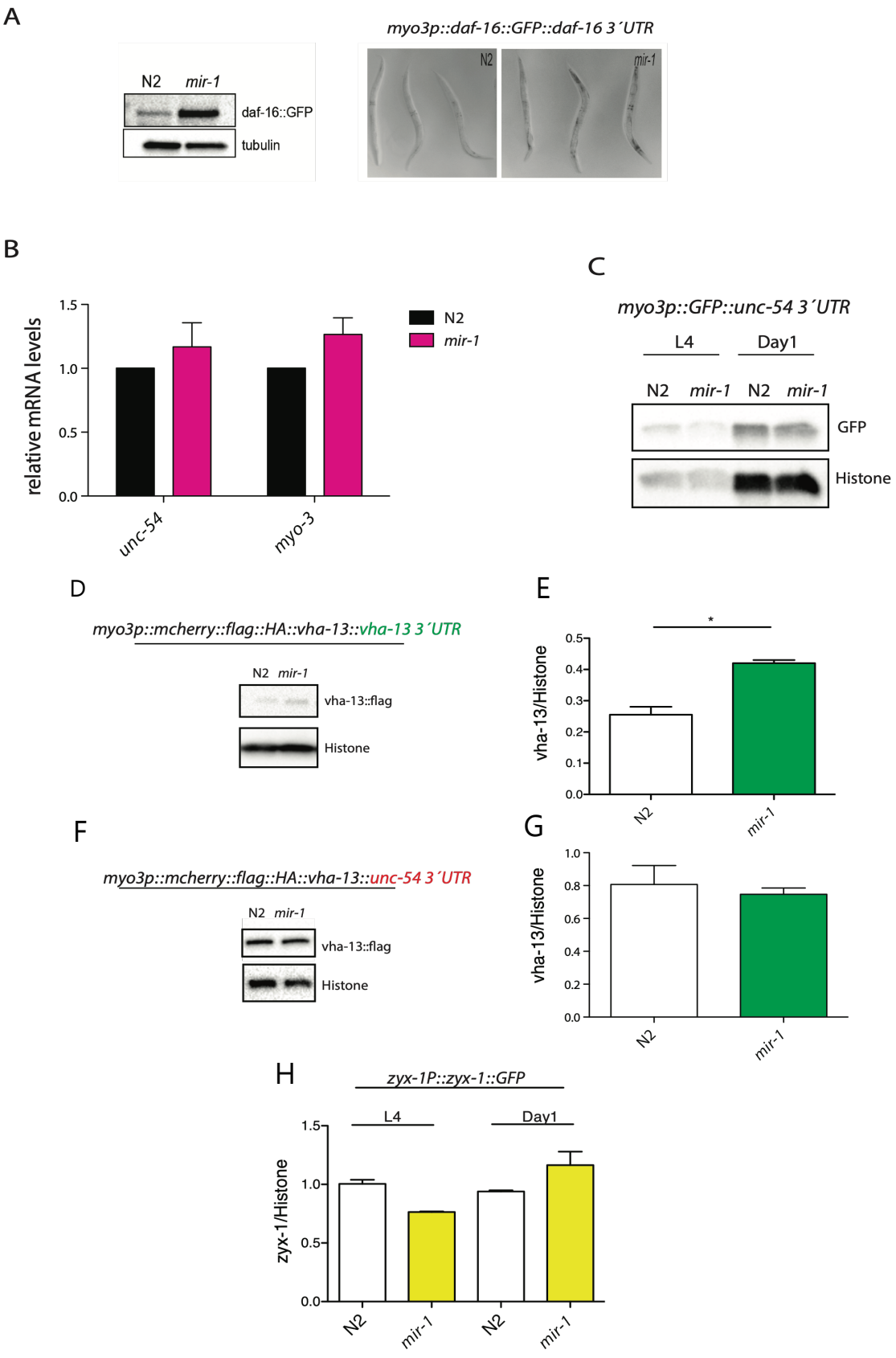


Figure 19. Muscle-specific *daf-16* containing its endogenous 3´UTR is regulated in *mir-1* mutant background, whereas muscle-specific *vha-13* without its endogenous 3´UTR cannot be regulated by *mir-1*. (A) Representative Western Blot of total protein extracts of synchronized day 1 *myo3p::daf-16::GFP::daf-16 3´UTR* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-GFP antibody (left). Representative pictures of muscle expression of *myo3p::daf-16::GFP::daf-16 3´UTR* transgenic worms in *mir-1* mutant and wild-type background(right). **(B)** qRT-PCR showing relative mRNA levels of *unc-54* and

myo3 normalized to *cdc-42* in *mir-1* mutants compared to wild-type controls, shown mean +SEM, N=3, individual t-tests, ns. **(C)** Western Blot of total protein extracts of synchronized L4 and day 1 *myo3p::GFP::unc-54 3´UTR* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-GFP antibody. **(D)** Representative Western Blot of total protein extracts of synchronized L4 *myo3p::mcherry::flag::HA::vha-13::vha-13 3´UTR* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody. **(E)**Quantification of Western Blot analysis showing *flag::vha-13* levels normalized to histone, shown mean +SEM, N=2, t-test, *, $p<0.05$. **(F)** Representative Western Blot of total protein extracts of synchronized L4 *myo3p::mcherry::flag::HA::vha-13::unc-54 3´UTR* transgenic worms in *mir-1* mutant and wild-type background incubated with anti-flag antibody. **(G)** Quantification of Western Blot analysis showing *flag::vha-13* levels normalized to histone, shown mean +SEM, N=3, t-test, ns. **(H)** Quantification of Western Blot analysis showing *zyx-1::GFP* levels in L4 and Day 1 adults in wild-type and *mir-1* mutant worms, normalized to histone, shown mean +SEM, N=2, t-test, ns.

2.8 *mir-1* affects lysosomal biogenesis

Although my data suggested regulation of v-ATPase subunit, *vha-13*, by *mir-1*, regulation of this one gene was subtle and difficult to see above background. On the other hand, *mir-1* target predictions indicate multiple subunits of the v-ATPase as potential regulatory targets. I therefore reasoned that *mir-1* might more generally affect lysosomal structure and function. To test this hypothesis, I first used a *lmp-1p::lmp-1::GFP* reporter strain. LMP-1 protein specifically localizes to the membrane of lysosomes and is therefore used as marker for lysosomal biogenesis (Hermann et al. 2005). Protein levels of LMP-1 were significantly increased in *mir-1* mutants compared to wild-type control, as measured by Western blot analysis (Figure 20a,b). The v-ATPase hydrolyzes ATP to pump protons across the membrane to acidify the lumen of the lysosome (Beyenbach and Wieczorek 2006). I therefore asked whether *mir-1* mutants affect the number of acidified lysosomes. Using Lysotracker Red, a dye that targets mild to strong acidic membranous structures such as lysosomes (Chazotte 2011), I observed an increase in staining intensity in *mir-1* mutants (Figure 20c,d). Due to technical limitations, lysotracker staining could only be observed in the worm intestine. This result leaves open the question as to whether lysosomes are also regulated in the muscle and whether *mir-1*, being expressed in muscle tissue, might have a non-autonomous effect. Nonetheless, the overall increase in lysosome biogenesis is consistent with the upregulation of lysosomal components, such as the v-ATPase subunits, and a possible cause of *mir-1*-dependent regulation of proteostasis.

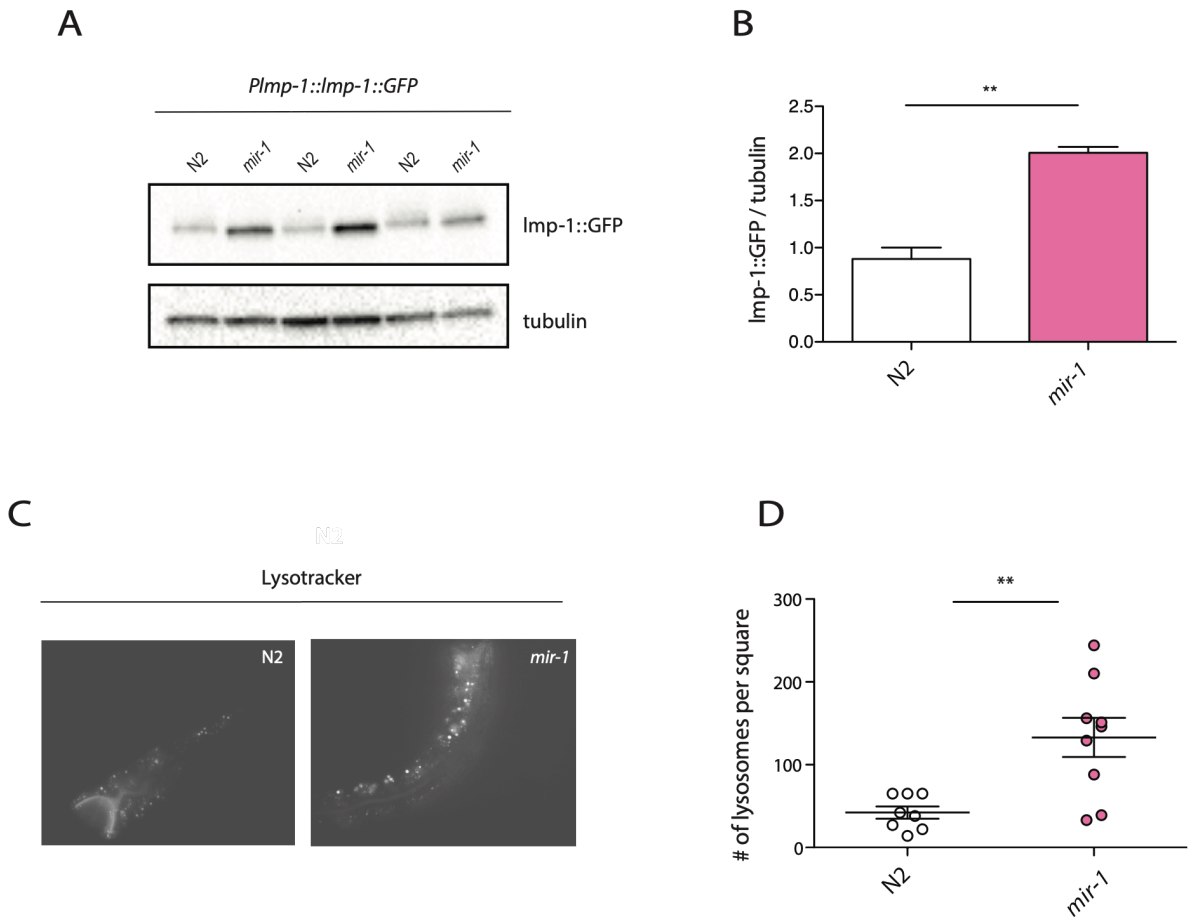


Figure 20. *mir-1* mutants present an increase in acidified lysosomes and increased lysosomal biogenesis. (A) Western Blot for *lmp-1::GFP* in wild-type and *mir-1* mutants at L4 stage. **(B)** Quantification of Western blot (like in (A)), N=3, line and error bars indicate mean ± SEM, t-test, **, p<0.01. **(C)** Representative images of lysotracker staining in wild-type and *mir-1* mutants at L4 stage. **(D)** Quantification of lysotracker images using a predefined squared area approximately spanning the second to fourth gut cell. Quantification was performed using Image J. N=3, line and error bars indicate mean ± SEM of one representative experiment, t-test, **, p<0.01.

2.9 *Mir-1* affects autophagic flux

One of the main functions of the lysosome is to promote autophagy through fusion with the autophagosome. Therefore, I measured autophagic flux using a transgenic reporter strain expressing GFP-tagged LGG-1. LGG-1::GFP associates with the inner and the outer membrane of the phagophore. While the LGG-1::GFP located in the outer membrane, is recycled to the cytosol, LGG-1::GFP attached to the inner membrane is degraded by lysosomal proteases. GFP is resistant to lysosomal pH and degrades more slowly. The ratio of free GFP to the sum of total GFP (free GFP and LGG-1::GFP) can be used to asses autophagic flux by Western Blot analysis (Lefebvre et al. 2014). In line with the increase in lysosomal mass, I

observed an increase in autophagic flux in *mir-1* mutants compared to wildtype controls (Figure 21a,b). Starvation was used as control to confirm that the ratio of free GFP/total GFP is increased upon autophagy-inducing conditions. To further investigate whether the increase in autophagic flux is causative for the increased motility of *mir-1* mutants, I knocked down components of the autophagy machinery in *mir-1;Q35* worms by RNAi in order to rescue the motility phenotype. RNAi against autophagy components did not cause any toxicity as measured by knocking down essential autophagy genes in wildtype worms not expressing Q35. (Figure 21d). Despite variability, motility was modestly but significantly reduced in *mir-1;Q35* worms treated with RNAi against *bec-1* and *vps-33.1* (Figure 21c). Knockdown of v-ATPase subunits caused a much more pronounced phenotype. V-ATPase knockdown is embryonal lethal and therefore I used RNAi treatment L4-onwards. RNAi against most autophagic genes does not cause embryonic lethality. For consistency, I used RNAi against autophagic genes L4 onwards instead of egg-on, which could have weakened the effect on motility even though worms were exposed to the RNAi for 8 days. RNAi clones were thus further tested for their ability to block autophagic flux using the above introduced LGG-1::GFP reporter strain. In this case, the ratio of GFP/total GFP was unchanged compared to luciferase control, sugesting the knockdown efficiency of autophagy genes might be insufficient to observe any interpretable results at least when RNAi was used L4-onwards (data not shown).

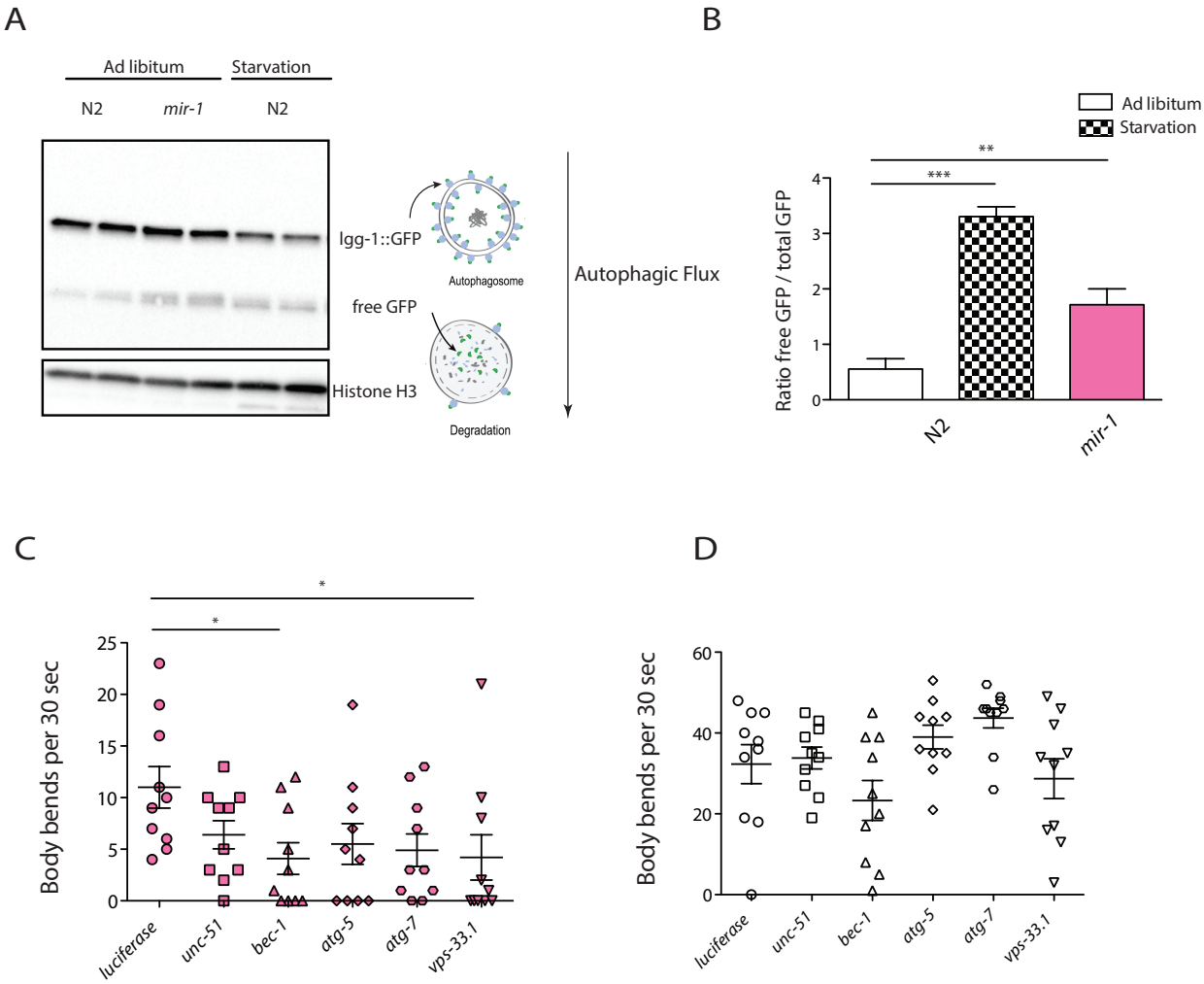


Figure 21. *mir-1* mutants present an increase in autophagic flux.
(A) Western blot of total protein extracts of synchronized young adult *GFP::LGG-1* transgenic worms incubated with anti-GFP antibody. Starvation was induced by transferring worms to unseeded NGM plates for 4h and was used as positive control for the induction of the autophagy reporter. The cleaved GFP forms (around 28 kDa) correspond to the GFP degradation products in the autophagolysosome. To analyze autophagic flux, the ratios of free GFP to total GFP (lgg-1::GFP + free GFP) were calculated.
(B) Quantification of Western Blot as in (A). N=2, line and error bars indicate mean ± SEM, individual t-tests, **, p<0.01, ***, p<0.001. **(C)** Quantification of *mir-1;Q35* motility grown on RNAi against components of autophagy machinery L4 onwards. Motility was scored on day 8 of adulthood using swimming assay, N=2, line and error bars indicate mean ± SEM, 1-way ANOVA, Dunnett´s multiple comparisons test,*, p<0.05. **(D)** Quantification of N2 motility grown on RNAi against autophagy machinery L4 onwards. Motility was scored on day 8 of adulthood using swimming assay, N=1, line and error bars indicate mean ± SEM, 1-way ANOVA, Dunnett´s multiple comparisons test.

To conclude, I generated evidence that *mir-1* regulates proteostasis through the regulation of lysosome biogenesis and autophagy. This effect might be mediated by *mir-1*-dependent regulation of *daf-16* and *vha-13*, and possibly via other v-ATPase subunits.

2.10 Non-autonomous regulation by *mir-1*

The finding that muscle *mir-1* regulates lysosomal acidification in the intestine of *C. elegans* made me wonder whether *mir-1* might function cell non-autonomously. Non-autonomous actions of miRNAs are highly debated in the field (O'Brien et al. 2018) (H. Scott 2017) (M. H. Sohel 2016) and it would therefore be interesting to shed some light on this controversially discussed topic. I formulated two hypotheses: Either *mir-1* was secreted from muscle to intestinal tissue to directly regulate target mRNAs in the intestine or *mir-1* was regulating factors in the muscle tissues, which indirectly affected gene expression in the intestine (Figure 22a). First, I aimed to exclude the possibility that *mir-1* is autonomously regulating gene expression by simply being expressed in the intestine. To test this possibility, I used a reporter line, which expresses GFP under the control of the *mir-1* promotor. This reporter solely showed GFP-expression in the body wall muscle and pharynx of *C. elegans* (Figure 22b). To further exclude that *mir-1* is expressed in the intestine of the worm, I also performed in-situ hybridization, to detect low abundant RNAs. As expected, a strong signal for *mir-1* in the pharynx and body wall muscle could be observed (Figure 22c). Unfortunately, even after intensive optimization of the technique, I was not able to fully conclude whether the subtle staining in the intestine of the worms was a real signal or background staining. At the same time, Yoshiki Andachi and Yuji Kohara published a method on in-situ hybridization of miRNAs in *C. elegans* (Andachi and Kohara 2016). Due to the very weak and inconsistent staining in the intestine, Andachi et al. reported that it was more likely that the staining was an artifact (personal communication). *Mir-1* seems to be specifically expressed in the muscle tissue of *C. elegans,* although I cannot fully exclude that *mir-1* might be secreted to gut tissue.

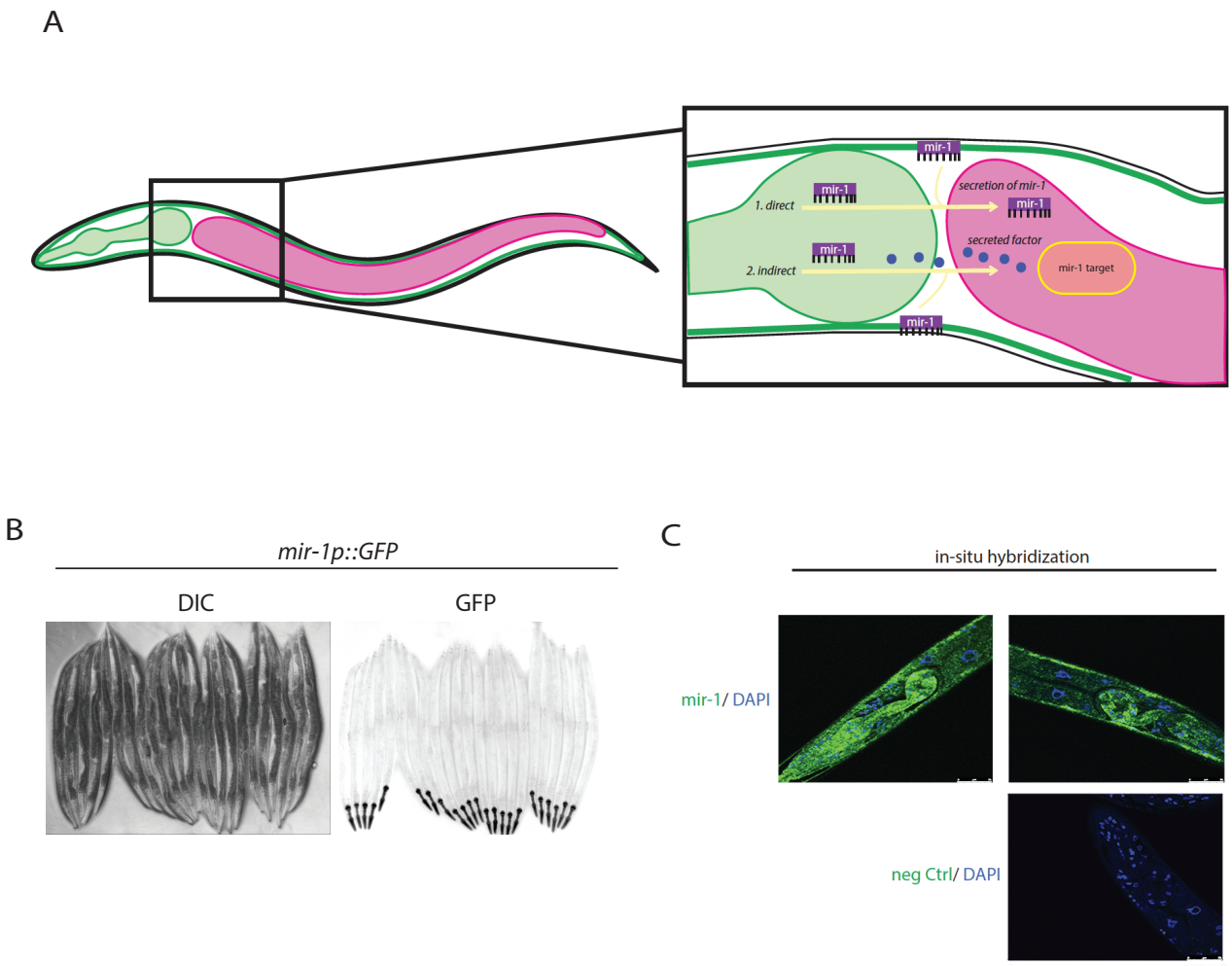


Figure 22. *Mir-1* is expressed in muscle tissue, but not gut tissue.
(A) Model showing two possible mechanisms of target regulation in the gut by *mir-1*: 1. direct regulation 2. indirect regulation. **(B)** Representative pictures showing *mir-1p::GFP* transgenic worms at day 1 of adulthood under basal conditions, N=2. **(C)** Representative pictures of ISH analysis for detection of *mir-1* (green) in day 1 adult worms, N=2.

As another test to see whether *mir-1* is secreted to the gut to directly regulate gene expression, I used a construct containing three copies of the seed sequence of *mir-1* in the 3´UTR (provided by Dr. Christoph Geisen). The 3´UTR was fused to GFP under the control of the gut specific *ges-1* promoter (Figure 23a). I injected the ges-1p::GFP::3xseed construct into *C. elegans* and crossed the respective strain into *mir-1* mutant background. Western Blot analysis of the GFP signal showed a mild de-repression of the construct in *mir-1* mutants (Figure 23b).

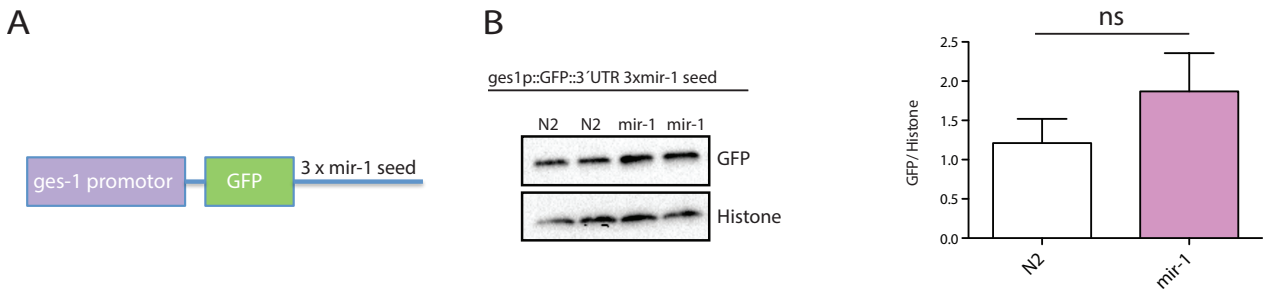
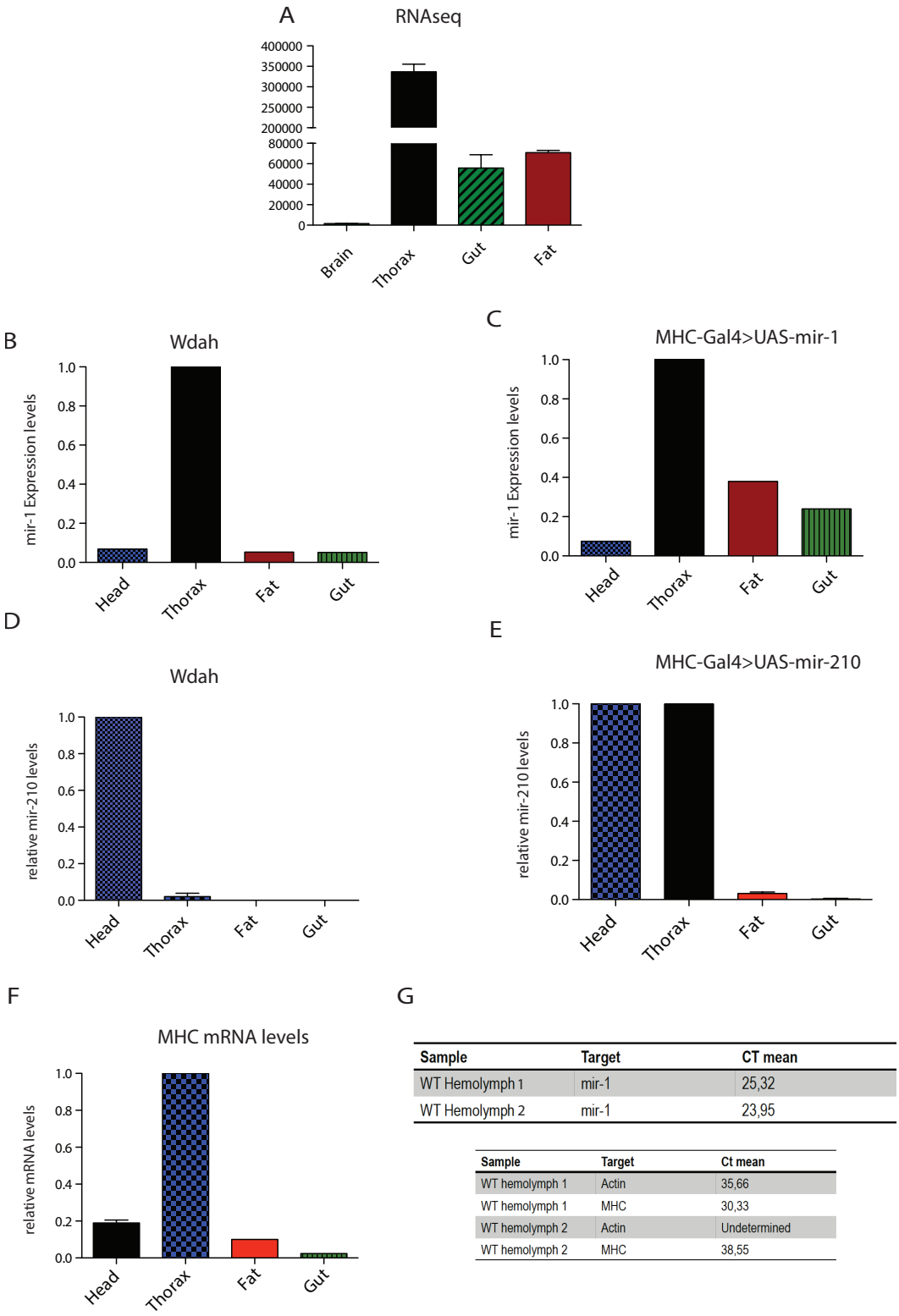


Figure 23. A gut-specific reporter for *mir-1* regulation seems to be regulated *C. elegans*. (A) Schematic overview of reporter plasmid showing GFP under the control of a 3´UTR including 3 copies of *mir-1* seed region expressed under *ges-1* promoter. **(B)** Western Blot of total protein extracts of day 1 *ges-1p::GFP::3´UTR 3x mir-1 seed* transgenic worms in wild-type or *mir-1* mutant background incubated with anti-GFP antibody, N=3 (left). Quantification of Western Blot showing GFP levels normalized to Histone, N=3, t-test, ns.

Although this initial finding suggested that *mir-1* might regulate gene expression non-autonomously, the effect was too mild for any interpretation and proper controls for this effect need to be carried out. I reasoned that other model organisms whose tissues are easier to dissect might allow me to address some of these issues more directly. Interestingly, RNAseq data of miRNAs in *Drosophila melanogaster* generated by the group of Prof. Linda Partridge showed that *mir-1* was not only detected in muscle tissue (thorax), but also in the gut and in fat tissue of the flies (Figure 24a). This result was quite interesting as *mir-1* was described to be specific for muscle tissue expression in *D. melanogaster* (Aboobaker et al. 2005). I confirmed the RNAseq results by RT-qPCR (Figure 24b) and obtained with the help of Dr. Carina Weigelt UAS-mir-1 flies that were crossed with a muscle-specific driver line (MHC-Gal4). Interestingly, overexpressing *mir-1* under a muscle-specific promoter not only increased *mir-1* levels in the thorax, but also in fat and gut tissue (Figure 24c). To exclude that this effect is due to a leakiness of the promoter, I overexpressed *mir-210*, a brain-specific miRNA (Figure 24d) under the same MHC-Gal4 promoter and observed an increase of *mir-210* in the thorax, but not in the gut (Figure 24e). *Mir-210* levels in fat tissue were slightly above detection limits in MHC-Gal4<UAS-mir-210 flies, raising the possibility of promotor leakiness in this tissue. I therefore dissected flies and checked MHC levels. In fat tissue and gut I could detect MHC expression levels slightly above detection limit (Figure 24f). At this point, it is difficult to resolve whether the slight expression of MHC in gut and fat tissue can account for the increase in *mir-1* levels in these tissues in MHC-Gal4<UAS-mir-1 flies.

To further investigate whether *mir-1* was transported extracellularly, I extracted hemolymph of wild-type flies and determined whether *mir-1* could be detected in the circulation of the flies. Interestingly, *mir-1* levels could be detected in the hemolymph (Figure 24g, upper panel), suggesting that *mir-1* was at least secreted and stably circulating in flies. To exclude that the *mir-1* levels detected in the hemolymph resulted from sheared cells during the hemolymph extraction process, I checked for cellular components like actin and MHC in the hemolymph, which were both below detection limits and therefore not present in hemolymph (Figure 24g, lower panel).

To conclude, we generated evidence that *mir-1* is circulating in Drosophila hemolymph. Furthermore, we could show that *mir-1* is present in tissues other than muscle tissue. However, whether *mir-1* is regulating gene expression in a non-autonomous fashion, either in a direct or indirect manner, remains to be seen.



G

Sample	Target	CT mean
WT Hemolymph 1	mir-1	25,32
WT Hemolymph 2	mir-1	23,95

Sample	Target	Ct mean
WT hemolymph 1	Actin	35,66
WT hemolymph 1	MHC	30,33
WT hemolymph 2	Actin	Undetermined
WT hemolymph 2	MHC	38,55

Figure 24. *mir-1* is detectable in Drosophila hemolymph and is increased in fat and gut tissue in flies overexpressing *mir-1* under a muscle-specific promotor.
(A) RNAseq Data provided by Linda Partridge shows *mir-1* expression in muscle tissue (thorax) but also in fat and gut tissue. **(B)** Expression levels of *mir-1* in head, thorax, fat and gut of wild-type flies as measured by TaqMan qPCR normalized to *SnoR442*, tissues extracted from 25 flies, N=1. **(C)** Expression levels of *mir-1* in head, thorax, fat and gut of MHC-Gal4>UAS-mir-1 flies as measured by TaqMan qPCR normalized to *SnoR442*, tissues

extracted from 25 flies, N=1. **(D)** Expression levels of *mir-210* in head, thorax, fat and gut of wild-type flies as measured by TaqMan qPCR normalized to *SnoR442*, tissues extracted from 25 flies, N=2. **(E)** Expression levels of *mir-210* in head, thorax, fat and gut of MHC-Gal4>UAS-mir-210 flies as measured by TaqMan qPCR normalized to *SnoR442*, tissues extracted from 25 flies, N=2. **(F)** Expression levels of MHC in head, thorax, fat and gut of wild-type flies as measured bei RT-qPCR normalized to actin, tissues extracted from 25 flies, N=1. **(G)** Expression levels of *mir-1* in hemolymph of wild-type flies as measured by TaqMan qPCR, N=2 (upper table). Expression levels of MHC and actin in hemolymph of wild-type flies as measured by qRT-PCR, N=2 (lower table).

3 Discussion

In the following chapter I will conclude and discuss the findings of my PhD thesis.

I present evidence that *mir-1* decreases VHA-13 protein level by directly suppressing *vha-13* mRNA in the muscle of wild-type *C. elegans.* This effect might be even more evident in the aged C. elegans. *Mir-1* also decreases DAF-16 protein level in the muscle, possibly through a direct mechanism that still needs to be investigated. Downregulation of VHA-13 and possibly other v-ATPase subunits as well as DAF-16 decreases lysosomal biogenesis, lysosomal acidification and autophagic flux and manifests in polyglutamine aggregation and a decline in motility. Furthermore, I could provide evidence that *mir-1* might act in a non-autonomous fashion.

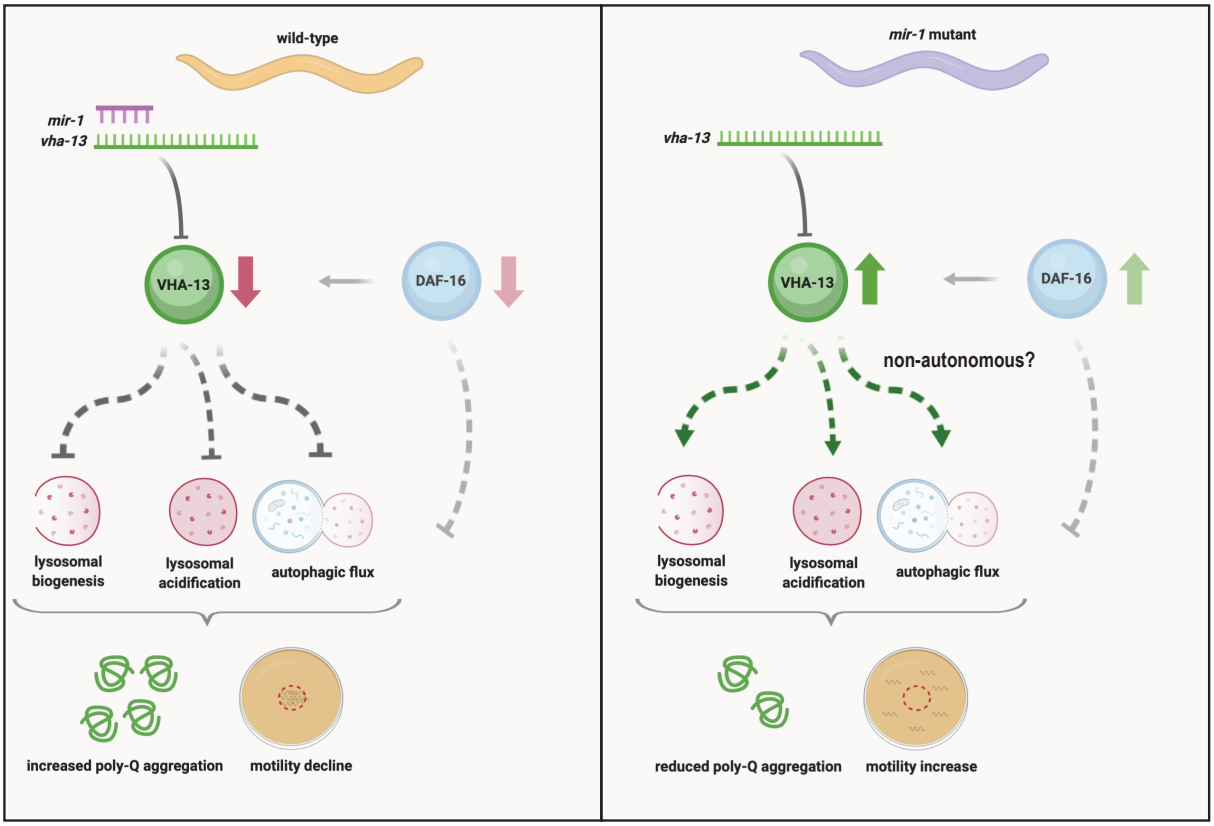


Figure 25. Working Model. In wild-type worms, *mir-1* represses *vha-13* mRNA levels in the muscle of *C. elegans*, causing decreased VHA-13 protein levels. In a possible parallel pathway, *mir-1* also represses DAF-16 protein level in the muscle of *C. elegans*. Downregulation of VHA-13 and possibly other v-ATPase subunits as well as DAF-16 decreases lysosomal biogenesis, lysosomal acidification and autophagic flux and manifests in polyglutamine aggregation and a decline in motility (left). In *mir-1* mutants, *vha-13* mRNA cannot be repressed by *mir-1* and VHA-13 protein levels as well as DAF-16 protein levels are increased. Upregulation of VHA-13 and possibly other v-ATPase subunits as well as DAF-16 increases lysosomal biogenesis, lysosomal acidification and autophagic flux and manifests in reduces polyglutamine aggregation and improved motility (right).

3.1 Muscle ageing and *mir-1* and its potential conservation

Skeletal muscle is a major site of metabolic activity, accounting for about 40% of the total body mass, making it the most abundant tissue in the human body. Muscle ageing is described as a gradual decline of muscle mass and strength. Although the mechanism involved is only partially understood, it is thought that the accumulation of several events lead to muscle ageing. An accumulation of dysfunctional cellular components, especially mitochondria (Carter et al. 2018) (Mass et al. 2017), probably due to a decrease in cellular repair processes like autophagy and ubiquitin-proteasome pathway in combination with decreased protein synthesis of muscle proteins (Masiero et al. 2009) (Bonaldo and Sandri 2013) (Piccirillo et al. 2014) play important roles in muscle ageing. Autophagy plays a crucial role in maintaining muscle mass (Masiero et al. 2009) (Escobar et al. 2019) as well as regenerative potential in geriatric satellite cells (García-prat et al. 2016). Recent reviews discuss autophagy as promoter of longevity (Hansen and Rubinsztein 2018) and as potential therapy to enhance aged muscle regeneration (David E. Lee et al. 2019), highlighting the importance of this research.

In my PhD, I identified the highly conserved muscle-enriched miRNA *mir-1* as negative regulator of lysosomal biogenesis and autophagy. *Mir-1* mutants were protected against polyglutamine- as well as human A-beta-induced paralysis, possibly via an upregulation of acidified lysosomes and an increase in autophagic flux. As I measured both, lysosomal biogenesis as well as autophagic flux only on day 1 of adulthood, I can not conclude whether both processes are also increased during the process of ageing or whether the protective effect against polyQ-induced proteotoxicity is rather due to an improved PQC only early in life. However, mRNA levels of v-ATPase subunits are maintained between day 1 and day 4 of adulthood in *mir-1* mutants, whereas the levels dramatically decline in wild-type worms, suggesting a better functioning PQC network throughout the process of ageing in

mir-1 mutants. Further research is needed to investigate whether the autophagic flux and lysosomal biogenesis/acidification are also increased in ageing. As the maintenance of mRNA levels of several v-ATPase subunits in *mir-1* mutants could potentially lead to an improved muscle health during ageing, it would be interesting to explore whether overall muscle health and function, including mitochondrial function, is increased in *mir-1* mutants. I obtained preliminary evidence by showing that worms lacking *mir-1* present a more juvenile posture and motility up to day 20 of adulthood when grown at 25°C. Further experiments focusing on muscle morphology and integrity are needed to strengthen the potential benefit of *mir-1* deletion on muscle ageing.

Whether *mir-1* is upregulated in the aged human muscle, is controversial, because only few studies analyzed microRNAs in aged muscle tissue and the heterogeneity in human studies is problematic to identify small quantitative changes in tightly controlled pathways (Nielsen et al. 2014) (Jung et al. 2018). Nonetheless, *mir-1* was recently reported as an interesting biomarker to measure frailty (Rusanova et al. 2019). Furthermore, it is well established that *mir-1* is downregulated upon exercise (Zacharewicz, Lamon, and Aaron 2013) in muscle and also in the circulation (Denham et al. 2018). Furthermore, autophagy declines in human skeletal muscle with ageing and this decrease contributes to sarcopenia, whereas exercise increases autophagy in skeletal muscle in older subjects (Park 2019). Given my results on the link between *mir-1* and autophagy, *mir-1* might be an interesting target to investigate in those studies.

Based on this mammalian data, it would be interesting to investigate the effect of exercise on *mir-1* levels in C. elegans to further understand the underlying mechanism of *mir-1* regulation during physical activity and its impact on PQC. Monica Driscoll established *C. elegans* as a useful model organism to study exercise- She could show that acute swimming-induced changes in *C. elegans* share common features with some acute exercise responses that are reported in humans (Laranjeiro et al. 2017). Interestingly, adaptations like body wall muscle improvements in structural gene expression, locomotory performance and mitochondrial morphology occurred after multiple daily swim sessions. Adaptions to exercise not only occurred in muscle tissue, but also extended neuromuscular and intestinal healthspan and enhanced learning ability and protected against neurodegeneration (Laranjeiro et al. 2019). The molecular processes that mediate these system-wide exercise benefits, especially as applies to “off targets” that do not actively participate in training directly, remain poorly understood. *Mir-1* would be an

interesting candidate to study in the regulation of exercise-induced autonomous as well as non-autonomous health benefits.

In *C. elegans*, *mir-1* is downregulated with age in wild-type worms (Lucanic et al. 2013)(Ibáñez-Ventoso et al. 2006). However, *mir-796*, a *mir-1* family member in *C. elegans* is one of the most upregulated miRNAs in *C. elegans* ageing (Kato et al. 2011). Although the group of Slack already suggested v-ATPase subunits as potential targets of *mir-1/mir-796* (Kato et al. 2011), no studies investigated the regulation of the v-ATPase by *mir-1* so far. Also, a possible link between *mir-1* and autophagy is still unclear. Only recently, one study by Hua et al showed that *mir-1* inhibits autophagy related 3-mediated (ATG3) autophagy in a cell culture model (Hua, Zhu, and Wei 2018). This study could hint towards an evolutionary conserved mechanism of *mir-1* regulating autophagy.

In conclusion, I generated evidence that *mir-1* regulates muscle PQC in *C. elegans* by regulating lysosomal biogenesis and thereby possibly affecting autophagic flux. Whether the data generated during my PhD is transferable to higher organisms still needs to be assessed but the strong effect on PQC regulation in the muscle by *mir-1* in *C. elegans* and the conservation of the *mir-1* binding sites in mammalian v-ATPase subunits suggests a possible conservation of this regulation in higher organisms. Finding interventions to modulate autophagy is of interest in ageing research (Hansen and Rubinsztein 2018) (David E. Lee et al. 2019) and *mir-1*, being a potential therapeutic target could open up novel opportunities.

3.2 Target regulation by *mir-1*

MiRNAs regulate thousands of mRNAs by means of complementarity to sequences in the 3´UTR of the transcripts. Complete complementary of the miRNA to the mRNA leads to degradation of the mRNA transcript. However, in most cases, the homology of the miRNA to the mRNA is incomplete and causes only a partial inhibition of mRNA translation, leading to a much milder effect on gene expression (Filipowicz, Bhattacharyya, and Sonenberg 2008). It is therefore expected that most miRNAs cause subtle changes on gene expression, making it rather difficult to identify regulation of defined targets by miRNAs (Selbach et al. 2008). Therefore, I undertook two independent and unbiased approaches to maximize the chances of finding candidates. Bioinformatic target prediction analysis clearly showed a strong clustering of v-ATPase subunits that I confirmed as potential mediators for *mir-1*-dependent PQC in my motility screen. Interestingly, proteomic analysis also identified

two v-ATPase subunits as significantly upregulated proteins in *mir-1* mutants. This finding was quite unexpected as only 56 proteins were upregulated in *mir-1* mutants compared to wild-type. Although, other v-ATPase subunits (including *vha-13*) were not changed in proteomics, detecting two subunits in the small set of changed genes made us confident that v-ATPase subunits could be targets of *mir-1*. Unfortunately, tagging of VHA-19, the most promising candidate, caused a lethal phenotype and could not be further investigated.

The tissue-specific expression of *mir-1* made it challenging to confirm the regulation of *vha-13* and *daf-16* by *mir-1*. However, I could confirm the de-repression of both candidates in the muscle of *mir-1* mutants and furthermore, could provide evidence that *mir-1* directly regulates *vha-13*. Exchanging the complete endogenous 3´UTR against one without *mir-1* binding sites (unc-54 3´UTR) completely abolished the regulation of *vha-13* reporter by *mir-1*, while mutating the *mir-1* binding site in the endogenous 3´UTR did not prevent regulation of the *vha-13* reporter by *mir-1*. It is conceivable that cryptic *mir-1* binding sites might be present in the *vha-13* 3´UTR that provide regulation even when the canonical site is mutated. Sequence analysis of the 3´UTR revealed at least one non-conserved, weaker binding site that we are currently mutating.

Although *vha-13* seems to be an interesting regulatory protein, being implicated in germline rejuvenation (Adam Bohnert and Kenyon 2017), I am speculating that *mir-1* might also be involved in the regulation of other v-ATPase subunits. It has been reported that miRNAs tend to regulate clusters of biological processes (Ribeiro et al. 2019). A striking number of v-ATPase subunits contain at least one *mir-1* binding site, which opens up the question whether *mir-1* might orchestrate the expression of the whole class of v-ATPase subunits. Alternatively, it is conceivable that *daf-16*, another potential target of *mir-1*, could regulate the expression of v-ATPase subunits, since the group of Carvalho showed that *daf-16* directly enhances transcription of v-ATPase subunits by binding to their promoter region (Baxi et al. 2017). Further experiments are needed to investigate whether there is crosstalk between the targets *daf-16* and *vha-13* or whether they work in parallel pathways to regulate *mir-1*-dependent muscle PQC. Furthermore, whether *daf-16* is a direct target of *mir-1* still needs to be elucidated.

Another interesting, yet puzzling finding in my PhD work is the organismal-wide maintenance of v-ATPase mRNA levels in *mir-1* mutants during the process of ageing, despite the tissue-specific expression of *mir-1*. *Vha-13* mRNA levels seem to be in particular stable during the ageing process in *mir-1* mutants. Furthermore, overall protein levels of VHA-13, (as measured by western blot of the endogenously

tagged protein), are slightly upregulated in *mir-1* mutants at day 4 of adulthood, although on day 1 of adulthood, the regulatory effect on VHA-13 protein can only be observed in muscle tissue. Whether this effect can be attributed to an organismal-wide improved PQC due to improved muscle health in the aged worms needs to be further investigated.

Another interesting aspect that I did not focus on during my PhD work is the regulation of retrograde signaling at the neuromuscular junction by *mir-1*. Gary Ruvkun, Joshua Kaplan and John Kim reported that *mir-1* alters muscle sensitivity to acetylcholine (ACh), by regulating the nicotinic acetylcholine receptors UNC-29 and UNC-63 in C. elegans. The same study could show that *mir-1* also regulates retrograde signaling by regulating the muscle transcription factor MEF-2, which results in altered pre-synaptic ACh secretion (Simon et al. 2008). Furthermore, Richard Morimoto showed also in *C. elegans* that a moderate increase in physiological cholinergic signaling at the neuromuscular junction (NMJ) induces the calcium (Ca^{2+})-dependent activation of HSF-1 in post-synaptic muscle cells, resulting in suppression of protein misfolding (Silva, Amaral, and Morimoto 2013). Interestingly, the group or Morimoto further reported that increased acetylcholine (ACh) signaling causes a general imbalance in protein homeostasis in the postsynaptic muscle cells, thereby leading to the premature appearance of polyQ35 aggregates in the muscle of *C. elegans* (Garcia et al. 2007).

It is therefore conceivable that *mir-1* could exert at least part of its function on muscle PQC by regulating neuromuscular communication.

In conclusion, I generated evidence that *vha-13* and *daf-16* play important roles in *mir-1* dependent PQC in the muscle. However, whether this effect is direct or indirect and whether other factors contribute to *mir-1* dependent muscle PQC needs to be further investigated.

3.3 Non-autonomous regulation by *mir-1*

Small RNAs that function in a non-cell autonomous manner are becoming increasingly recognized as regulatory molecules with the potential to transmit information between cells or even organs (Sarkies and Miska 2014).

During the course of my work, I made several observations that could be consistent with such cell-non-autonomous regulation by *mir-1*:

First, due to technical limitations, lysotracker staining was only measurable in the gut of *C. elegans* and was surprisingly increased in the gut of *mir-1* mutants. Second,

lmp-1::GFP level and autophagic flux seem to be increased ubiquitously and third, at least in middle-aged worms (day 4), v-ATPase subunits seem to be increased ubiquitously as well.

These findings raise the question whether the muscle-specific *mir-1* might regulate PQC non-autonomously.

Several decades ago, it was already reported that plant small-interference RNAs (siRNAs), could spread from one part of the plant to cause gene silencing (PGTS) in another part of the plant (Voinnet and Baulcombe 1997) (Palauqui et al. 1997). While the mobility of siRNAs and its consequences are meanwhile well documented, less is known about the mechanisms underlying non-autonomous effects of other classes of sRNAs, such as miRNAs. Interestingly, miRNAs are not only present in the circulation (Weber et al. 2010) (Chen et al. 2008) (Cogswell et al. 2008), but circulating miRNAs are also reported to be immensely stable (Mitchell et al. 2008). This is attributed to the fact that many circulating miRNAs are found in vesicles like exosomes (Gallo et al. 2012), suggesting a regulated release process. Although some studies reported that circulating miRNAs can be taken up and even regulate gene expression in recipient cells, less is known about the process of uptake and whether there is a directed release or if miRNAs are randomly taken up by cells through endocytosis (O'Brien et al. 2018).

C. elegans, being an easily dissectible model organism, could help to shed some light on the exact mechanism of miRNA secretion, uptake and gene regulation in recipient cells.

Using a *mir-1* translational reporter, I could show that *mir-1* is indeed solely expressed in muscle tissue of *C. elegans*. However, although *in situ* hybridization showed an expected intense staining in body wall muscle and pharynx, I could neither include or exclude whether there might be traces of *mir-1* present in the gut of *C. elegans*. In addition, the gut-specific reporter containing three strong *mir-1* binding sites was visibly derepressed in *mir-1* mutants, but quantitative analysis of fluorescence showed that the difference did not reach significance.

Drosophila data revealed that *mir-1* is circulating in Drosophila hemolymph, but it is not clear whether the elevated *mir-1* levels found in gut and fat in MHC-Gal4>UAS-mir-1 flies are due to leakiness of the promoter.

In summary, despite several approaches, my attempts to decipher the possibility of a non-autonomous regulation in *C. elegans* by *mir-1* were inconclusive due to technical limitations.

However, given the fact that the muscle makes up about 40% of the body mass and that the muscle serves as a source of amino acids to be utilized for energy

production by various organs during catabolic periods, it is conceivable that overall improved muscle health in *mir-1* mutants positively affects processes like the PQC in other tissues. In Drosophila, several studies showed that interventions like muscle-specific upregulation of AMPK (Manley 2013; Stenesen et al. 2013) or mTOR (Patel and Tamanoi 2006) improves systemic stress responses and even lifespan (Stenesen et al. 2013). Furthermore, muscle-specific activation of FoxO/4E-BP signaling in Drosophila promotes proteostasis not only in muscle but also in other tissues such as brain, fat tissue and retina via a systemic decrease in insulin signaling and upregulation of basal autophagy (Demontis and Perrimon 2010). How these effects are communicated between muscle and recipient tissues are not entirely clear. In *C. elegans*, it has even been observed that entire lysosomes can be transferred directly between tissues (Kaoru Yasuda et al. 2011) (Nussbaum-Krammer et al. 2013).

In conclusion, I could show potential tissue non-autonomous effects of *mir-1*. However, whether the *mir-1* mediated effect on gut lysosome levels is a direct or indirect effect needs to be determined. It is conceivable that the non-autonomous effect by *mir-1* is due to improved PQC in the *C. elegans* muscle, which in turn may have a positive effect on organismal PQC. How this improved organismal PQC is communicated (if it is not *mir-1* directly), is yet another challenging variable that would be exciting to investigate in future studies.

4 Future perspectives

My results presented in this thesis open up a number of important questions, as discussed in the previous chapter. These questions could initiate interesting follow-up experiments. In this final chapter, I would like to highlight some future directions.

4.1 Analyzing muscle morphology in ageing *mir-1* mutants

In my PhD work, I showed that *mir-1* deletion was highly beneficial in a muscle-specific polyQ proteotoxicity model. Not only was the load of aggregates significantly decreased but motility was also maintained into high age when control animals were already completely paralyzed. As aggregating proteins are only one of the many causes of muscle ageing, it would be interesting to better characterize the overall health benefit of *mir-1* loss/loss-of-function in the ageing muscle, independent of the polyQ model. A recently published book chapter and a review summarizing recent findings regarding age-related changes in C. elegans are outstanding resources for the age-dependent anatomical changes of *C. elegans* (Herndon, Wolkow, Driscoll & Hall 2017) (Son et al. 2019). Based on these resources, I would suggest the characterization of several aspects of muscle ageing; motility (without polyQ), muscle morphology, mitochondrial morphology in the muscle as well as muscle structural gene expression. A special focus should also be on autophagy and lysosomal acidification in aged *mir-1* mutants to investigate whether they maintain their increased levels of autophagy and lysosomal biogenesis/acidification that I reported in young worms. Mitochondrial function and structure is known to decline with age (Kayo Yasuda et al. 2006) and it would therefore be interesting to investigate mitochondrial muscle physiology in *mir*-1 mutants. As it was described that muscle myofilaments may remain unaffected during ageing (Mergoud, Molin, and Solari 2014), one could focus on measuring pharyngeal morphology and pumping rate as a measure of muscle health as it is described that pumping rate declines with age (Huang, Xiong, and Kornfeld 2004) and that sarcopenia can be observed in the pharynx of *C. elegans* (Chow et al. 2008). Moreover, the degree of sarcopenia correlates with muscle contraction as measured by pumping rate (Chow et al. 2008). Although I did observe a similar decline in pumping rate between *mir-1* mutants and

wild-type worms until day 8 of adulthood, it would be interesting to include more timepoints and measure pumping rate beyond day 8.

As exercise is reported to improve healthy muscle ageing, all the above-mentioned parameter should be investigated in exercised *C. elegans*. First of all, one would need to establish a regulation of *mir-1* upon exercise and whether the presence or absence of *mir-1* influences adaptation to exercise. Monica Driscoll established swimming as a useful physical activity that mimics endurance sports in humans (Laranjeiro et al. 2017). Furthermore, she could observe exercise-induced adaptations to muscle gene expression and mitochondrial morphology and even health improvements in tissues that do not actively contribute to physical activity, like neurons and gut (Laranjeiro et al. 2019). It is conceivable, that *mir-1* might play a role in these adaptive responses to physical activity.

In conclusion, studying different cellular and molecular parameters of the muscle in aged worms would help paint a better picture of muscle aging in worm in general, as well as the role of *mir-1* in this.

4.2 Confirming *mir-1* targets and finding potential upstream regulator

In my PhD work, I provided evidence that *mir-1* directly regulates *vha-13* in the muscle of *C. elegans*, by binding to its 3´UTR. This data is promising, but the expression levels of the reporter lines are not exactly the same, and the difference in regulatory ability could thus be attributed to the different expression levels, rather than the 3´UTR itself. Similarly, it could be argued that the expression levels of *unc-54P*::*vha-13::unc-54 3´UTR* construct exceed the range of *mir-1* regulatory capacity. While this is latter explanation is unlikely, since both constructs are expressed at a relatively low level compared to the highly abundant *mir-1*, it would be advisable to explicitly exclude this possibility by creating more independent lines with more similar expression levels. In addition, by including a co-injection marker that encodes for a fluorescent protein in another tissue I could normalize regulation to this internal control. Furthermore, assessing the levels of *unc-54P::vha-13::vha-13 3´UTR* and *unc-54P*::*vha-13::unc-54 3´UTR* transgenes in a *C. elegans* strain that overexpresses *mir-1* could give further evidence for a direct or indirect regulation. Assessing why regulation of *vha-13* by *mir-1* is prevented upon exchange of the entire 3´UTR, while *vha-13* can still be regulated when the *mir-1* binding site is only mutated, would be another interesting follow-up experiment. To this end we are

currently mutating the non-conserved, cryptic binding site that we recently detected in the *vha-13* 3´UTR.

Furthermore, it would be interesting to investigate whether other potential targets are directly regulated by *mir-1*. As the most interesting other subunit, *vha-19*, cannot be endogenously tagged due to embryonic lethality, extrachromosomal lines could be generated. *Vha-5*, another subunit which is strongly expressed in the pharynx and contains *mir-1* binding sites, is another interesting candidate. Endogenously tagged *vha-5* is viable and is currently investigated as potential *mir-1* target.

Although I could show that a muscle-specific *daf-16* transgene is de-repressed in the muscle of *mir-1* mutants, it is still unclear whether this regulation is direct or indirect and needs to be addressed.

4.3 Molecular crosstalk and potential upstream regulators

In addition, to studying direct targets of *mir-1* it would be interesting to assess if there is also additional crosstalk between the targets themselves. Such crosstalk could exist between *daf-16* and the v-ATPase subunits, given that *daf-16* was shown to directly regulate v-ATPase subunits at the transcriptional level (Baxi et al. 2017).

In addition to crosstalk between putative downstream targets, it is also possible that different miRNAs modulate the effect of *mir-1*. In particular, it has been reported that the *mir-1* family member *mir-796* is one of the strongest upregulated miRNAs in aged *C. elegans* (Ibáñez-Ventoso et al. 2006). As both miRNAs belong to the same family and therefore share the same targets, the presence of *mir-796* in *mir-1* mutants could be compensating and thereby alleviating the full effect of *mir-1* deletion on target mRNA. Although the *mir-1/mir-796* double mutant did not further decrease aggregate number nor further increase motility (data not shown), it would be of interest to assess whether the double mutant might further de-repress VHA-13 or further increase lysosomal function, autophagic flux or even lifespan.

Finally, finding potential upstream regulators would be helpful to further complete the genetical pathway in which *mir-1* regulates muscle PQC. RNAseq data from our lab suggests that *mir-1* is downregulated in *daf-2* mutants. Interestingly, activation of the insulin pathway was reported to downregulate the expression of *mir-1* in human skeletal muscle. (Granjon et al. 2009). Furthermore, *mir-1* is dysregulated in the muscle in type-2 diabetes (Ducluzeau et al. 2001). It would therefore be interesting to further investigate *daf-2* as upstream regulator of *mir-1*.

4.4 Inspecting a link between muscle PQC and overall organismal PQC

Interestingly, I observed increased lysosomal acidification in the gut of *mir-1* mutants. This finding, together with the systemic increase in lysosomal biogenesis and autophagy suggests a possible systemic effect of muscle-specific *mir-1* deletion. Several studies showed that by increasing muscle health, systemic health is also improved and can prolong lifespan (Demontis and Perrimon 2010) (Manley 2013) (Stenesen et al. 2013) (Patel and Tamanoi 2006). Therefore, increased protein quality control in the muscle caused by de-repression of *mir-1* targets could have a systemic effect on *C. elegans* health.

The intestinal biomass is converted into Vitellogenin proteins throughout the ageing process, so that they finally make up 30% - 40% of total worm content (Ezcurra et al. 2018). Interestingly, my proteomics data showed a decrease in several vitellogenin proteins in *mir-1* mutants. It would therefore be interesting to determine fat content as measure of gut ageing.

Fertility as a measure for germline ageing or memory tests as measure for neuronal ageing could be assessed to address whether the healthspan effect in *mir-1* mutants is limited to gut health or whether it is rather a systemic effect.

In addition to assessing whether *mir-1* affects protein quality control outside of the muscle, it will be of interest to understand how such an increase is communicated towards other tissues. *Mir-1* might act cell non-autonomously by being secreted to the gut and directly regulating intestinal gene expression. I provided some preliminary evidence for this hypothesis; but fully answering this question requires a battery of *in vivo* as well as *in vitro* experiments and well-designed controls.

Firstly, one could re-analyze the Drosophila tissue samples. So far, I could not exclude promoter leakiness as cause for elevated *mir-1* levels in fat and gut tissue in Drosophila overexpressing *mir-1* under a muscle-specific promotor. As it has been suggested that mostly the mature forms of miRNAs are secreted and found in circulation (M. Sohel 2016) (Bayraktar, Van Roosbroeck, and Calin 2017) (Sarkies and Miska 2014), one could measure levels of the mature versus the pri- or pre-miRNA in the different tissues to determine whether the miRNA is transcribed in the tissue or might indeed be taken up from the circulation.

Secondly, one could overexpress *mir-1* in a cell system and measure whether *mir-1* is detectable in the supernatant. If *mir-1* is detectable in supernatant, one could

further investigate whether *mir-1* might be circulating in a particular form, like in exosomes. Furthermore, donor cells could be incubated with the *mir-1*-containing supernatant and intracellular *mir-1* levels could be investigated by qRT-PCR. Expressing a *mir-1* reporter in the donor cell line, could answer the question whether secreted *mir-1* is able to regulate gene expression in recipient cells.

Finally, the gut-specific promoter construct containing *3xmir-1* binding site could further be investigated. So far, I explored whether I could observe a de-repression of the reporter in *mir-1* mutants, showing a slight but not significant effect. I could perform the converse experiment, investigating whether muscle-specific overexpression of *mir-1* can suppress the gut reporter.

4.5 Conservation in mammals

Ultimately, it would be interesting to investigate whether the findings of my PhD work are evolutionarily conserved. Based on alignment analysis, I found that the mammalian homolog of VHA-13, ATP6V1A, also contains *mir-1* binding sites in its 3´UTR.

Furthermore, it has been shown that *mir-1* directly regulates expression of ATP6V1A through its 3´UTR as measured by luciferase constructs expressed in a human cancer cell line (Peng et al. 2018), suggesting such conservation may exist. It would therefore be of interest to further investigate this link between *mir-1* and v-ATPase subunits in mammals. For example by performing cell culture experiments, preferably in muscle cell lines (C2C12 cells) to address whether modulation of *mir-1* expression by siRNA or overexpression affects the abundance of ATP6V1A, autophagic flux and lysosomal acidification.

5 Material & Methods

5.1 Genetics and C. elegans handling

5.1.1 Maintenance of *C. elegans*

All strains were maintained at 20°C on nematode growth plates seeded with OP50 strain of *E.coli*. The strains used in experiments were outcrossed at least 4 times to N2 Bristol strain, which was also used as control strain.

To decontaminate the strains, worms were washed off the NGM plates and collected in a falcon tube with M9. After the worms settled, supernatant was removed and worms were bleached for 10 minutes shaking in bleach solution (sodium hypochlorite, potassium hydroxide and water) until worms completely dissolved and only eggs remained. Samples were washed at least two times with M9 buffer and eggs were transferred to fresh culture plates.

5.1.2 Synchronization of worm population

For all experiments, worms were synchronized using egglays. Gravid adult worms were transferred to fresh NGM plates and allowed to lay eggs for 4 hours. Worms were then removed and eggs were allowed to develop until L4, Day 1 or Day 4/5 adults and collected as indicated elsewhere. Egglays were always performed in the mornings to account for circadian rhythm.

5.1.3 List of *C. elegans* Strains used

Table 1. List of *C. elegans* strains

N2	Bristol (wild-type)
AA2508	*mir-1(gk276) I*
AA4575	*mir-1(dh1111)*
SJ4100	*N2; zcIs13[hsp-6::GFP] V*
AA4404	*mir-1(gk276); zcIs13[hsp-6::GFP] V*
SJ4005	*N2; zcIs4[hsp-4::GFP] V*
AA4405	*mir-1(gk276); zcIs4[hsp-4::GFP] V*
AM141	*N2; rmIs133[P(unc-54) Q40::YFP]*
AA3112	*mir-1(gk276) I; rmIs133[P(unc-54) Q40::YFP]*
AM140	rmIs132[P(unc-54) Q35::YFP]
AA4403	*mir-1(gk276); rmIs132[P(unc-54) Q35::YFP]*
AA4577	*mir-1(dh1111);rmIs132[P(unc-54) Q35::YFP]*
AA4809	*N2; rmIs132[P(unc-54) Q35::YFP]; dhEx965[mir-1p::mir-1, myo-2p::mCherry]*
AA4810	*mir-1(gk276); rmIs132[P(unc-54) Q35::YFP]; dhEx965[mir-1p::mir-1, myo-2p::mCherry]*
AA2794	*N2; rmEx135(F25B3.3p::Q86::YFP)*
	mir-1(gk276); rmEx135(F25B3.3p::Q86::YFP)
CL2006	*dvIs2[pCL12(Punc-54::human Abeta peptide 1-42 minigene; pRF4(rol-6(su1006))*
AA4811	*mir-1 (gk276); dvIs2[pCL12(Punc-54::human Abeta peptide 1-42 minigene; pRF4(rol-6(su1006))*
	daf-16(mu86);mir-1(gk276); rmIs132[P(unc-54) Q35::YFP]
RT258	*unc-119(ed3) III; pwIs50pwIs50[lmp-1::GFP + Cbr-unc-119(+)].*
AA4812	*mir-1(gk276); pwIs50pwIs50[lmp-1::GFP + Cbr-unc-119(+)]*
AA4583	*adIs2122(lgg-1::GFP; rol-6(su1006))*
AA2532	*mir-1(gk276) I; adIs2122(lgg-1::GFP; rol-6(su1006))*
PHX506	*daf-16(syb506)*
AA4808	*mir-1 (gk276); daf-16(syb506)*
PHX502	*daf-16(syb502)*
AA4807	*mir-1 (gk276); daf-16(syb502)*
PHX586	*vha-13(syb586)*

AA4813	mir-1 (gk276); vha-13(syb586)
PHX587	*vha-13(syb587 syb504)*
AA4814	mir-1 (gk276); vha-13(syb587 syb504)
AA4850	vha-13(syb586); dhEx965[mir-1p::mir-1, myo-2p::mCherry]
AA4851	vha-13(syb587 syb504); dhEx965[mir-1p::mir-1, myo-2p::mCherry]
AA2511	*mir-1(gk276) I; daf-16(mu86) I; muEx212(myo-3p::GFP::daf-16; rol-6(su1006))*
AA2512	*daf-16(mu86) I; muEx212(myo-3p::GFP::daf-16; rol-6(su1006))*
AA4865	N2;Ex[myo3p::flag::HA::mCherry::vha-13cDNA::unc-54 3´UTR, my2p::GFP]
AA4866	*mir-1(gk276);Ex[myo3p::flag::HA::mCherry::vha-13cDNA::unc-54 3´UTR, my2p::GFP]*
	N2;Ex[myo3p::flag::HA::mCherry::vha-13cDNA::vha-13 3´UTR, my2p::GFP]
	mir-1(gk276);Ex[myo3p::flag::HA::mCherry::vha-13cDNA::vha-13 3´UTR, my2p::GFP]
PP1375	uthEx633(myo3p::GFP)
AA4816	mir-1 (gk276); uthEx633(myo3p::GFP)
AA3569	*maIs251[unc-119(+) + Pmir-1::GFP]*
AA3939	*N2; dhEx1176[daf-16 3´UTR-3xmir-1-si-pCF110+ myo-2p::mCherry] G3*
	mir-1(gk276); dhEx1176[daf-16 3´UTR-3xmir-1-si-pCF110+ myo-2p::mCherry]
AA3569	*maIs251[unc-119(+) + Pmir-1::GFP]*
AA4987	*N2; rmIs132[P(unc-54) Q35::YFP; myo3p::flag::HA::mCherry::vha-13cDNA::unc-54 3´UTR, my2p::GFP]*

5.1.4 Genotyping

In case genotyping was not possible by phenotypic assessment, taq polymerase chain reaction (PCR) and agarose gel electrophoresis were conducted. As template served the lysate obtained from incubating a single worm in 10 µl (for PCR) single worm lysis buffer (10mM Tris pH 8.3, 50mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, 0.45% Triton X-100 (all v/v), 1mg/ml proteinase K (NEB)) for 60 min at 65°C and subsequently 15 min at 95°C.

Table 2. List PCR primer

Genotype	PCR primer	PCR product
mir-1 (gk276)	FW CCGCATCGCGAAAAGTCATC RV CCAAATCTTTCACGAGTCGT	WT: 500 bp MUT: 300 bp
daf-16 (mu86) Source: Hansen, 2003	Pr. 1 (MH39) CCGTTCAAGCTGCTGCCTTCACTCT PR. 2 (MH40) CAGCATCTTCTTCAGGAATTTGTTC Pr. 3 (MH41) GCCTTTGTCTCTCTATCGGCCACCA Pr. 4 (MH42) CGGAAAGATGATGGAACGTT	WT: 473 bp MUT: 657 bp

5.1.5 Gene knockdown by RNAi

Worms were either grown egg-on or L4 onwards on *E. coli* HT115 (DE3) bacteria expressing dsRNA of the target gene under the control of an IPTG-inducible promotor. NGM plates for RNAi experiments contained a final concentration of 100 µg/µl ampicillin and 1mM IPTG to select for vector carrying HT115 bacteria and induce dsRNA expression. All RNAi clones used for this thesis were selected either from the Ahringer or the Vidal library (Kamath and Ahringer 2003; Rual et al. 2004). All RNAi clones, except of excluded candidates from the RNAi screens were confirmed by sequencing.

5.1.6 Lifespan assay

Lifespan assays were either performed at 20°C or 25°C. N2 Bristol lifespan was used as control. Synchronization of worms was, as previously described, done by a 4h egglay. A minimum of 100 worms used per strain and transferred to fresh plates every day till the end of their reproductive period. Worms were scored every second day for their survival whereby worms that had undergone internal hatching or vulval bursting were censored. Data were plotted to calculate mean, median and maximum lifespan.

5.1.7 Brood size measurement

Synchronized young adult worms were singled to 3 cm plates containing OP50. Worms were transferred to fresh plates every 24h and progeny was counted during a period of 5 days. A minimum of 10 worms were used for each genotype.

5.1.8 Pharyngeal pumping rate assay

Synchronized Day 1 worms were singled to 3 cm plates and pumping rate was measured by by counting grinder movements on a stereomicroscope on Day 1, Day 4 and Day 8.

5.1.9 Stress assays

5.1.9.1 Heat stress

Worms were grown to young adults under standard conditions. 50 worms were transferred to 6-cm NGM plates containing OP50 and exposed to 35°C for 8h. Survival was scored every 2h.

5.1.9.2 Oxidative stress

Worms were grown to young adults under standard conditions. Unseeded 3-cm plates were covered with 5 ul of a 1M H_2O_2 solution and plates were dried at room temperature. Final H_2O_2 was expected to be around 10mM. Worms were transferred to freshly prepared plates and scored every 2h.

5.1.10 Worm imaging

Standard worm maintenance was performed using a Leica M80 stereomicroscope. For analysing fluorescent intensity at a lower magnification, stacks of worms were aligned on unseeded, iced NGM plates and imaged on a Leica M165FC fluorescence microscope using a Leica DFC 3000G camera and Leica Application Suite.

Images of single worms were either obtained on a Carl Zeiss Axio Imager Z1 connected to a Zeiss Axiocam 506 mono camera using AxioVision software or on a Leica TCS SP8-X confocal microscope at 60 times magnification. Quantification of florescent intensity was determined using Adobe Photoshop CS5 (Microsoft office).

5.1.11 PolyQ aggregate quantification

Quantification of polyQ aggregates of unc-54P::Q35::YFP worms were done from images obtained from synchronized Day 4 adults taken on a Leica M165FC fluorescence microscope (see 5.1.7) at a magnification of about 8 fold. Images of at least 15 worms were taken for each biological replicate. To avoid bias, worms were arranged for analysis on a Leica M80 light microscope. To further minimize bias, quantification of aggregates was done using Image J software. Images were inverted and the same threshold was used to subtract background. Image J particle analysis was then used to determine the number of aggregates per worm.

5.1.12 Motility Assay

5.1.12.1 Circle Test

To assess motility in a swimming assay worms were synchronized to a 4 hours time window and were cultured at standard conditions on OP50, unless mentioned otherwise. A standardized circle was drawn on the bottom of unseeded NGM plates. Worms that were able to move at least slightly after touch stimulus on culture plates were transferred into the middle of the circled region. Worms that made it out of the circle within 1 minute were counted as mobile fraction.

5.1.12.2 Swimming assay

To assess motility in a swimming assay worms were synchronized to a 4 hours time window and were cultured at standard conditions on OP50, unless mentioned otherwise. Worms that were able to move at least slightly after touch stimulus on culture plates were transferred to M9 buffer on unseeded NGM plates and were allowed to adjust for about 30 sec. Body bends were then measured within a 30 sec interval by counting. 15 worms per genotype and condition were analyzed for each biological replicate. To avoid bias all experiments were carried out blinded.

5.1.13 Fractionation of SDS soluble and SDS insoluble polyQ peptides

Synchronized day 1 adult worms were harvested and washed in M9 and lysed in RIPA buffer (50 mM Tris pH 8, 150 mM NaCl, 5 mM EDTA, 0.5% SDS, 1% NP40, complete Mini Protease and phosphatase Inhibitor Cocktail (Roche)) by freeze/thaw cycles and sonication. The insoluble fraction was pelleted by 20 min centrifugation at 4°C and 16,000 x g. The supernatant was subsequently collected as the soluble fraction and the pellet was washed in RIPA buffer and after centrifugation

solubilized in urea/SDS buffer (8 M urea, 50 mM Tris pH 8, 2% SDS, 50 mM DTT). 4x LDS sample buffer (Thermo Fisher) was added to protein fractions and samples were separated and visualized by SDS PAGE and immunoblotting.
To detect total amounts of SDS soluble and insoluble polyQ fragments worms were directly collected in urea/SDS buffer and snap frozen.

5.1.14 Selective RNAi screen for bioinformatic and proteomics candidates

Approximately 40 synchronized *mir-1;Q35* worms were transferred L4 onwards to NGM plates containing 40 µM 5-Fluoro-2′- deoxyuridine (FUDR, Sigma), seeded with HT115 bacteria expressing select RNAi clones. *Daf-2* RNAi was used as control that RNAi was properly induced, as knockdown of *daf-2* is known to increase motility. At day 4 of adulthood worms were transferred to fresh culture plates and at day 8 motility was assessed on plates by comparing to *luciferase* as well as polyQ in wild-type background. Motility was tested on day 8 using the circle test described in (see 5.1.9.1). RNAi clones that completely reversed *mir-1;Q35* motility on *luciferase* back to *Q35* motility on *luciferase* were selected as primary hits and were re-tested using the swimming assay and without FUDR in the culture plates (see 5.1.9.2). The effect of the candidate RNAi clones was next measured in polyQ in wild-type background as counter screen. The worms were grown to day 5 of adulthood and a motility assay was performed as described above. If the motility on specific RNAi clones was below the range of error of *luciferase*, the candidates were not considered secondary hits and excluded from further analysis.

5.1.15 Microinjections

N2 bristol strain was used for injections. L4 larvae were placed in a drop of about 50 µl halocarbon oil (Sigma) on a 2% agarose pad. Injection Mix used to create *myo3p::vha-13cDNA::unc-54 3´UTR* extrachromosomal strains constisted of 40ng/ul of plasmid, 5 ng/ul of co-injection marker myo3p::GFP and 55 ng/ul of TOPO empty vector. Injections were done using a Carl Zeiss Axio Imager Z1 microscope installed with a manual micromanipulator which was connected to a microinjector (Femtojet4).

5.1.16 CRISPR mir-1 alleles

Design of CRISPR guides was done using services of http:://crispr.mit.edu/guides. NeBio sgRNA Designer was used to design primer. Engen sgRNA synthesis kit was finally used to synthesize guides. Guides were analyzed by gel electrophoresis and tape station. Injection mix contained the following components: Cas9 EnGen (NEB), mir-1 #3 sgRNA, mir-1 #6 sgRNA, mir-1 #9 sgRNA, mir-1 #10 sgRNA, dpy-10

sgRNA, KCl, Hepes pH 7.4, water. Mixture was incubated for 10 minutes at 37°C to allow activation of Cas9 and worms were injected as described in 5.1.11. In the F1 generation worms with dpy phenotype were singled and genotyped for *mir-1* deletion using *mir-1* genotyping primer (table 2).

Table 3. List CRISPR Guides

Guide Name	Target Sequence
dpy-10	CGCTACCATAGGCACCACG
mir-1 #3	AAGAAGTATGTAGAACGGGG
mir-1 #6	GTAAAGAAGTATGTAGAACG
mir-1 #9	TATAGAGTAGAATTGAATCT
mir-1 #10	ATATAGAGTAGAATTGAATC

5.2 Molecular Biology

5.2.1 Molecular cloning

All restriction digest reactions were performed with enzymes provided by NEB according to their user's manual. T4 DNA Ligase (NEB) was used for ligation reactions. Chemically competent DH5α *Escherichia coli* (LifeTechnologies) were used for transformation of ligation reactions or plasmids following the manufacturer's instructions. For selection of positive clones, a suitable antibiotic was used, for example 100 µg/mL ampicillin in LB plates. To purify plasmids out of bacteria the QIAprep Miniprep or Midiprep Kits (Qiagen) were used. Cloning success was verified by PCR followed by gel electrophoresis and Sanger sequencing at Eurofins Genomics (Germany). Cloning strategies and primers used are described below (Table 4).

Table 4. Cloning strategy for vectors generated in this study including primers

Plasmid Name	Backbone	Primer	Method
myo3p::mcherry::flag::HA::vha-13cDNA::unc-54 3´UTR	pDESTR4-R3	FW GGTGGTGGTACCATGGCCGCAGAATGTTCGTA RV ACCACCGGTACCTCCTCGAGGTTTCTGAAAGCGTTA	vha-13 was amplified from cDNA with KpnI overhangs. Plasmid and insert were digested with KpnI and ligated.
myo3p::mcherry::flag::HA::vha-13cDNA::vha-13 3´UTR	myo3p::mcherry::flag::HA::vha-13cDNA::unc-54 3´UTR	FW GGTGGTGCGGCCGCATTTTAAAATTGATTCTCTTG RV CACCGCCCGTACGGCGAAGTTTAGAAATCTACAAAAG	vha-13 3´UTR was amplified from cDNA with NotI and BglI overhangs. Plasmid and insert were digested with NotI and BglI and ligated.
PmirGLO-vha-13 3´UTR	PmirGLO	FW GGTGGTGAGCTCATTTTAAAATTGATTCTTCTCTTG RV ACCACCCCTCGAGGAAGTTTAGAAATCTACAAAAG	vha-13 3´UTR was amplified from cDNA with SacI and XhoI overhangs. Plasmid and insert were digested with SacI and XhoI and ligated.

5.2.2 RNA extraction and quantitative RT-PCR

Populations of about 500 day 1 and day 4 adult *C. elegans* were harvested in M9 and washed twice in cold M9. The worm pellet was taken up in 700 µl QIAzol reagent (Qiagen) and snap frozen in liquid nitrogen. The samples were subjected to 4 freeze/thaw cycles (liquid nitrogen/37°C water bath) and homogenization with 1.0 mm Zirconia/Silica beads (FisherScientific) in a TissueLyser LT (Qiagen) for 15 min at full speed to crack the worms´ cuticle and release RNA. After homogenization, supernatant was transferred to fresh eppendorff tubes and 120 µl Chloroform were added to 600 µl QIAzol solution. The components were mixed by vortexing and incubated for 2 min at RT. After 15 min centrifugation at 12000 x g and 4°C the aqueous phase was collected for total RNA extraction using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. The RNA quantitiy and quality were determined on a NanoDrop 2000c (peqLab) and cDNA was prepared using the iScript cDNA Synthesis Kit (BioRad). To quantifiy cDNA Power SYBR Green Master Mix (Applied Biosystems) was used and reaction was measured on a ViiA 7 Real-Time PCR system (Applied Biosystems). Four technical replicates were pipetted on a 384-well plate using the JANUS automated workstation (PerkinElmer). Expression of target RNA was calculated from comparative CT values, normalized to ama-1 and cdc-42 as internal controls using the corresponding ViiA7 software. All unpublished primers were validated by determination of their standard curves and melting properties.

Table 5. List quantitative RT-PCR primer

ama-1	FW GGATGGAATGTGGGTTGAGA RV CGGATTCTTGAATTTCGCGC
cdc-42	FW CTGCTGGACAGGAAGATTACG RV CTCGGACATTCTCGAATGAAG
vha-5	FW GCTCTTTCTCTTGCTCACGCTCA RV TCCGGTAACGAACACCATGTGC
vha-10	FW TGGCTGGAAACAAGCAAGCTGTC RV CTCGGCTTGATGTCGCAAACG
vha-13	FW AAGCTCTTCGTGAGATTTCTGGA RV ACGGATCCTTCACGTTCTGGAG
vha-19	FW AACAAGGCTTCGGCCAGTAATC RV GTCAACGGAGGCACTCTTATATG
MHC	FW GCAGAAGCGTCACAGCACTTCATTG RV AGTTGCTCGAAACCGTTGTACTC

Table 6. List Taqman Probes

Target Gene	Species	Taqman Probe Number
U18	*C. elegans*	001764
mir-1	Human, Drosophila, *C.elegans*	000385
SnoR442	Drosophila	001742
mir-210	Drosophila	005997

5.2.3 In-situ hybridization

Populations of 5000 day 1 adults were harvested in PBS and washed twice with PBS before Fixation solution (PBS+4%PFA) was added to the worm pellet. Worms were fixed for 1h at room temperature shaking and subsequently washed twice with PBS and incubated in 70% EtOH overnight at 4°C. The next day, worms were washed twice with PBS and incubated with 1% H_2O_2 in PBS twice for 15 minutes to stop endogenous peroxidase activity. After three washing steps with PBST (PBS+0.1% Tween-20), worms were digested with proteinase K at a concentration of 20ug/ml in PBST for 20 minutes at 37°C. After washing twice with PBS, worms were dehydrated by emerging them in 70% EtOH for one minute, 96% EtOH for one minute, 99.9% EtOH for a minute. Worms were equilibrated in 1xISH buffer (Exiqon, #90000). Hybridization was performed by adding either 62 nM of mir-1-LNA probe or scrambled LNA control (Exiqon) in 1xISH buffer an incubating the worms for 2h at hybridization temperature of 44°C in a thermocycler. After hybridization, worms were washed in a dilution series of SSC buffer (Sigma): 5xSSC, 1xSSC and 0.2xSSC for 5 minutes at hybridization temperature. After a last 5 minute incubation period of worms in 0.2xSSC buffer at RT, blocking solution (PBST+ 2% sheep serum+ 1% BSA) was added and incubated for 30 minutes at RT. Blocking solution was removed and anti-DIG-POD (1:400 in dilutant buffer) (Roche) was added and worms were incubated at RT for 1h. Worms were then washed twice with PBS-T for 3 minutes and twice with PBS for 3 minutes. For signal detection, worms were incubated in TSA-plus substrate (TSA Plus System, Perkin Elmer) for 10 minutes at RT.After 4 washing steps of 5 minutes each in PBS, worms were finally mounted on a slide using SlowFade Gold antifade reagent with DAPI (Life technology) and kept overnight at RT in a dark box. The next day, worms were imaged on a Leica TCS SP8-X confocal microscope.

Table 7. List LNA microRNA probes

MicroRNA	Sequence	Exiqon #
cel-miR-1-3p miRCURY LNA miRNA	5'UGGAAUGUAAAGAAGUAUGUA	00614276
Scrambled miRCURY LNA miRNA Control	5'GTGTAACACGTCTATACGCCCA	99004-15

5.3 Biochemistry

5.3.1 Proteomic analysis

For sample collection, population of at least 5000 synchronized day 1 wild-type and *mir-1* mutant worms were collected in M9 buffer, washed three times in M9 and directly frozen in liquid nitrogen and stored at -80°C until all 5 replicates were collected. *S*amples for proteomic analysis were then boiled in lysis buffer (100 mM Tris, 6 M Guanidinium chloride, 10 mM Tris(2-carboxyethyl)phosphine hydrochloride, 40 mM 2-Chloroacetamide) for 10 min, lysed at high performance in 10 cycles of 30 s soncation intervals in a Bioruptor Plus sonication device (diagenode) and boiled again. The samples were centrifuged at 20000 x g for 20 min to get rid of debris. The samples were diluted 1:10 in 20 mM Tris pH 8.3 / 10% acetonitrile (ACN) and after the protein concentration was measured using BCA Protein Assays (Thermo Fisher) the samples were digested over night with rLys-C (Promega).The peptides were cleaned on a Supelco Visiprep SPE Vacuum Manifold (Sigma) using OASIS HLB Extraction cartridges (Waters). The columns were conditioned twice with Methanol, equilibrated twice with 0.1% formic acid, loaded with the sample, washed three times with 0.1% formic acid and the peptides eluted with 60% ACN / 0.1% formic acid. The samples were dried at 30°C for roughly 4 h in a Concentrator plus speedvac (Eppendorf) set for volatile aqueous substances. The dried peptides were taken up in an adequate volume of 0.1% formic acid (usually in 20 µl) and the samples were analyzed by the Max Planck Proteomic Core facility for analysis according to the following protocol (by Ilian Atanassov):

For protein identification and quantification, the raw data were analyzed with MaxQuant version 1.5.2.8 (Cox and Mann, 2008) using the integrated Andromeda search engine (Cox et al., 2011). Peptide fragmentation spectra were searched against the canonical and isoform sequences of the *C. elegans* reference proteome (proteome ID UP000001940, downloaded May 2017 from UniProt). Methionine oxidation and protein N-terminal acetylation were set as variable modifications; cysteine carbamidomethylation was set as fixed modification. The digestion parameters were set to “specific” and “Trypsin/P,” The minimum number of peptides and razor peptides for protein identification was 1; the minimum number of unique peptides was 0. Protein identification was performed at a peptide spectrum matches and protein false discovery rate of 0.01. The “second peptide” option was on. Successful identifications were transferred between the different raw files using the “Match between runs” option. Label-free quantification (LFQ) (Cox et al., 2014) was performed using an LFQ minimum ratio count of 2. LFQ intensities were filtered for at least four valid values in at least one group and imputed from a normal distribution with a width of 0.3 and down shift of 1.8. Differential expression analysis was performed using limma (Ritchie et al., 2015). Functional category annotation and enrichment analysis was performed using Perseus version 1.5.0.0 (Tyanova, Temu et al., 2016). The column “Majority protein IDs” was used for GOCC, GOBP, and GOMF annotation. Category enrichment analysis was done using Fisher exact test using an FDR threshold of 0.02.

5.3.2 Western Blot analysis

For Western blot analysis synchronized young adult or gravid day 1 adult worms were picked into eppendorf tubes containing M9, snap frozen in liquid nitrogen and lysed in 4x LDS sample buffer (Thermo Fisher) containing 50 mM DTT. After boiling and sonication, equal volumes were subjected to reducing SDS-PAGE and transferred to nitrocellulose membranes. The membranes were then blocked for two hours at room temperature in 5 % milk in Tris-buffered Saline and Tween20 (TBST) and probed with the primary antibodies in TBST with 5% milk overnight at 4 °C. Specific secondary antibodies (mouse or rabbit) were used at a concentration of 1:5000 in TBST with 5 % milk at room temperature for two hours. The membranes were developed with Western Lightening Plus- Enhanced Chemiluminescence Substrate (PerkinElmer). Bands were detected on a ChemiDoc MP Imaging System (BioRad) and the intensity quantified using the corresponding Image Lab software (BioRad).

5.3.3 Antibodies

The following primary antibodies were used in this study: GFP (JL-8 Living Colors, mouse monoclonal), Histone H3 (ab1791 abcam, rabbit polyclonal), α-Tubulin (T6199 Sigma, mouse monoclonal), FLAG (F1804 Sigma, mouse monoclonal).The following horseradish peroxidase conjugated antibodies were used in this study: anti-mouse IgG (G-21040 Invitrogen, goat polyclonal), anti-rabbit IgG (G-21234 Invitrogen, goat polyclonal).

5.4 Drosophila work

5.4.1 Maintenance of Drosophila

Fly stocks were kept at 25°C on a 12h light and 12h dark cycle and fed a standard 1x SYA diet (5% sugar, 10% yeast, 1.5% agar, 0.3% Nipagen (10%in EtOH) and 0.03% Propionic Acid).

5.4.2 Drosophila synchronization and dissection

For all experiments, an equal number of eggs were distributed to new flasks to control for larval density. Females and males were allowed to mate for 48h ("once-mated") before each experiment. For dissections flies were first immobilizing by cooling them shortly on ice and subsequently tissues were dissected in phosphate-buffered saline (PBS). Tissues were directly frozen on dry ice.

5.4.3 Hemolymph extraction

Hemolymph extraction was performed by cutting of the head of synchronized flies and placing them in a 0.5 ml eppendorf tube in which a small whole was poked in the bottom, just allowing liquid to pass. The eppendorf was then placed in a 1.5ml eppendorf tube and centrifuged at 10,000 rpm for 5 minutes.

5.4.4 List of Drosophila strains used

Table 8. List of Drosophila strains

Name	Source
wDah	Grönke et al. 2010
MHC-Gal4	Partridge lab
UAS-mir-1	Bloomington #41125
UAS-mir-210	Partridge lab (Bejarano et al. 2012)

5.6 Cell Culture

5.6.1 Luciferase assays

HEK393T cells were seeded in a density of $5x10^4$ cells per well in a 24-well plate. The following day, when cells reached >90% confluence, cells were transfected using Lipofectamine 2000. Cell were transfected either with 500 ng of PmirGLO-vha-13 3´UTR and 250ng of pEGFP-C2 empty vector or 500 ng of PmirGLO-vha-13 3´UTR and 250 ng of pEGFP-C2-hmir-1. Medium was changed 12h after transfection and transfection efficiency was assessed 24h after transfection using fluorescent microscopy. Cells were harvested after 24h and cell lysis was achieved rocking cells for 15 minutes in passive lysis buffer (provided in Promega Dual-reporter kit). A spectrometer that injected substrates automatically was used to determine signals of renilla luciferase and firefly luciferase. Firefly was measured after 1.5 seconds and renilla was measured after 15 seconds. Substrates were used from the Promega Dual-reporter kit. Controls were untransfected lysed cells for background and cells transfected with PmirGLO not containing any 3´UTR and either pEGFP-C2 empty vector or pEGFP-C2-hmir-1 to account for any regulation other than by the vha-13 3´UTR.

5.7 Statistical analysis

Results are presented as mean + SD or SEM or mean ± SD or SEM as indicated. Statistical analyses were performed using 1way ANOVA with Tukey´s or Dunnett´s multiple comparisons test or unpaired t-test. The program used was GraphPad Prism (GraphPad software). Significance levels are *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$ versus respective control.

5.8 Software

Most graphs were produced and statistical analyses performed using GraphPad PRISM 6.Data from proteomic experiments was graphically displayed using Instant Clue (Nolte et al., 2018).
Bands on Western blots were quantified using Image Lab Software (Bio-Rad Laboratories GmbH).
DNAstar software was used for in-silico cloning.
ImageJ software was used for counting polyQ aggregates.

References

Aboobaker, A Aziz, Pavel Tomancak, Nipam Patel, Gerald M Rubin, and Eric C Lai. 2005. "MicroRNAs Exhibit Diverse Spatial Expression Patterns during Embryonic Development." *Proceedings of the National Academy of Sciences of the United States of America* 102 (50): 18017 LP – 18022. https://doi.org/10.1073/pnas.0508823102.

Adam Bohnert, K., and Cynthia Kenyon. 2017. "A Lysosomal Switch Triggers Proteostasis Renewal in the Immortal C. Elegans Germ Lineage." *Nature* 551 (7682): 629–33. https://doi.org/10.1038/nature24620.

Alberts B, Johnson A, Lewis J, et al. 2002. *Molecular Biology of the Cell. 4th Edition.*

Alexander, Adanna G., Vanessa Marfil, and Chris Li. 2014. "Use of C. Elegans as a Model to Study Alzheimer's Disease and Other Neurodegenerative Diseases." *Frontiers in Genetics* 5 (JUL): 1–21. https://doi.org/10.3389/fgene.2014.00279.

Andachi, Yoshiki, and Yuji Kohara. 2016. " A Whole-Mount in Situ Hybridization Method for MicroRNA Detection in Caenorhabditis Elegans ." *Rna* 22 (7): 1099–1106. https://doi.org/10.1261/rna.054239.115.

Andrew, Fire, SiQun Xu, Mary K. Montgomery, Kostas Steven A., and Driver Samuel E. & Mello Craig C. 1998. "Potent Andspecific Genetic Interferenceby Double-StrandedRNA in Caenorhabditiselegans" 391 (February): 806–11.

Antebi, Adam. 2007. "Genetics of Aging in Caenorhabditis Elegans." *PLoS Genetics* 3 (9): 1565–71. https://doi.org/10.1371/journal.pgen.0030129.

Apfeld, Javier, and Cynthia Kenyon. 1998. "Cell Nonautonomy Of." *Cell* 95: 199–210.

Avan Aihie Sayer, Sian M. Robinson, Harnish P. Patel, Tea Shavlakadze, Cyrus Cooper, Miranda D. Grounds. 2013. "New Horizons in the Pathogenesis, Diagnosis and Management of Sarcopenia." *Age and Ageing* 42 (2): 145–50.

Axe, Elizabeth L., Simon A. Walker, Maria Manifava, Priya Chandra, H. Llewelyn Roderick, Anja Habermann, Gareth Griffiths, and Nicholas T. Ktistakis. 2008. "Autophagosome Formation from Membrane Compartments Enriched in Phosphatidylinositol 3-Phosphate and Dynamically Connected to the Endoplasmic Reticulum." *Journal of Cell Biology* 182 (4): 685–701. https://doi.org/10.1083/jcb.200803137.

Ayyadevara, Srinivas, Meenakshisundaram Balasubramaniam, Pooja Suri, Samuel G. Mackintosh, Alan J. Tackett, Dennis H. Sullivan, Robert J. Shmookler Reis, and Richard A. Dennis. 2016. "Proteins That Accumulate with Age in Human Skeletal-Muscle Aggregates Contribute to Declines in Muscle Mass and Function in Caenorhabditis Elegans." *Aging* 8 (12): 3486–97. https://doi.org/10.18632/aging.101141.

Balch, William E., Andrew Dillin, Evan T. Powers, Jeffery W. Kelly, and Richard I. Morimoto.

2009. “Biological and Chemical Approaches to Diseases of Proteostasis Deficiency.” *Annual Review of Biochemistry* 78 (1): 959–91. https://doi.org/10.1146/annurev.biochem.052308.114844.

Bartel, David P. 2009. “MicroRNAs: Target Recognition and Regulatory Functions.” *Cell* 136 (2): 215–33. https://doi.org/10.1016/j.cell.2009.01.002.

Baxi, Kunal, Ata Ghavidel, Brandon Waddell, Troy A. Harkness, and Carlos E. de Carvalho. 2017. “Regulation of Lysosomal Function by the Daf-16 Forkhead Transcription Factor Couples Reproduction to Aging in Caenorhabditis Elegans.” *Genetics* 207 (1): 83–101. https://doi.org/10.1534/genetics.117.204222.

Bayraktar, Recep, Katrien Van Roosbroeck, and George A. Calin. 2017. “Cell-to-Cell Communication: MicroRNAs as Hormones.” *Molecular Oncology* 11 (12): 1673–86. https://doi.org/10.1002/1878-0261.12144.

Becker, Thomas, Gerrit Loch, Marc Beyer, Ingo Zinke, Anna C. Aschenbrenner, Pilar Carrera, Therese Inhester, Joachim L. Schultze, and Michael Hoch. 2010. “FOXO-Dependent Regulation of Innate Immune Homeostasis.” *Nature* 463 (7279): 369–73. https://doi.org/10.1038/nature08698.

Behrends, Christian, Mathew E. Sowa, Steven P. Gygi, and J. Wade Harper. 2010. “Network Organization of the Human Autophagy System.” *Nature* 466 (7302): 68–76. https://doi.org/10.1038/nature09204.

Berg, Trond Olav, Monica Fengsrud, Per Eivind Strømhaug, Trond Berg, and Per O. Seglen. 1998. “Isolation and Characterization of Rat Liver Amphisomes: Evidence for Fusion of Autophagosomes with Both Early and Late Endosomes.” *Journal of Biological Chemistry* 273 (34): 21883–92. https://doi.org/10.1074/jbc.273.34.21883.

Betel, Doron, Manda Wilson, Aaron Gabow, Debora S Marks, and Chris Sander. 2008. “The MicroRNA . Org Resource : Targets and Expression” 36: 149–53. https://doi.org/10.1093/nar/gkm995.

Beyenbach, Klaus W., and Helmut Wieczorek. 2006. “The V-Type H+ ATPase: Molecular Structure and Function, Physiological Roles and Regulation.” *Journal of Experimental Biology* 209 (4): 577–89. https://doi.org/10.1242/jeb.02014.

Blommaart, E.F., Luiken, J.J., Blommaart, P.J., van Woerkom, G.M. & Meijer, A.J. 1995. “Phosphorylation of Ribosomal Protein S6 Is Inhibitory for Autophagy in Isolated Rat Hepatocytes.” *J. Biol. Chem.*, no. 270: 2320–26.

Blüher, Matthias, Matthias Blu, Barbara B Kahn, and C Ronald Kahn. 2013. “Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue.” *Science* 572 (2003): 2000–2003. https://doi.org/10.1126/science.1078223.

Boehm, Michelle, and Frank Slack. 2005. “Supp - A Developmental Timing MicroRNA and Its Target Regulate Life Span in C. Elegans.” *Science (New York, N.Y.)* 310 (5756): 1954–

57. https://doi.org/10.1126/science.1115596.

Bonafè, Massimiliano, Michelangela Barbieri, Francesca Marchegiani, Fabiola Olivieri, Emilia Ragno, Claudia Giampieri, Elena Mugianesi, Matteo Centurelli, Claudio Franceschi, and Giuseppe Paolisso. 2003. “Polymorphic Variants of Insulin-like Growth Factor I (IGF-I) Receptor and Phosphoinositide 3-Kinase Genes Affect IGF-I Plasma Levels and Human Longevity: Cues for an Evolutionarily Conserved Mechanism of Life Span Control.” *Journal of Clinical Endocrinology and Metabolism* 88 (7): 3299–3304. https://doi.org/10.1210/jc.2002-021810.

Bonaldo, Paolo, and Marco Sandri. 2013. “Cellular and Molecular Mechanisms of Muscle Atrophy” 39: 25–39. https://doi.org/10.1242/dmm.010389.

Boulias, Konstantinos, and H. Robert Horvitz. 2012. “The C. Elegans MicroRNA Mir-71 Acts in Neurons to Promote Germline-Mediated Longevity through Regulation of DAF-16/FOXO.” *Cell Metabolism* 15 (4): 439–50. https://doi.org/10.1016/j.cmet.2012.02.014.

Boutros, Michael, and Julie Ahringer. 2008. “The Art and Design of Genetic Screens: RNA Interference.” *Nature Reviews Genetics* 9 (7): 554–66. https://doi.org/10.1038/nrg2364.

Bowers, William E. 1998. “Christian de Duve and the Discovery of Lysosomes and Peroxisomes” 8 (August): 330–33.

Brault, Jeffrey J., Andreas Schild, Alfred L. Goldberg, Stefano Schiaffino, Stewart H. Lecker, Marco Sandri, Peirang Cao, and Jinghui Zhao. 2007. “FoxO3 Coordinately Activates Protein Degradation by the Autophagic/Lysosomal and Proteasomal Pathways in Atrophying Muscle Cells.” *Cell Metabolism* 6 (6): 472–83. https://doi.org/10.1016/j.cmet.2007.11.004.

Brenner, S. 1974. “The Genetics of Caenorhabditis Elegans.” *Genetics* 77 (1): 71–94. https://doi.org/10.1002/cbic.200300625.

Brignull, H. R., Morley, J.F., Garcia, S.M., Morimoto, R.I. 2006. “Modeling Polyglutamine Pathogenesis in C . Elegans.” *Methods in Enzymology* 412 (06): 256–82. https://doi.org/10.1016/S0076-6879(06)12016-9.

Brisseau, Guy F., Sergio Grinstein, David J. Hackam, Tommy Nordström, Morris F. Manolson, Aye Aye Khine, and Ori D. Rotstein. 1996. “Interleukin-1 Increases Vacuolar-Type H +-ATPase Activity in Murine Peritoneal Macrophages.” *Journal of Biological Chemistry* 271 (4): 2005–11. https://doi.org/10.1074/jbc.271.4.2005.

Budenholzer, Lauren, Chin Leng Cheng, Yanjie Li, and Mark Hochstrasser. 2017. “Proteasome Structure and Assembly.” *Journal of Molecular Biology* 429 (22): 3500–3524. https://doi.org/10.1016/j.jmb.2017.05.027.

Bulck, Michiel Van, Ana Sierra-Magro, Jesus Alarcon-Gil, Ana Perez-Castillo, and Jose A. Morales-Garcia. 2019. “Novel Approaches for the Treatment of Alzheimer’s and Parkinson’s Disease.” *International Journal of Molecular Sciences* 20 (3).

https://doi.org/10.3390/ijms20030719.

Calvin, Robin C Friedman, J Graham Ruby, and David P Bartel. 2011. “Formation, Regulation and Evolution of Caenorhabditis Elegans 3′UTRs.” *Nature* 469 (7328): 97–101. https://doi.org/10.1038/nature09616.

Campos, Juliane C., Leslie M. Baehr, Kátia M.S. Gomes, Luiz R.G. Bechara, Vanessa A. Voltarelli, Luiz H.M. Bozi, Márcio A.C. Ribeiro, et al. 2018. “Exercise Prevents Impaired Autophagy and Proteostasis in a Model of Neurogenic Myopathy.” *Scientific Reports* 8 (1): 1–14. https://doi.org/10.1038/s41598-018-30365-1.

Cartee, Gregory D., Russell T. Hepple, Marcas M. Bamman, and Juleen R. Zierath. 2016. “Exercise Promotes Healthy Aging of Skeletal Muscle.” *Cell Metabolism* 23 (6): 1034–47. https://doi.org/10.1016/j.cmet.2016.05.007.

Carter, Heather N, Yuho Kim, Avigail T Erlich, Dorrin Zarrin-khat, David A Hood, Scott Powers, and Karyn Hamilton. 2018. “Autophagy and Mitophagy Flux in Young and Aged Skeletal Muscle Following Chronic Contractile Activity” 16: 3567–84. https://doi.org/10.1113/JP275998.

Caygill, Elizabeth E., and Laura A. Johnston. 2008. “Temporal Regulation of Metamorphic Processes in Drosophila by the Let-7 and MiR-125 Heterochronic MicroRNAs.” *Current Biology*. https://doi.org/10.1016/j.cub.2008.06.020.

Chalfie, Martin, Yuan Tu, Ghia Euskirchen, W. Ward William, and C. Douglas, Prasher. 1994. “Green Fluorescent Protein as a Marker for Gene Expression.” *Science* 263 (February): 802–5. http://science.sciencemag.org/.

Chazotte, B. 2011. “Labeling Lysosomes in Live Cells with LysoTracker.” *Cold Spring Harb Protoc.* 2.

Chen, Jian-Fu, Mandel Elizabeth M, Thomson Michael J et al. 2006. “The Role of MicroRNA-1 and MicroRNA-133 in Skeletal Muscle Proliferation and Differentiation.” *Nature Genetics* 38 (2): 228–33. https://doi.org/10.1111/j.1582-4934.2011.01513.x.Small.

Chen, Xi, Yi Ba, Lijia Ma, Xing Cai, Yuan Yin, Kehui Wang, Jigang Guo, et al. 2008. “Characterization of MicroRNAs in Serum: A Novel Class of Biomarkers for Diagnosis of Cancer and Other Diseases.” *Cell Research* 18 (10): 997–1006. https://doi.org/10.1038/cr.2008.282.

Chen, Xi, Hongwei Liang, Junfeng Zhang, Ke Zen, and Chen Yu Zhang. 2012. “Secreted MicroRNAs: A New Form of Intercellular Communication.” *Trends in Cell Biology* 22 (3): 125–32. https://doi.org/10.1016/j.tcb.2011.12.001.

Chen, Yong, Marjan Gucek, Rosa Puertollano, and Jose a. Martina. 2012. “MTORC1 Functions as a Transcriptional Regulator of Autophagy by Preventing Nuclear Transport of TFEB.” *Autophagy* 8 (6): 877–876. https://doi.org/10.4161/auto.19653.

Chow, DK, CF Glenn, JL Johnston, IG Goldberg, and CA Wolkow. 2008. “Muscle Contraction

Rate Over Lifespan" 41 (3): 252–60. https://doi.org/10.1016/j.exger.2005.12.004.Sarcopenia.

Clague, Michael J., and Sylvie Urbé. 2010. "Ubiquitin: Same Molecule, Different Degradation Pathways." *Cell* 143 (5): 682–85. https://doi.org/10.1016/j.cell.2010.11.012.

Clancy, David J., Lawrence G. Harshman, Sean Oldham, Hugo Stocker, David Gems, Lawrence G. Harshman, Sean Oldham, et al. 2001. "Extension of Life-Span by Loss of CHICO, a Drosophila Insulin Receptor Substrate Protein." *Science* 292 (5514): 104–6. https://doi.org/https://doi.org/10.1126/science.1057991.

Clauss, Sebastian, Reza Wakili, Bianca Hildebrand, Stefan Kääb, Eva Hoster, Ina Klier, Eimo Martens, et al. 2016. "MicroRNAs as Biomarkers for Acute Atrial Remodeling in Marathon Runners (The MiRathon Study - A Sub-Study of the Munich Marathon Study)." *PLoS ONE* 11 (2): 1–23. https://doi.org/10.1371/journal.pone.0148599.

Cogswell, John P., James Ward, Ian A. Taylor, Michelle Waters, Yunling Shi, Brian Cannon, Kevin Kelnar, et al. 2008. "Identification of MiRNA Changes in Alzheimer's Disease Brain and CSF Yields Putative Biomarkers and Insights into Disease Pathways." *Journal of Alzheimer's Disease* 14 (1): 27–41. https://doi.org/10.3233/JAD-2008-14103.

Cohen, Ehud, Jan Bieschke, Rhonda M. Perciavalle, Jeffery W. Kelly, and Andrew Dillin. 2006. "Opposing Activities Protect against Age-Onset Proteotoxicity." *Science* 313 (5793): 1604–10. https://doi.org/10.1126/science.1124646.

Collins, Galen Andrew, and Alfred L Goldberg. 2017. "Review The Logic of the 26S Proteasome."

Consortium, The C. elegans Sequencing. 1998. "Genome Sequence of the Nematode C . Elegans : A Platform for Investigating Biology." *Science* 283 (5398): 2012–19.

Cotter, Kristina, Laura Stransky, Christina McGuire, and Michael Forgac. 2015. "Recent Insights into the Structure, Regulation, and Function of the V-ATPases." *Trends in Biochemical Sciences* 40 (10): 611–22. https://doi.org/10.1016/j.tibs.2015.08.005.

Coulson, Alan, John Sulston, Sydney Brenner, and Jonathan Karn. 1986. "Toward a Physical Map of the Genome of the Nematode Caenorhabditis Elegans (Ordered Clone Bank/Genomic Data Base/Clone Matching)." *Proc. Nati. Acad. Sci. USA* 83 (October): 7821–25.

Cruz-Jentoft, A. J., J. P. Baeyens, J. M. Bauer, Y. Boirie, T. Cederholm, F. Landi, F. C. Martin, et al. 2010. "Sarcopenia: European Consensus on Definition and Diagnosis: Report of the European Working Group on Sarcopenia in Older People." *Age and Ageing* 39 (4): 412–23. https://doi.org/10.1093/ageing/afq034.

Cuervo, Ana María, Amparo Palmer, A. Jennifer Rivett, and Erwin Knecht. 1995. "Degradation of Proteasomes by Lysosomes in Rat Liver." *European Journal of Biochemistry*. https://doi.org/10.1111/j.1432-1033.1995.0792p.x.

Cui, Shufang, Biao Sun, Xin Yin, Xia Guo, Dingming Chao, Chunni Zhang, Chen Yu Zhang, Xi Chen, and Jizheng Ma. 2017. “Time-Course Responses of Circulating MicroRNAs to Three Resistance Training Protocols in Healthy Young Men.” *Scientific Reports* 7 (1): 1–13. https://doi.org/10.1038/s41598-017-02294-y.

D, Accili, and Arden Kc. 2004. “FoxOs at the Crossroads of Cellular Metabolism, Differentiation, and Transformation.” *Cell* 117: 421–26.

D, Garigan, Hsu AL, Fraser AG, Kamath RS, Ahringer J, and Kenyon C. 2002. “Genetic Analysis of Tissue Aging in Caenorhabditis Elegans: A Role for Heat-Shock Factor and Bacterial Proliferation.” *Genetics* 161 (3): 1101–12. http://www.wormbase.org/db/misc/paper?name=WBPaper00005342.

Daitoku, Hiroaki, Jun ichi Sakamaki, and Akiyoshi Fukamizu. 2011. “Regulation of FoxO Transcription Factors by Acetylation and Protein-Protein Interactions.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1813 (11): 1954–60. https://doi.org/10.1016/j.bbamcr.2011.03.001.

David E. Lee, Akshay Bareja, David B. Bartlett, and James P. White. 2019. “Cells Autophagy as a Therapeutic Target to Enhance Aged Muscle Regeneration.” https://doi.org/10.3390/cells8020183.

Demontis, Fabio, and Norbert Perrimon. 2010. “FOXO/4E-BP Signaling in Drosophila Muscles Regulates Organism-Wide Proteostasis during Aging.” *Cell* 143 (5): 813–25. https://doi.org/10.1016/j.cell.2010.10.007.

Demontis, Fabio, Rosanna Piccirillo, Alfred L. Goldberg, and Norbert Perrimon. 2013. “Mechanisms of Skeletal Muscle Aging: Insights from Drosophila and Mammalian Models.” *DMM Disease Models and Mechanisms* 6 (6): 1339–52. https://doi.org/10.1242/dmm.012559.

Denham, Joshua, Adrian Gray, John Scott-Hamilton, and Amanda D. Hagstrom. 2018. “Sprint Interval Training Decreases Circulating MicroRNAs Important for Muscle Development.” *International Journal of Sports Medicine* 39 (1): 67–72. https://doi.org/10.1055/s-0043-120763.

Dice, J. 2007. “Chaperone-Mediated Autophagy.” *Autophagy* 8627: 295–99. https://doi.org/10.4161/auto.4144.

Diepholz, Meikel, David Venzke, Simone Prinz, Claire Batisse, Beate Flörchinger, Manfred Rössle, Dmitri I. Svergun, Bettina Böttcher, and James Féthière. 2008. “A Different Conformation for EGC Stator Subcomplex in Solution and in the Assembled Yeast V-ATPase: Possible Implications for Regulatory Disassembly.” *Structure* 16 (12): 1789–98. https://doi.org/10.1016/j.str.2008.09.010.

Distefano, Giovanna, and Bret H. Goodpaster. 2018. “Effects of Exercise and Aging on Skeletal Muscle.” *Cold Spring Harbor Perspectives in Medicine* 8 (3).

https://doi.org/10.1101/cshperspect.a029785.

Douglas, Peter M., and Andrew Dillin. 2010. “Protein Homeostasis and Aging in Neurodegeneration.” *Journal of Cell Biology* 190 (5): 719–29. https://doi.org/10.1083/jcb.201005144.

Drummond, D Allan, and Claus O Wilke. 2010. “NIH Public Access.” *Nature Reviews Genetics* 10 (10): 715–24. https://doi.org/10.1038/nrg2662.The.

Drummond, M. J., J. J. McCarthy, C. S. Fry, K. A. Esser, and B. B. Rasmussen. 2008. “Aging Differentially Affects Human Skeletal Muscle MicroRNA Expression at Rest and after an Anabolic Stimulus of Resistance Exercise and Essential Amino Acids.” *AJP: Endocrinology and Metabolism* 295 (6): E1333–40. https://doi.org/10.1152/ajpendo.90562.2008.

Drummond, Micah J., Odessa Addison, Lucille Brunker, Paul N. Hopkins, Donald A. McClain, Paul C. Lastayo, and Robin L. Marcus. 2014. “Downregulation of E3 Ubiquitin Ligases and Mitophagy-Related Genes in Skeletal Muscle of Physically Inactive, Frail Older Women: A Cross-Sectional Comparison.” *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 69 (8): 1040–48. https://doi.org/10.1093/gerona/glu004.

Duan, Ming Jing, Mei Ling Yan, Qin Wang, Meng Mao, Dan Su, Lin Lin Sun, Ke Xin Li, et al. 2018. “Overexpression of MiR-1 in the Heart Attenuates Hippocampal Synaptic Vesicle Exocytosis by the Posttranscriptional Regulation of SNAP-25 through the Transportation of Exosomes.” *Cell Communication and Signaling* 16 (1): 1–17. https://doi.org/10.1186/s12964-018-0303-5.

Ducluzeau, Pierre Henri, Noël Perretti, Martine Laville, Fabrizio Andreelli, Nathalie Vega, Jean Paul Riou, and Hubert Vidal. 2001. “Regulation by Insulin of Gene Expression in Human Skeletal Muscle and Adipose Tissue: Evidence for Specific Defects in Type 2 Diabetes.” *Diabetes* 50 (5): 1134–42. https://doi.org/10.2337/diabetes.50.5.1134.

Durieux, Jenni, Suzanne Wolff, and Andrew Dillin. 2011. “The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity.” *Cell* 144 (1): 79–91. https://doi.org/10.1016/j.cell.2010.12.016.

Duve, C. de, B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans. 1955. “Tissue Fractionation Studies.” *Biochemical Journal* 60: 604–17. https://doi.org/10.1042/bj0600604.

Duve C., Wattiaux R. De. 1966. “Functions of Lysosomes.” *Annu Rev Physiol.*, no. 28: 435–92.

Elbaz-Alon, Yael, Eden Rosenfeld-Gur, Vera Shinder, Anthony H. Futerman, Tamar Geiger, and Maya Schuldiner. 2014. “A Dynamic Interface between Vacuoles and Mitochondria in Yeast.” *Developmental Cell* 30 (1): 95–102. https://doi.org/10.1016/j.devcel.2014.06.007.

Elia, Leonardo, Riccardo Contu, Manuela Quintavalle, Francesca Varrone, Cristina Chimenti, Matteo Antonio Russo, Vincenzo Cimino, et al. 2009. “Reciprocal Regulation of Microrna-1 and Insulin-like Growth Factor-1 Signal Transduction Cascade in Cardiac and Skeletal Muscle in Physiological and Pathological Conditions.” *Circulation* 120 (23): 2377–85. https://doi.org/10.1161/CIRCULATIONAHA.109.879429.

Ellis, Ronald E, Dean M Jacobson, and H Robert Horvitz. 1991. “Genes Required for the Engulfment of Cell Corpses During Programmed Cell Death In.” *Genetics* 59.

Escobar, Kurt A, Nathan H Cole, Christine M Mermier, and Trisha A Vandusseldorp. 2019. “Autophagy and Aging : Maintaining the Proteome through Exercise and Caloric Restriction,” no. February 2018. https://doi.org/10.1111/acel.12876.

Ezcurra, Marina, Alexandre Benedetto, Thanet Sornda, Ann F. Gilliat, Catherine Au, Qifeng Zhang, Sophie van Schelt, et al. 2018. “C. Elegans Eats Its Own Intestine to Make Yolk Leading to Multiple Senescent Pathologies.” *Current Biology* 28 (16): 2544-2556.e5. https://doi.org/10.1016/j.cub.2018.06.035.

Fichtlscherer, Stephan, Andreas M. Zeiher, and Stefanie Dimmeler. 2011. “Circulating MicroRNAs: Biomarkers or Mediators of Cardiovascular Diseases?” *Arteriosclerosis, Thrombosis, and Vascular Biology* 31 (11): 2383–90. https://doi.org/10.1161/ATVBAHA.111.226696.

Fidani, Liana, John Hardy, Andrew Warren, Penelope Roques, David Hughes, Alison Goate, Martin Rossor, et al. 2003. “Early-Onset Alzheimer’s Disease Caused by Mutations at Codon 717 of the β-Amyloid Precursor Protein Gene.” *Nature* 353 (6347): 844–46. https://doi.org/10.1038/353844a0.

Filipowicz, Witold, Suvendra N. Bhattacharyya, and Nahum Sonenberg. 2008. “Mechanisms of Post-Transcriptional Regulation by MicroRNAs: Are the Answers in Sight?” *Nature Reviews Genetics* 9 (2): 102–14. https://doi.org/10.1038/nrg2290.

Finger, Fabian, Franziska Ottens, Alexander Springhorn, Tanja Drexel, Lucie Proksch, Sophia Metz, Luisa Cochella, and Thorsten Hoppe. 2018. “Olfaction Regulates Organismal Proteostasis and Longevity via MicroRNA-Dependent Signaling.” *BioRxiv* 1 (March): 420687. https://doi.org/10.1038/s42255-019-0033-z.

Finley, Daniel. 2009. “Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome.” *Annual Review of Biochemistry* 78 (1): 477–513. https://doi.org/10.1146/annurev.biochem.78.081507.101607.

Fisher, J. M., and R. H. Scheller. 1988. “Prohormone Processing and the Secretory Pathway.” *Journal of Biological Chemistry*.

Folick, Andrew, Holly D. Oakley, Yong Yu, Eric H. Armstrong, Manju Kumari, Lucas Sanor, David D. Moore, Eric A. Ortlund, Rudolf Zechner, and Meng C. Wang. 2015. “Lysosomal Signaling Molecules Regulate Longevity in Caenorhabditis Elegans.” *Science* 347

(6217): 83–86. https://doi.org/10.1126/science.1258857.

Frost, R. J. A., and E. N. Olson. 2011. “Control of Glucose Homeostasis and Insulin Sensitivity by the Let-7 Family of MicroRNAs.” *Proceedings of the National Academy of Sciences* 108 (52): 21075–80. https://doi.org/10.1073/pnas.1118922109.

Gallo, Alessia, Mayank Tandon, Ilias Alevizos, and Gabor G. Illei. 2012. “The Majority of MicroRNAs Detectable in Serum and Saliva Is Concentrated in Exosomes.” *PLoS ONE* 7 (3). https://doi.org/10.1371/journal.pone.0030679.

García-prat, Laura, Pura Muñoz-cánoves, Marta Martinez-vicente, and Laura Garc. 2016. “Dysfunctional Autophagy Is a Driver of Muscle Stem Cell Functional Decline with Aging.” *Autophagy* 12 (3): 612–13. https://doi.org/10.1080/15548627.2016.1143211.

Garcia, Susana M., M. Olivia Casanueva, M. Catarina Silva, Margarida D. Amara, and Richard I. Morimoto. 2007. “Neuronal Signaling Modulates Protein Homeostasis in Caenorhabditis Elegans Post-Synaptic Muscle Cells.” *Genes and Development*. https://doi.org/10.1101/gad.1575307.

Gething, Mary-jane. 1999. “Role and Regulation of the ER Chaperone BiP.” *Cell & Developmental Biology* 10: 465–72.

Ghildiyal, Megha, and Phillip D. Zamore. 2009. “Small Silencing RNAs: An Expanding Universe.” *Nature Reviews Genetics* 10 (2): 94–108. https://doi.org/10.1038/nrg2504.

Gibbings, Derrick J., Constance Ciaudo, Mathieu Erhardt, and Olivier Voinnet. 2009. “Multivesicular Bodies Associate with Components of MiRNA Effector Complexes and Modulate MiRNA Activity.” *Nature Cell Biology* 11 (9): 1143–49. https://doi.org/10.1038/ncb1929.

Gomes, Clarissa P C, Getúlio P. Oliveira-Jr, Bibiano Madrid, Jeeser A. Almeida, Octávio L. Franco, and Rinaldo W. Pereira. 2014. “Circulating MiR-1, MiR-133a, and MiR-206 Levels Are Increased after a Half-Marathon Run.” *Biomarkers* 19 (7): 585–89. https://doi.org/10.3109/1354750X.2014.952663.

Gomez-Orte, Eva, Eric Cornes, Angelina Zheleva, Beatriz Sáenz-Narciso, María de Toro, María Iñiguez, Rosario Lopez, et al. 2018. “Effect of the Diet Type and Temperature on the *C. Elegans* Transcriptome.” *Oncotarget* 9 (11): 9556–71. https://doi.org/10.18632/oncotarget.23563.

Grad, Yonatan, John Aach, Gabriel D. Hayes, Brenda J. Reinhart, George M. Church, Gary Ruvkun, and John Kim. 2003. “Computational and Experimental Identification of C. Elegans MicroRNAs.” *Molecular Cell* 11 (5): 1253–63. https://doi.org/10.1016/S1097-2765(03)00153-9.

Granjon, Aurélie, Marie Paule Gustin, Jennifer Rieusset, Etienne Lefai, Emmanuelle Meugnier, Isabelle Güller, Catherine Cerutti, et al. 2009. “The MicroRNA Signature in Response to Insulin Reveals Its Implication in the Transcriptional Action of Insulin in

Human Skeletal Muscle and the Role of a Sterol Regulatory Element-Binding Protein-1c/Myocyte Enhancer Factor 2C Pathway." *Diabetes* 58 (11): 2555–64. https://doi.org/10.2337/db09-0165.

Graves, Paul, and Yan Zeng. 2012. "Biogenesis of Mammalian MicroRNAs: A Global View." *Genomics, Proteomics and Bioinformatics* 10 (5): 239–45. https://doi.org/10.1016/j.gpb.2012.06.004.

Greer, Eric L., Dara Dowlatshahi, Max R. Banko, Judit Villen, Kimmi Hoang, Daniel Blanchard, Steve P. Gygi, and Brunet Anna. 2008. "An AMPK-FOXO Pathway Mediates the Extension of Lifespan Induced by a Novel Method of Dietary Restriction in C. Elegans." *Current Biology*. https://doi.org/10.1037/a0013262.Open.

Gu, Shuo, and Mark A. Kay. 2010. "How Do MiRNAs Mediate Translational Repression?" *Silence* 1 (1): 1–5. https://doi.org/10.1186/1758-907X-1-11.

Gui, YaXing, Hai Liu, LiShan Zhang, Wen Lv, and XingYue Hu. 2015. "Altered MicroRNA Profiles in Cerebrospinal Fluid Exosome in Parkinson Disease and Alzheimer Disease." *Oncotarget* 6 (35). https://doi.org/10.18632/oncotarget.6158.

Guo, Zhiyun, Miranda Maki, Ruofan Ding, Yalan Yang, Bao Zhang, and Lili Xiong. 2014. "Genome-Wide Survey of Tissue-Specific MicroRNA and Transcription Factor Regulatory Networks in 12 Tissues." *Scientific Reports* 4: 1–9. https://doi.org/10.1038/srep05150.

Hailey, Dale W., Angelika S. Rambold, Prasanna Satpute-Krishnan, Kasturi Mitra, Rachid Sougrat, Peter K. Kim, and Jennifer Lippincott-Schwartz. 2010. "Mitochondria Supply Membranes for Autophagosome Biogenesis during Starvation." *Cell* 141 (4): 656–67. https://doi.org/10.1016/j.cell.2010.04.009.

Halaschek-Wiener, Julius, Jaswinder S. Khattra, Sheldon McKay, Anatoli Pouzyrev, Jeff M. Stott, George S. Yang, Robert A. Holt, et al. 2005. "Analysis of Long-Lived C. Elegans Daf-2 Mutants Using Serial Analysis of Gene Expression." *Genome Research*. https://doi.org/10.1101/gr.3274805.

Hansen, Malene, and David C Rubinsztein. 2018. "Autophagy as a Promoter of Longevity : Insights from Model Organisms." *Nature Reviews Molecular Cell Biology*. https://doi.org/10.1038/s41580-018-0033-y.

Harber, Matthew P., Adam R. Konopka, Matthew D. Douglass, Kiril Minchev, Leonard A. Kaminsky, Todd A. Trappe, and Scott Trappe. 2009. "Aerobic Exercise Training Improves Whole Muscle and Single Myofiber Size and Function in Older Women." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 297 (5): R1452–59. https://doi.org/10.1152/ajpregu.00354.2009.

Harries, Lorna W. 2014. "MicroRNAs as Mediators of the Ageing Process." *Genes* 5 (3): 656–70. https://doi.org/10.3390/genes5030656.

Hayashi-Nishino, Mitsuko, Naonobu Fujita, Takeshi Noda, Akihito Yamaguchi, Tamotsu Yoshimori, and Akitsugu Yamamoto. 2009. “A Subdomain of the Endoplasmic Reticulum Forms a Cradle for Autophagosome Formation.” *Nature Cell Biology* 11 (12): 1433–37. https://doi.org/10.1038/ncb1991.

Hermann, Greg J., Lena K. Schroeder, Caroline A. Hieb, Aaron M. Kershner, Beverley M. Rabbitts, Paul Fonarev, Barth D. Grant, and James R. Priess. 2005. “Genetic Analysis of Lysosomal Trafficking in Caenorhabditis Elegans.” *Molecular Biology of the Cell* 16 (7): 3273–88. https://doi.org/10.1091/mbc.E05-01-0060.

Herndon, L. A., Wolkow, C. A., Driscoll, M., & Hall, D. H. 2017. “No Title.” In *In A. Olsen, & M. S. Gill (Eds.), Ageing: Lessons from C. Elegans*, 9–39. Cham, Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-44703-2.

Herndon, Laura A, Peter J Schmeissner, Justyna M Dudaronek, Paula A Brown, Kristin M Listner, Yuko Sakano, Marie C Paupard, David H Hall, and Monica Driscoll. 2002. “Stochastic and Genetic Factors Influence Tissue-Specific Decline in Ageing C . Elegans” 419 (October).

Hershko A., Ciechanover A. 1998. “The Ubiquitin System.” *Trends in Biochemical Sciences* 22 (10): 383–87. https://doi.org/10.1016/S0968-0004(97)01122-5.

Hipkiss, Alan R. 2007. “On Why Decreasing Protein Synthesis Can Increase Lifespan.” *Mechanisms of Ageing and Development* 128 (5–6): 412–14. https://doi.org/10.1016/j.mad.2007.03.002.

Hönscher, Carina, Muriel Mari, Kathrin Auffarth, Maria Bohnert, Janice Griffith, Willie Geerts, Martin van der Laan, Margarita Cabrera, Fulvio Reggiori, and Christian Ungermann. 2014. “Cellular Metabolism Regulates Contact Sites between Vacuoles and Mitochondria.” *Developmental Cell* 30 (1): 86–94. https://doi.org/10.1016/j.devcel.2014.06.006.

Horvath, Steve. 2013. “DNA Methylation Age of Human Tissues and Cell Types.” *Genome Biology* 14 (10). https://doi.org/10.1186/gb-2013-14-10-r115.

Hsu, AL, CT Murphy, and C Kenyon. 2003. “Regulation of Aging and Age-Related Disease by DAF-16 and Heat-Shock Factor.” *Science* 300 (May): 1142–45. http://www.wormbase.org/db/misc/paper?name=WBPaper00005851.

Hua, Li, Guirong Zhu, and Jianguo Wei. 2018. “MicroRNA-1 Overexpression Increases Chemosensitivity of Non-Small Cell Lung Cancer Cells by Inhibiting Autophagy Related 3-Mediated Autophagy.” *Cell Biology International*. https://doi.org/10.1002/cbin.10995.

Huang, C., C. Xiong, and K. Kornfeld. 2004. “Measurements of Age-Related Changes of Physiological Processes That Predict Lifespan of Caenorhabditis Elegans.” *Proceedings of the National Academy of Sciences* 101 (21): 8084–89. https://doi.org/10.1073/pnas.0400848101.

Hughes, Adam L., and Daniel E. Gottschling. 2012. “An Early Age Increase in Vacuolar PH Limits Mitochondrial Function and Lifespan in Yeast.” *Nature* 492 (7428): 261–65. https://doi.org/10.1038/nature11654.

Huotari J., Helenius A. 2011. “Endosome Maturation.” *EMBO Journal* 30: 3481–3500.

Huynh, K. K., and S. Grinstein. 2007. “Regulation of Vacuolar PH and Its Modulation by Some Microbial Species.” *Microbiology and Molecular Biology Reviews* 71 (3): 452–62. https://doi.org/10.1128/mmbr.00003-07.

Hwang, Kou M., Linda C. Yang, Christine K. Carrico, Rose A. Schulz, John B. Schenkman, and Alan C. Sartorelli. 1974. “Production of Membrane Whorls in Rat Liver by Some Inhibitors of Protein Synthesis.” *Journal of Cell Biology* 62 (1): 20–31. https://doi.org/10.1083/jcb.62.1.20.

Hwangbo, Dae Sung, Boris Gersham, Meng-ping Tu, and Michael Palmer. 2004. “Drosophila DFOXO Controls.Pdf” 429 (June): 562–67. https://doi.org/10.1038/nature02549.

Ibáñez-Ventoso, Carolina, Maocheng Yang, Suzhen Guo, Harlan Robins, Richard W. Padgett, and Monica Driscoll. 2006. “Modulated MicroRNA Expression during Adult Lifespan in Caenorhabditis Elegans.” *Aging Cell* 5 (3): 235–46. https://doi.org/10.1111/j.1474-9726.2006.00210.x.

Isik, Ahmet Turan. 2010. “Clinical Interventions in Aging Dovepress Late Onset Alzheimer’s Disease in Older People.” *Clinical Interventions in Aging*, 5–307. https://doi.org/10.2147/CIA.S11718.

Jiang, Xuejun, Ian G. Ganley, She Chen, Junru Wang, Xiaojun Ding, and Du H. Lam. 2009. “ULK1·ATG13·FIP200 Complex Mediates MTOR Signaling and Is Essential for Autophagy.” *Journal of Biological Chemistry* 284 (18): 12297–305. https://doi.org/10.1074/jbc.m900573200.

Jing, Zhao, Weidong Han, Xinbing Sui, Jiansheng Xie, and Hongming Pan. 2015. “Interaction of Autophagy with MicroRNAs and Their Potential Therapeutic Implications in Human Cancers.” *Cancer Letters* 356 (2): 332–38. https://doi.org/10.1016/j.canlet.2014.09.039.

Jung, Hwa Jin, Kwang-pyo Lee, Ki-sun Kwon, and Yousin Suh. 2018. “MicroRNAs in Skeletal Muscle Aging : Current Issues and Perspectives” XX (Xx): 1–7. https://doi.org/10.1093/gerona/gly207.

Kamath, Ravi S, and Julie Ahringer. 2003. “Genome-Wide RNAi Screening in Caenorhabditis Elegans” 30: 313–21. https://doi.org/10.1016/S1046-2023(03)00050-1.

Kanamoto, Takashi, Koji Terada, Hideki Yoshikawa, and Takahisa Furukawa. 2006. “Cloning and Regulation of the Vertebrate Homologue of Lin-41 That Functions as a Heterochronic Gene in Caenorhabditis Elegans.” *Developmental Dynamics*. https://doi.org/10.1002/dvdy.20712.

Karin, M., R. Birse, G. A. Perkins, T. E. Kim, J. H. Lee, A. V. Budanov, M. H. Ellisman, et al.

2010. “Sestrin as a Feedback Inhibitor of TOR That Prevents Age-Related Pathologies.” *Science* 327 (5970): 1223–28. https://doi.org/10.1126/science.1182228.

Katewa, Subhash D., Marysia C. Kolipinski, Aric N. Rogers, Seymour Benzer, Tony Au Lu, Misha A. Vargas, Pankaj Kapahi, and Brian M. Zid. 2009. “4E-BP Extends Lifespan upon Dietary Restriction by Enhancing Mitochondrial Activity in Drosophila.” *Cell* 139 (1): 149–60. https://doi.org/10.1016/j.cell.2009.07.034.

Kato, Masaomi, Xiaowei Chen, Sachi Inukai, Hongyu Zhao, and Frank J Slack. 2011. “Age-Associated Changes in Expression of Small , Noncoding RNAs , Including MicroRNAs , in C . Elegans,” 1804–20. https://doi.org/10.1261/rna.2714411.wide.

Kenyon, Cynthia, Jean Chang, and Erin Gensch. 1993. “A C. Elegans Mutant That Lives Twice as Long as Wild Type.” *Nature* 366 (December): 461–64.

Kenyon, Cynthia J. 2010. “The Genetics of Ageing.” *Nature* 464 (7288): 504–12. https://doi.org/10.1038/nature08980.

Kenyon, Cynthia, Seung-Jae Lee, Malene Hansen, Nataliya Libina, Stefan Taubert, and Douglas Crawford. 2007. “Lifespan Extension by Conditions That Inhibit Translation in Caenorhabditis Elegans.” *Aging Cell* 6 (1): 95–110. https://doi.org/10.1111/j.1474-9726.2006.00267.x.

Khvorova, Anastasia, Angela Reynolds, and Sumedha D. Jayasena. 2003. “Functional SiRNAs and MiRNAs Exhibit Strand Bias.” *Cell* 115 (2): 209–16. https://doi.org/10.1016/S0092-8674(03)00801-8.

Kirkin, Vladimir, Trond Lamark, Yu Shin Sou, Geir Bjørkøy, Jennifer L. Nunn, Jack Ansgar Bruun, Elena Shvets, et al. 2009. “A Role for NBR1 in Autophagosomal Degradation of Ubiquitinated Substrates.” *Molecular Cell* 33 (4): 505–16. https://doi.org/10.1016/j.molcel.2009.01.020.

Kirkin, Vladimir, David G. McEwan, Ivana Novak, and Ivan Dikic. 2009. “A Role for Ubiquitin in Selective Autophagy.” *Molecular Cell* 34 (3): 259–69. https://doi.org/10.1016/j.molcel.2009.04.026.

Kirkwood, Thomas B L, and Steven N Austad. 2000. “Why Do We Age?” 408 (November): 233–38.

Klass, Michael R. 1977. “Aging in the Nematode Caenorhabditis Elegans: Major Biological and Environmental Factors Influencing Life Span.” *Mechanisms of Ageing and Development* 6: 413–29.

Komatsu, Masaaki, Satoshi Waguri, Masato Koike, Yu-shin Sou, Takashi Ueno, Taichi Hara, Noboru Mizushima, et al. 2007. “Homeostatic Levels of P62 Control Cytoplasmic Inclusion Body Formation in Autophagy-Deficient Mice,” 1149–63. https://doi.org/10.1016/j.cell.2007.10.035.

Korolchuk, Viktor I., Fiona M. Menzies, and David C. Rubinsztein. 2010. “Mechanisms of

Cross-Talk between the Ubiquitin-Proteasome and Autophagy-Lysosome Systems." *FEBS Letters* 584 (7): 1393–98. https://doi.org/10.1016/j.febslet.2009.12.047.

Kosaka, Nobuyoshi, Haruhisa Iguchi, Yusuke Yoshioka, Fumitaka Takeshita, Yasushi Matsuki, and Takahiro Ochiya. 2010. "Secretory Mechanisms and Intercellular Transfer of MicroRNAs in Living Cells." *Journal of Biological Chemistry* 285 (23): 17442–52. https://doi.org/10.1074/jbc.M110.107821.

Krek, Azra, Dominic Grün, Matthew N Poy, Rachel Wolf, Lauren Rosenberg, Eric J Epstein, Philip MacMenamin, et al. 2005. "Combinatorial MicroRNA Target Predictions." *Nature Genetics* 37 (5): 495–500. https://doi.org/10.1038/ng1536.

Kunz, Joachim B, Heinz Schwarz, and Andreas Mayer. 2004. "Determination of Four Sequential Stages during Microautophagy in Vitro *" 279 (11): 9987–96. https://doi.org/10.1074/jbc.M307905200.

Lagos-Quintana, M., R. Rauhut, W. Lendeckel, and T. Tuschl. 2001. "Identification of Novel Genes Coding for Small Expressed RNAs." *Science* 294 (5543): 853–58. https://doi.org/10.1126/science.1064921.

Lapierre, Louis R., Sara Gelino, Alicia Meléndez, and Malene Hansen. 2011. "Autophagy and Lipid Metabolism Coordinately Modulate Life Span in Germline-Less C. Elegans." *Current Biology* 21 (18): 1507–14. https://doi.org/10.1016/j.cub.2011.07.042.

Laranjeiro, Ricardo, Girish Harinath, Daniel Burke, Bart P. Braeckman, and Monica Driscoll. 2017. "Single Swim Sessions in C. Elegans Induce Key Features of Mammalian Exercise." *BMC Biology* 15 (1). https://doi.org/10.1186/s12915-017-0368-4.

Laranjeiro, Ricardo, Girish Harinath, Jennifer E Hewitt, Jessica H Hartman, Mary Anne Royal, Joel N Meyer, Siva A Vanapalli, and Monica Driscoll. 2019. "Swim Exercise in C. Elegans Extends Neuromuscular and Intestinal Healthspan, Enhances Learning Ability, and Protects against Neurodegeneration." *BioRxiv*, 633776. https://doi.org/10.1101/633776.

Larsen, P L, P S Albert, and D L Riddle. 1995. "Genes That Regulate Both Development and Longevity in Caenorhabditis Elegans." *Genetics* 139 (4): 1567–83. http://www.ncbi.nlm.nih.gov/pubmed/7789761%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1206485.

Lee, R C, R L Feinbaum, and V Ambros. 1993. "The C. Elegans\rheterochronic Gene Lin-4 Encodes Small RNAs with Antisense\rcomplementarity to Lin-14." *Cell* 75: 843–85: 843–54. https://doi.org/10.1016/0092-8674(93)90529-Y.

Lee, Yoontae, Minju Kim, Jinju Han, Kyu-Hyun Yeom, Sanghyuk Lee, Sung Hee Baek, and V Narry Kim. 2004. "MicroRNA Genes Are Transcribed by RNA Polymerase II." *The EMBO Journal* 23 (20): 4051–60. https://doi.org/10.1038/sj.emboj.7600385.

Lefebvre, Christophe, Michael Trichet, Claire Boulogne, Marion Manil-se, Renaud Legouis,

Marion Manil-Ségalen, Christophe Lefebvre, et al. 2014. “The C. Elegans LC3 Acts Downstream of GABARAP to Degrade Autophagosomes by Interacting with the HOPS Subunit VPS39.” *Developmental Cell*. https://doi.org/10.1016/j.devcel.2013.11.022.

Levine, Beth, Guido Kroemer, and Institut Gustave Roussy. 2008. “Review Autophagy in the Pathogenesis of Disease,” 27–42. https://doi.org/10.1016/j.cell.2007.12.018.

Lewis, Benjamin P., I. Hung Shih, Matthew W. Jones-Rhoades, David P. Bartel, and Christopher B. Burge. 2003. “Prediction of Mammalian MicroRNA Targets.” *Cell* 115 (7): 787–98. https://doi.org/10.1016/S0092-8674(03)01018-3.

Li, Chuanwei, Fang Pei, Xiaoshan Zhu, Dayue D Duan, and Chunyu Zeng. 2012. “Circulating MicroRNAs as Novel and Sensitive Biomarkers of Acute Myocardial Infarction.” *Clinical Biochemistry* 45 (0): 727–32. https://doi.org/10.1016/j.clinbiochem.2012.04.013.

Li, Jianzhe, Xiaomin Dong, Zhongping Wang, and Jianhua Wu. 2014. “MicroRNA-1 in Cardiac Diseases and Cancers.” *Korean Journal of Physiology and Pharmacology* 18 (5): 359–63. https://doi.org/10.4196/kjpp.2014.18.5.359.

Li, Yan, Shengjie Zhang, Lei Zhao, Hao Ying, Wei Liu, Hui Wang, Jingjing Jiang, et al. 2018. “MicroRNA-378 Promotes Autophagy and Inhibits Apoptosis in Skeletal Muscle.” *Proceedings of the National Academy of Sciences* 115 (46): E10849–58. https://doi.org/10.1073/pnas.1803377115.

Liebl, Martina P., and Thorsten Hoppe. 2016. “It’s All about Talking: Two-Way Communication between Proteasomal and Lysosomal Degradation Pathways via Ubiquitin.” *American Journal of Physiology - Cell Physiology* 311 (2): C166–78. https://doi.org/10.1152/ajpcell.00074.2016.

Link, C. D. 1995. “Expression of Human Beta-Amyloid Peptide in Transgenic Caenorhabditis Elegans.” *Proceedings of the National Academy of Sciences* 92 (20): 9368–72. https://doi.org/10.1073/pnas.92.20.9368.

Liou, Willisa, Hans J. Geuze, M. J H Geelen, and Jan W. Slot. 1997. “The Autophagic and Endocytic Pathways Converge at the Nascent Autophagic Vacuoles.” *Journal of Cell Biology* 136 (1): 61–70. https://doi.org/10.1083/jcb.136.1.61.

Liu, Wei, Hongchao Cao, Cheng Ye, Cunjie Chang, Minghua Lu, Yanyan Jing, Duo Zhang, et al. 2014. “Hepatic MiR-378 Targets P110Î± and Controls Glucose and Lipid Homeostasis by Modulating Hepatic Insulin Signalling.” *Nature Communications* 5 (May). https://doi.org/10.1038/ncomms6684.

Livneh, Ido, Victoria Cohen-Kaplan, Chen Cohen-Rosenzweig, Noa Avni, and Aaron Ciechanover. 2016. “The Life Cycle of the 26S Proteasome: From Birth, through Regulation and Function, and onto Its Death.” *Cell Research* 26 (8): 869–85. https://doi.org/10.1038/cr.2016.86.

Locke, M., and A. K. Sykes. 1975. “The Role of the Golgi Complex in the Isolation and

Digestion of Organelles." *Tissue and Cell* 7 (1): 143–58. https://doi.org/10.1016/S0040-8166(75)80012-7.

López-Otín, Carlos, Maria A. Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. 2013. "The Hallmarks of Aging." *Cell* 153 (6). https://doi.org/10.1016/j.cell.2013.05.039.

Lucanic, Mark, Jill Graham, Gary Scott, Dipa Bhaumik, Christopher C Benz, Alan Hubbard, Gordon J Lithgow, and Simon Melov. 2013. "Age - Related Micro - RNA Abundance in Individual C . Elegans." *Aging* 5 (6): 394–411.

Ma, J. C., M. J. Duan, L. L. Sun, M. L. Yan, T. Liu, Q. Wang, C. D. Liu, et al. 2015. "Cardiac Over-Expression of MicroRNA-1 Induces Impairment of Cognition in Mice." *Neuroscience* 299: 66–78. https://doi.org/10.1016/j.neuroscience.2015.04.061.

Ma, Yanjun, and Linda M Hendershot. 2004. "ER Chaperone Functions during Normal and Stress Conditions" 28: 51–65. https://doi.org/10.1016/j.jchemneu.2003.08.007.

Majeski, Amy E., and J. Fred Dice. 2004. "Mechanisms of Chaperone-Mediated Autophagy." *International Journal of Biochemistry and Cell Biology* 36 (12): 2435–44. https://doi.org/10.1016/j.biocel.2004.02.013.

Malta, C. Di, P. Colella, M. G. Arencibia, D. C. Rubinsztein, S. Erdin, S. U. Erdin, F. Vetrini, et al. 2011. "TFEB Links Autophagy to Lysosomal Biogenesis." *Science* 332 (6036): 1429–33. https://doi.org/10.1126/science.1204592.

Mammucari, Cristina, Stefano Schiaffino, Eva Masiero, Vanina Romanello, Alfred L. Goldberg, Marco Sandri, Claudia Sandri, et al. 2007. "FoxO3 Controls Autophagy in Skeletal Muscle In Vivo." *Cell Metabolism* 6 (6): 458–71. https://doi.org/10.1016/j.cmet.2007.11.001.

Manley, Gerard. 2013. "Public Access NIH Public Access" 71 (2): 233–36. https://doi.org/10.1038/mp.2011.182.doi.

Martinez-lopez, Nuria, Diana Athonvarangkul, Rajat Singh, and Forchheimer Building. 2015. "Longevity Genes" 847: 73–87. https://doi.org/10.1007/978-1-4939-2404-2.

Martinez, V.G., Javadi, C.S., Ngo, E., Lagow, R.D., Zhang, B. 2007. "Age-Related Changes in Climbing Behaviour and Neural Circuit Physiology in Drosophila." *Developmental Neurobiology* 67: 778–91.

Masiero, Eva, Lisa Agatea, Cristina Mammucari, Bert Blaauw, Emanuele Loro, Masaaki Komatsu, Daniel Metzger, Carlo Reggiani, Stefano Schiaffino, and Marco Sandri. 2009. "Autophagy Is Required to Maintain Muscle Mass." *Cell Metabolism* 10 (6): 507–15. https://doi.org/10.1016/j.cmet.2009.10.008.

Mass, Muscle, Metabolic Homeostasis, Epithelial Senescence, Caterina Tezze, Vanina Romanello, Maria Andrea Desbats, Leonardo Salviati, et al. 2017. "Age-Associated Loss of OPA1 in Muscle Impacts Article Age-Associated Loss of OPA1 in Muscle Impacts Muscle Mass , Metabolic Homeostasis , Systemic Inflammation , and Epithelial

Senescence," 1374–89.

McGee, Matthew D., Darren Weber, Nicholas Day, Cathy Vitelli, Danielle Crippen, Laura A. Herndon, David H. Hall, and Simon Melov. 2011. "Loss of Intestinal Nuclei and Intestinal Integrity in Aging C. Elegans." *Aging Cell* 10 (4): 699–710. https://doi.org/10.1111/j.1474-9726.2011.00713.x.

Meijer, Wiebe H., Ida J. Van Der Klei, Marien Veenhuis, and Jan A.K.W. Kiel. 2007. "ATG Genes Involved in Non-Selective Autophagy Are Conserved from Yeast to Man, but the Selective Cvt and Pexophagy Pathways Also Require Organism-Specific Genes." *Autophagy* 3 (2): 106–16. https://doi.org/10.4161/auto.3595.

Mellman, I., Fuchs R., Helenius A. 1986. "Acidification of the Endocytic and Exocytic Pathways." *Annual Review of Biochemistry*, no. 55: 663–700.

Mergoud, Adeline, Laurent Molin, and Florence Solari. 2014. "UNC - 120 / SRF Independently Controls Muscle Aging and Lifespan in Caenorhabditis Elegans."

Miller, Mark S., Panagiotis Lekkas, Joan M. Braddock, Gerrie P. Farman, Bryan A. Ballif, Thomas C. Irving, David W. Maughan, and Jim O. Vigoreaux. 2008. "Aging Enhances Indirect Flight Muscle Fiber Performance yet Decreases Flight Ability in Drosophila." *Biophysical Journal* 95 (5): 2391–2401. https://doi.org/10.1529/biophysj.108.130005.

Mishima, Yuichiro, Cei Abreu-Goodger, Alison A. Staton, Carlos Stahlhut, Chong Shou, Chao Cheng, Mark Gerstein, Anton J. Enright, and Antonio J. Giraldez. 2009. "Zebrafish MiR-1 and MiR-133 Shape Muscle Gene Expression and Regulate Sarcomeric Actin Organization." *Genes and Development* 23 (5): 619–32. https://doi.org/10.1101/gad.1760209.

Mitchell, Patrick S, Rachael K Parkin, Evan M Kroh, Brian R Fritz, Stacia K Wyman, Era L Pogosova-agadjanyan, Amelia Peterson, et al. 2008. "Mitchell et Al. - 2008 - Circulating MicroRNAs as Stable Blood-Based Markers for Cancer Detection.Pdf." https://doi.org/10.1073/pnas.0804549105.

Mittelbrunn, María, Cristina Gutiérrez-Vázquez, Carolina Villarroya-Beltri, Susana González, Fátima Sánchez-Cabo, Manuel Ángel González, Antonio Bernad, and Francisco Sánchez-Madrid. 2011. "Unidirectional Transfer of MicroRNA-Loaded Exosomes from T Cells to Antigen-Presenting Cells." *Nature Communications* 2 (1). https://doi.org/10.1038/ncomms1285.

Moon, Jangsup, Soon Tae Lee, Il Gyu Kong, Jung Ick Byun, Jun Sang Sunwoo, Jung Won Shin, Ji Young Shim, et al. 2016. "Early Diagnosis of Alzheimer's Disease from Elevated Olfactory Mucosal MiR-206 Level." *Scientific Reports* 6 (December 2015): 1–9. https://doi.org/10.1038/srep20364.

Morimoto, R.I. 2008. "Proteotoxic Stress and Inducible Chaperone Networks in Neurodegenerative Disease and Aging." *Genes and Development*.

https://doi.org/10.1101/gad.1657108.

Moriyama, Y., M. Maeda, and M. Futai. 1992. “The Role of V-ATPase in Neuronal and Endocrine Systems.” *The Journal of Experimental Biology* 172: 171–78.

Morley, J. F., H. R. Brignull, J. J. Weyers, and R. I. Morimoto. 2002. “The Threshold for Polyglutamine-Expansion Protein Aggregation and Cellular Toxicity Is Dynamic and Influenced by Aging in Caenorhabditis Elegans.” *Proceedings of the National Academy of Sciences* 99 (16): 10417–22. https://doi.org/10.1073/pnas.152161099.

Mortimore, GE, Schworer, CM. 1977. “Induction of Autophagy by Amino-Acid Deprivation in Perfused Rat Liver.” *Nature* 270 (November): 174–76.

Muench, Stephen P., Markus Huss, Chun Feng Song, Clair Phillips, Helmut Wieczorek, John Trinick, and Michael A. Harrison. 2009. “Cryo-Electron Microscopy of the Vacuolar ATPase Motor Reveals Its Mechanical and Regulatory Complexity.” *Journal of Molecular Biology* 386 (4): 989–99. https://doi.org/10.1016/j.jmb.2009.01.014.

Murata, Shigeo, Isei Tanida, Satoshi Waguri, Yasuo Uchiyama, Eiki Kominami, Keiji Tanaka, Masato Koike, et al. 2006. “Loss of Autophagy in the Central Nervous System Causes Neurodegeneration in Mice.” *Nature* 441 (7095): 880–84. https://doi.org/10.1038/nature04723.

Murphy, C. T., S.-J. Lee, and C. Kenyon. 2007. “Tissue Entrainment by Feedback Regulation of Insulin Gene Expression in the Endoderm of Caenorhabditis Elegans.” *Proceedings of the National Academy of Sciences* 104 (48): 19046–50. https://doi.org/10.1073/pnas.0709613104.

Nair, K Sreekumaran. 2005. “Aging Muscle 1–5.” *American Journal of Clinical Nutrition* 81: 953–63.

Nascimento, C M, M Ingles, M R Cominetti, and J Viña. 2019. “Sarcopenia , Frailty and Their Prevention by Exercise.” *Free Radical Biology and Medicine* 132 (August 2018): 42–49. https://doi.org/10.1016/j.freeradbiomed.2018.08.035.

Niccoli, Teresa, and Linda Partridge. 2012. “Ageing as a Risk Factor for Disease.” *Current Biology* 22 (17): R741–52. https://doi.org/10.1016/j.cub.2012.07.024.

Nielsen, Søren, Thine Hvid, Meghan Kelly, Birgitte Lindegaard, Christine Dethlefsen, Kamilla Winding, Neha Mathur, Camilla Scheele, Bente K Pedersen, and Matthew J Laye. 2014. “Muscle Specific MiRNAs Are Induced by Testosterone and Independently Upregulated by Age” 4 (January): 1–11. https://doi.org/10.3389/fphys.2013.00394.

Nishi, Tsuyoshi, and Michael Forgac. 2002. “The Vacuolar (H+)-ATPases - Nature’s Most Versatile Proton Pumps.” *Nature Reviews Molecular Cell Biology* 3 (2): 94–103. https://doi.org/10.1038/nrm729.

Nussbaum-Krammer, Carmen I., Kyung Won Park, Liming Li, Ronald Melki, and Richard I. Morimoto. 2013. “Spreading of a Prion Domain from Cell-to-Cell by Vesicular Transport

in Caenorhabditis Elegans." *PLoS Genetics* 9 (3): 21–23. https://doi.org/10.1371/journal.pgen.1003351.

O'Brien, Jacob, Heyam Hayder, Yara Zayed, and Chun Peng. 2018. "Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation." *Frontiers in Endocrinology* 9 (AUG): 1–12. https://doi.org/10.3389/fendo.2018.00402.

Oakes, Jonathan A., Elijah D. Johnston, Brian K. Kennedy, Diana N. Pak, Nick Dang, Di Hu, Mitsuhiro Tsuchiya, et al. 2008. "Yeast Life Span Extension by Depletion of 60S Ribosomal Subunits Is Mediated by Gcn4." *Cell* 133 (2): 292–302. https://doi.org/10.1016/j.cell.2008.02.037.

Ogg, S, S Paradis, Gottlieb, GI Patterson, L Lee, HA Tissenbaum, and GB Ruvkun. 1997. "The Fork Head Transcription Factor DAF-16 Transduces Insulin-like Metabolic and Longevity Signals in C. Elegans." *Nature* 389 (October): 994–99. https://doi.org/10.1038/40194.

Oosten-Hawle, Patricija Van, Robert S. Porter, and Richard I. Morimoto. 2013. "Regulation of Organismal Proteostasis by Transcellular Chaperone Signaling." *Cell* 153 (6): 1366. https://doi.org/10.1016/j.cell.2013.05.015.

Palauqui, Jean Christophe, Taline Elmayan, Jean Marie Pollien, and Hervé Vaucheret. 1997. "Systemic Acquired Silencing: Transgene-Specific Post-Transcriptional Silencing Is Transmitted by Grafting from Silenced Stocks to Non-Silenced Scions." *EMBO Journal* 16 (15): 4738–45. https://doi.org/10.1093/emboj/16.15.4738.

Panwar, Bharat, Gilbert S. Omenn, and Yuanfang Guan. 2017. "MiRmine: A Database of Human MiRNA Expression Profiles." *Bioinformatics* 33 (10): 1554–60. https://doi.org/10.1093/bioinformatics/btx019.

Park, Sung Sup. 2019. "Sarcopenia Targeting with Autophagy Mechanism by Exercise" 52 (1): 64–69.

Patel, P. H., and F. Tamanoi. 2006. "Increased Rheb-TOR Signaling Enhances Sensitivity of the Whole Organism to Oxidative Stress." *Journal of Cell Science* 119 (20): 4285–92. https://doi.org/10.1242/jcs.03199.

Peng, Shanxin, Jing Wang, Songtao Wei, Changfei Li, Kai Zhou, Jun Hu, Xin Ye, et al. 2018. "Endogenous Cellular MicroRNAs Mediate Antiviral Defense against Influenza A Virus." *Molecular Therapy - Nucleic Acids* 10 (March): 361–75. https://doi.org/10.1016/j.omtn.2017.12.016.

Pfeifer, U. 1977. "Inhibition by Insulin of the Physiological Autophagic Breakdown of Cell Organelles. Title." *Acta Biol. Med. Ger.*, no. 36: 1691–94.

Piccirillo, Rosanna, Fabio Demontis, Norbert Perrimon, and Alfred L Goldberg. 2014. "Mechanisms of Muscle Growth and Atrophy in Mammals and Drosophila," no. August 2013: 201–15. https://doi.org/10.1002/dvdy.24036.

Pickford, Fiona, Eliezer Masliah, Markus Britschgi, Kurt Lucin, Ramya Narasimhan, Philipp A. Jaeger, Scott Small, et al. 2008. “The Autophagy-Related Protein Beclin 1 Shows Reduced Expression in Early Alzheimer Disease and Regulates Amyloid β Accumulation in Mice.” *Journal of Clinical Investigation* 118 (6): 2190–99. https://doi.org/10.1172/JCI33585.

Pollock, Ross D., Katie A. O’Brien, Lorna J. Daniels, Kathrine B. Nielsen, Anthea Rowlerson, Niharika A. Duggal, Norman R. Lazarus, Janet M. Lord, Andrew Philp, and Stephen D.R. Harridge. 2018. “Properties of the Vastus Lateralis Muscle in Relation to Age and Physiological Function in Master Cyclists Aged 55–79 Years.” *Aging Cell*. https://doi.org/10.1111/acel.12735.

Prahlad, Veena, Tyler Cornelius, and Richard I. Morimoto. 2008. “Regulation of the Cellular Heat Shock Response in Caenorhabditis Elegans by Thermosensory Neurons.” *Science* 320 (5877): 811–14. https://doi.org/10.1126/science.1156093.

Pu, Jing, Carlos M. Guardia, Tal Keren-Kaplan, and Juan S. Bonifacino. 2016. “Mechanisms and Functions of Lysosome Positioning.” *Journal of Cell Science* 129 (23): 4329–39. https://doi.org/10.1242/jcs.196287.

Rajawat, Yogendra S., Zoe Hilioti, and Ioannis Bossis. 2009. “Aging: Central Role for Autophagy and the Lysosomal Degradative System.” *Ageing Research Reviews* 8 (3): 199–213. https://doi.org/10.1016/j.arr.2009.05.001.

Ravikumar B., Duden R., Rubinsztein DC. 2002. “Aggregate-Prone Proteins with Polyglutamine and Polyalanine Expansions Are Degraded by Autophagy.” *Human Molecular Genetics* 11 (9): 1107–17. https://doi.org/10.1093/hmg/11.9.1107.

Ravikumar, Brinda, Kevin Moreau, Luca Jahreiss, Claudia Puri, and David C. Rubinsztein. 2010. “Erratum: Plasma Membrane Contributes to the Formation of Pre-Autophagosomal Structures (Nature Cell Biology (2010) 12 (747-757)).” *Nature Cell Biology* 12 (10): 1021. https://doi.org/10.1038/ncb1010-1021c.

Ravikumar, Brinda, Coralie Vacher, Zdenek Berger, Janet E Davies, Shouqing Luo, and Lourdes G Oroz. 2004. “Inhibition of MTOR Induces Autophagy and Reduces Toxicity of Polyglutamine Expansions in Fly and Mouse Models of Huntington Disease Abstract About This Article,” no. May.

Rawet Slobodkin, Moran, and Zvulun Elazar. 2013. “The Atg8 Family: Multifunctional Ubiquitin-like Key Regulators of Autophagy.” *Essays in Biochemistry* 55 (1): 51–64. https://doi.org/10.1042/BSE0550051.

Reinhart, BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, and Ruvkun GB. 2000. “The 21-Nucleotide Let-7 RNA Regulates Developmental Timing in Caenorhabditis Elegans.” *Nature* 403: 901–6.

Ribeiro, Amanda O, Vinicius F Campos, Ney Lemke, and Danillo Pinhal. 2019.

"Understanding the Modus Operandi of MicroRNA Regulatory Clusters," 1–18. https://doi.org/10.3390/cells8091103.

Richly, Holger, Michael Rape, Sigurd Braun, Sebastian Rumpf, Carsten Hoege, and Stefan Jentsch. 2005. "A Series of Ubiquitin Binding Factors Connects CDC48/P97 to Substrate Multiubiquitylation and Proteasomal Targeting." *Cell* 120 (1): 73–84. https://doi.org/10.1016/j.cell.2004.11.013.

Robida-Stubbs, Stacey, Kira Glover-Cutter, Dudley W. Lamming, Masaki Mizunuma, Sri Devi Narasimhan, Elke Neumann-Haefelin, David M. Sabatini, and T. Keith Blackwell. 2012. "TOR Signaling and Rapamycin Influence Longevity by Regulating SKN-1/Nrf and DAF-16/FoxO." *Cell Metabolism* 15 (5): 713–24. https://doi.org/10.1016/j.cmet.2012.04.007.

Roso, Alessandra Del, Simona Vittorini, Gabriella Cavallini, Alessio Donati, Zina Gori, Matilde Masini, Maria Pollera, and Ettore Bergamini. 2003. "Ageing-Related Changes in the in Vivo Function of Rat Liver Macroautophagy and Proteolysis." *Experimental Gerontology* 38 (5): 519–27. https://doi.org/10.1016/S0531-5565(03)00002-0.

Rual, Jean-françois, Julian Ceron, John Koreth, Tong Hao, Anne-sophie Nicot, Tomoko Hirozane-kishikawa, Jean Vandenhaute, et al. 2004. "Toward Improving Caenorhabditis Elegans Phenome Mapping With an ORFeome-Based RNAi Library," 2162–68. https://doi.org/10.1101/gr.2505604.7.

Rubinsztein, David C., Guillermo Mariño, and Guido Kroemer. 2011. "Autophagy and Aging." *Cell* 146 (5): 682–95. https://doi.org/10.1016/j.cell.2011.07.030.

Rusanova, Iryna, José Fernández-Martínez, Marisol Fernández-Ortiz, Paula Aranda-Martínez, Germaine Escames, Francisco J. García-García, Leocadio Mañas, and Darío Acuña-Castroviejo. 2019. "Involvement of Plasma MiRNAs, Muscle MiRNAs and Mitochondrial MiRNAs in the Pathophysiology of Frailty." *Experimental Gerontology* 124 (March). https://doi.org/10.1016/j.exger.2019.110637.

Russ, David W., Iva M. Boyd, Katherine M. McCoy, and Katherine W. McCorkle. 2015. "Muscle-Specificity of Age-Related Changes in Markers of Autophagy and Sphingolipid Metabolism." *Biogerontology* 16 (6): 747–59. https://doi.org/10.1007/s10522-015-9598-4.

Sarkies, Peter, and Eric A Miska. 2014. "Small RNAs Break out : The Molecular Cell Biology of Mobile Small RNAs." *Nature Reviews Molecular Cell Biology* 15 (July): 525–35.

Schreiber, Anne, and Matthias Peter. 2014. "Substrate Recognition in Selective Autophagy and the Ubiquitin-Proteasome System." *Biochimica et Biophysica Acta - Molecular Cell Research* 1843 (1): 163–81. https://doi.org/10.1016/j.bbamcr.2013.03.019.

Scott, Cameron C, and Jean Gruenberg Ã. 2010. "Prospects & Overviews Ion Flux and the Function of Endosomes and Lysosomes: PH Is Just the Start." *Bioessays* 33: 103–10. https://doi.org/10.1002/bies.201000108.

Scott, Hannah. 2017. “Extracellular MicroRNAs as Messengers in the Central and Peripheral Nervous System.” *Neuronal Signaling* 1 (4): NS20170112. https://doi.org/10.1042/NS20170112.

Selbach, Matthias, Björn Schwanhäusser, Nadine Thierfelder, Zhuo Fang, Raya Khanin, and Nikolaus Rajewsky. 2008. “Widespread Changes in Protein Synthesis Induced by MicroRNAs.” *Nature* 455 (7209): 58–63. https://doi.org/10.1038/nature07228.

Sengupta, Arunima, Jeffery D. Molkentin, and Katherine E. Yutzey. 2009. “FoxO Transcription Factors Promote Autophagy in Cardiomyocytes.” *Journal of Biological Chemistry* 284 (41): 28319–31. https://doi.org/10.1074/jbc.M109.024406.

Shukla, Girish C, Jagjit Singh, and Sailen Barik. 2011. “MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions.” *Molecular and Cellular Pharmacology* 3 (3): 83–92. http://www.ncbi.nlm.nih.gov/pubmed/22468167%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3315687.

Silva, M. Catarina, Margarida D. Amaral, and Richard I. Morimoto. 2013. “Neuronal Reprograming of Protein Homeostasis by Calcium-Dependent Regulation of the Heat Shock Response.” Edited by Alejandro Aballay. *PLoS Genetics* 9 (8): e1003711. https://doi.org/10.1371/journal.pgen.1003711.

Simon, David J, Jon M Madison, Annie L Conery, Katherine L Thompson-, Michael Soskis, Gary B Ruvkun, Joshua M Kaplan, and John K Kim. 2008. “The MicroRNA MiR-1 Regulates a MEF-2 Dependent Retrograde Signal at Neuromuscular Junctions.” *Cell.* 133 (5): 903–15. https://doi.org/10.1016/j.cell.2008.04.035.The.

Simonsen, Anne, Robert C. Cumming, Andreas Brech, Pauline Isakson, David R. Schubert, and Kim D. Finley. 2008. “Promoting Basal Levels of Autophagy in the Nervous System Enhances Longevity and Oxidant Resistance in Adult Drosophila.” *Autophagy* 4 (2): 176–84. https://doi.org/10.4161/auto.5269.

Siparsky, Patrick N., Donald T. Kirkendall, and William E. Garrett. 2014. “Muscle Changes in Aging: Understanding Sarcopenia.” *Sports Health* 6 (1): 36–40. https://doi.org/10.1177/1941738113502296.

Siracusa, Julien, Nathalie Koulmann, and Sébastien Banzet. 2018. “Circulating MyomiRs: A New Class of Biomarkers to Monitor Skeletal Muscle in Physiology and Medicine.” *Journal of Cachexia, Sarcopenia and Muscle*. https://doi.org/10.1002/jcsm.12227.

Smith, Daniel L., Stefan Bekiranov, Jeffrey S. Smith, Xuewen Pan, Mirela Matecic, Jef D. Boeke, and Nazif Maqani. 2010. “A Microarray-Based Genetic Screen for Yeast Chronological Aging Factors.” *PLoS Genetics* 6 (4): e1000921. https://doi.org/10.1371/journal.pgen.1000921.

Sohel, Mahmodul Hasan. 2016. “Circulating MicroRNAs : Release Mechanisms , Functions

and Challenges." *Achievements in the Life Sciences* 10: 175–86.

Sohel, MH. 2016. "Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges." *Achievements in the Life Sciences* 10 (2): 175–86. https://doi.org/10.1016/J.ALS.2016.11.007.

Son, Heehwa G., Ozlem Altintas, Eun Ji E. Kim, Sujeong Kwon, and Seung Jae V. Lee. 2019. "Age-Dependent Changes and Biomarkers of Aging in Caenorhabditis Elegans." *Aging Cell* 18 (2): 1–11. https://doi.org/10.1111/acel.12853.

Soon-Tae, Lee, Chu Kon, Jung Keun-Hwa, Kim Jin Hee, Huh Ji-Young, Yoon Hyejin, Park Dong-Kyu, et al. 2012. "MiR-206 Regulates Brain-derived Neurotrophic Factor in Alzheimer Disease Model." *Annals of Neurology*. https://doi.org/10.1002/ana.23588.

Stenesen, Drew, Jae Myoung Suh, Jin Seo, Kweon Yu, Kyu-sun Lee, Kyung-jin Min, and Jonathan M Graff. 2013. "Dietary Adenine Controls Adult Lifespan via Adenosine Nucleotide Biosynthesis and AMPK, and Regulates the Longevity Benefit of Caloric Restriction." *Cell Metabolism* 17 (1): 101–12. https://doi.org/10.1016/j.cmet.2012.12.006.Dietary.

Stolz, Alexandra, Andreas Ernst, and Ivan Dikic. 2014. "Cargo Recognition and Trafficking in Selective Autophagy." *Nature Cell Biology* 16 (6): 495–501. https://doi.org/10.1038/ncb2979.

Su, Ya, Katherine G. Blake-Palmer, Sara Sorrell, Babak Javid, Katherine Bowers, Aiwu Zhou, Simon H. Chang, Seema Qamar, and Fiona E. Karet. 2008. "Human H+ ATPase A4 Subunit Mutations Causing Renal Tubular Acidosis Reveal a Role for Interaction with Phosphofructokinase-1." *American Journal of Physiology - Renal Physiology* 295 (4): 950–58. https://doi.org/10.1152/ajprenal.90258.2008.

Su, Ya, Aiwu Zhou, Rafia S. Al-Lamki, and Fiona E. Karet. 2003. "The A-Subunit of the V-Type H+-ATPase Interacts with Phosphofructokinase-1 in Humans." *Journal of Biological Chemistry* 278 (22): 20013–18. https://doi.org/10.1074/jbc.M210077200.

Sulston, J. E., E. Schierenberg, J. G. White, and J. N. Thomson. 1983. "The Embryonic Cell Lineage of the Nematode *Caenorhabditis Elegans*." *Developmental Biology* 100 (1): 64–119. https://doi.org/10.1016/0012-1606(83)90201-4.

Syntichaki, P, K Troulinaki, and N Tavernarakis. 2007. "EIF4E Function in Somatic Cells Modulates Ageing in Caenorhabditis Elegans." *Nature* 445 (7130): 922–26. https://doi.org/10.1038/nature05603.

Takács-Vellai, Krisztina, György Csikós, Péter Erdélyi, János Barna, Éva Borsos, Attila L. Kovács, Tibor Vellai, et al. 2014. " Longevity Pathways Converge on Autophagy Genes to Regulate Life Span in Caenorhabditis Elegans ." *Autophagy* 4 (3): 330–38. https://doi.org/10.4161/auto.5618.

Tani, Saori, Shigehiro Kuraku, Hiroshi Sakamoto, Kunio Inoue, and Rie Kusakabe. 2013.

"Developmental Expression and Evolution of Muscle-Specific MicroRNAs Conserved in Vertebrates." *Evolution and Development* 15 (4): 293–304. https://doi.org/10.1111/ede.12039.

Tatar, M., S. K. Kim, R. Nusse, D. A. Peterson, J. I. Gordon, Kyung-Ah Lee, Joo-Heon Yoon, Ji-Hwan Ryu, and Won-Jae Lee. 2001. "A Mutant Drosophila Insulin Receptor Homolog That Extends Life-Span and Impairs Neuroendocrine Function." *Science* 292 (5514): 107–10. https://doi.org/10.1126/science.1057987.

Taylor, Rebecca C., and Andrew Dillin. 2013. "XXBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity." *Cell* 153 (7): 1435. https://doi.org/10.1016/j.cell.2013.05.042.

Tsukada, Miki. 1993. "Isofation and Charact ~~ Zation of Autophagy-Defective Mutants Of." *October* 333 (1): 169–74. https://doi.org/10.1038/modpathol.2015.6.

Ui-Tei, Kumiko, Kenji Nishi, Tomoko Takahashi, and Tatsuya Nagasawa. 2012. "Thermodynamic Control of Small RNA-Mediated Gene Silencing." *Frontiers in Genetics* 3 (JUN): 1–12. https://doi.org/10.3389/fgene.2012.00101.

Vickers, Kasey C., Brian T. Palmisano, Bassem M. Shoucri, Robert D. Shamburek, and Alan T. Remaley. 2011. "MicroRNAs Are Transported in Plasma and Delivered to Recipient Cells by High-Density Lipoproteins." *Nature Cell Biology* 13 (4): 423–35. https://doi.org/10.1038/ncb2210.

Voinnet, O., and Dc. Baulcombe. 1997. "Systemic Signalling in Gene Silencing." *Nature* 389 (October): 553.

Wahid, Fazli, Adeeb Shehzad, Taous Khan, and You Young Kim. 2010. "MicroRNAs: Synthesis, Mechanism, Function, and Recent Clinical Trials." *Biochimica et Biophysica Acta - Molecular Cell Research* 1803 (11): 1231–43. https://doi.org/10.1016/j.bbamcr.2010.06.013.

Wang, J., L. Z. Yang, J. S. Zhang, J. X. Gong, Y. H. Wang, C. L. Zhang, H. Chen, and X. T. Fang. 2018. "Effects of MicroRNAs on Skeletal Muscle Development." *Gene* 668 (April): 107–13. https://doi.org/10.1016/j.gene.2018.05.039.

Washietl, Stefan, Ivo L. Hofacker, Melanie Lukasser, Alexander Hüttenhofer, and Peter F. Stadler. 2005. "Mapping of Conserved RNA Secondary Structures Predicts Thousands of Functional Noncoding RNAs in the Human Genome." *Nature Biotechnology* 23 (11): 1383–90. https://doi.org/10.1038/nbt1144.

Weber, Jessica A., David H. Baxter, Shile Zhang, David Y. Huang, Kuo How Huang, Ming Jen Lee, David J. Galas, and Kai Wang. 2010. "The MicroRNA Spectrum in 12 Body Fluids." *Clinical Chemistry* 56 (11): 1733–41. https://doi.org/10.1373/clinchem.2010.147405.

White, John Graham, Eileen Southgate, J N Thomson, and Sydney Brenner. 1986. "The

Structure of the Nervous System of the Nematode Caenorhabditis Elegans." *Philos Trans R Soc Lond B Biol Sci.*, no. November 1986.

Wild, Philipp, David G. McEwan, and Ivan Dikic. 2014. "The LC3 Interactome at a Glance." *Journal of Cell Science* 127 (1): 3–9. https://doi.org/10.1242/jcs.140426.

Wilkens, Stephan, Zhenyu Zhang, and Yesha Zheng. 2005. "A Structural Model of the Vacuolar ATPase from Transmission Electron Microscopy." *Micron* 36 (2): 109–26. https://doi.org/10.1016/j.micron.2004.10.002.

Winter, Julia, Stephanie Jung, Sarina Keller, Richard I. Gregory, and Sven Diederichs. 2009. "Many Roads to Maturity: MicroRNA Biogenesis Pathways and Their Regulation." *Nature Cell Biology* 11 (3): 228–34. https://doi.org/10.1038/ncb0309-228.

Wong, E., S. Sridhar, U. Bandyopadhyay, S. J. Orenstein, S. Kaushik, A. M. Cuervo, M. Martinez-Vicente, R. Kiffin, and M. Kon. 2011. "Chaperone-Mediated Autophagy at a Glance." *Journal of Cell Science* 124 (4): 495–99. https://doi.org/10.1242/jcs.073874.

Xinran Xu, Fangfei Deng. 2014. "The Role of MiR-1 in the Heart: From Cardiac Morphogenesis to Physiological Function." *Human Genetics & Embryology* 04 (01): 1–4. https://doi.org/10.4172/2161-0436.1000119.

Yang, Z., Klionsky, D.J. 2009. "An Overview of the Molecular Mechanism of Autophagy." *Curr Top Microbiol Immunol* 335: 1–32.

Yang, Jin-Ming, Xingcong Ren, Yun Chen, Hua Zhu, Xiuping Liu, Chang-Gong Liu, Biao Li, and Hao Wu. 2014. "Regulation of Autophagy by a Beclin 1-Targeted MicroRNA, MiR-30a, in Cancer Cells." *Autophagy* 5 (6): 816–23. https://doi.org/10.4161/auto.9064.

Yasuda, Kaoru, Anupama Khandare, Leonid Burianovskyy, Shoichi Maruyama, Frank Zhang, Alberto Nasjletti, and Michael S. Goligorsky. 2011. "Tunneling Nanotubes Mediate Rescue of Prematurely Senescent Endothelial Cells by Endothelial Progenitors: Exchange of Lysosomal Pool." *Aging* 3 (6): 597–608. https://doi.org/10.18632/aging.100341.

Yasuda, Kayo, Takamasa Ishii, Hitoshi Suda, Akira Akatsuka, Philip S. Hartman, Sataro Goto, Masaki Miyazawa, and Naoaki Ishii. 2006. "Age-Related Changes of Mitochondrial Structure and Function in Caenorhabditis Elegans." *Mechanisms of Ageing and Development* 127 (10): 763–70. https://doi.org/10.1016/j.mad.2006.07.002.

Yong, William H., Tomoo Matsutani, Kazuhiro Tanaka, Ingo K. Mellinghoff, Kenta Masui, Genaro R. Villa, Feng Liu, et al. 2013. "MTOR Complex 2 Controls Glycolytic Metabolism in Glioblastoma through FoxO Acetylation and Upregulation of C-Myc." *Cell Metabolism*. https://doi.org/10.1016/j.cmet.2013.09.013.

Zacharewicz, Evelyn, Séverine Lamon, and P Aaron. 2013. "MicroRNAs in Skeletal Muscle and Their Regulation with Exercise , Ageing , and Disease" 4 (September): 1–11. https://doi.org/10.3389/fphys.2013.00266.

Zetterberg, Henrik, and Niklas Mattsson. 2014. “Understanding the Cause of Sporadic Alzheimer’s Disease.” *Expert Review of Neurotherapeutics* 14 (6): 621–30. https://doi.org/10.1586/14737175.2014.915740.

Zhang, Cong, and Ana Maria Cuervo. 2008. “Restoration of Chaperone-Mediated Autophagy in Aging Liver Improves Cellular Maintenance and Hepatic Function.” *Nature Medicine* 14 (9): 959–65. https://doi.org/10.1038/nm.1851.

Zhang, Ran, Xu Wang, Jia Hua Qu, Bing Liu, Peng Zhang, Tao Zhang, Peng Cheng Fan, et al. 2019. “Caloric Restriction Induces MicroRNAs to Improve Mitochondrial Proteostasis.” *IScience* 17: 155–66. https://doi.org/10.1016/j.isci.2019.06.028.

Zhang, Yujing, Kehui Wang, Junfeng Zhang, Zhen Bian, Qiang Wang, Fei Sun, Limin Li, et al. 2010. “Secreted Monocytic MiR-150 Enhances Targeted Endothelial Cell Migration.” *Molecular Cell* 39 (1): 133–44. https://doi.org/10.1016/j.molcel.2010.06.010.

Zhao, Yong, Joshua F. Ransom, Ankang Li, Vasanth Vedantham, Morgan von Drehle, Alecia N. Muth, Takatoshi Tsuchihashi, Michael T. McManus, Robert J. Schwartz, and Deepak Srivastava. 2007. “Dysregulation of Cardiogenesis, Cardiac Conduction, and Cell Cycle in Mice Lacking MiRNA-1-2.” *Cell* 129 (2): 303–17. https://doi.org/10.1016/j.cell.2007.03.030.

Zhou, Huihui, Hu Hu, and Maode Lai. 2010. “Non-Coding RNAs and Their Epigenetic Regulatory Mechanisms.” *Biology of the Cell*. https://doi.org/10.1042/bc20100029.

Zoncu, Liron Bar-Peled, Alejo Efeyan, Shuyu Wang, Yasemin, Sancak, and David M. Sabatini. (2011) mTORC1 Senses Lysosomal Amino Acids Through an Inside-Out mechanism that requires the Vacuolar H+-ATPase. Science. 2011. “NIH Public Access” 334 (6056): 678–83. https://doi.org/10.1126/science.1207056.mTORC1.

Zoncu, Roberto, Liron Bar-Peled, Alejo Efeyan, Shuyu Wang, Yasemin Sancak, and David M. Sabatini. 2011. “MTORC1 Senses Lysosomal Amino Acids through an Inside-out Mechanism That Requires the Vacuolar H+-ATPase.” *Science* 334 (6056): 678–83. https://doi.org/10.1126/science.1207056.

Supplementary Materials

Table 9: Lifespan analysis, p-values for statistical analyses were calculated using Mantel-Cox Log Rank test. Worms that escaped the dishes, had internal hatching or had bursting of vulva were censored from the experiment.

Strain/Treatment	Median survival (days)	Censored	Deaths	Sig*	Ref. Control
20°C Lifespans					
N2	24	21	67		
mir-1 (gk276)	24	19	56	ns	N2
mir-1 (dh1111)	22	21	53	ns	N2
N2	20	7	115		
mir-1 (gk276)	23	1	127	ns	N2
25° Lifespans					
N2	15	4	77		
mir-1 (gk276)	15	5	91	ns	N2
mir-1 (dh1111)	17	1	72	0.0042 **	N2
N2	15	3	45		
mir-1 (gk276)	17	3	52	ns	N2
mir-1 (dh1111)	17	3	48	0.0167 *	N2
N2	15	0	48		
mir-1 (gk276)	17	1	43	ns	N2
mir-1 (dh1111)	15	2	55	ns	N2

Table 10: List of all genes tested in the bioinformatic-based selective RNAi suppressor screen for motility improvement

Gene name	Wormbase ID
vha-1	R10E11.8
ace-1	W09B12.1
unc-44	B0350.2
vha-14	F55H2.2
vha-15	T14F9.1
ifb-2	F10C1.7
gly-13	B0416.6
dpy-23	R160.1
egl-46	K11G9.4
let-363	B0261.2
vha-8	C17H12.14
egl-8	B0348.4
vha-5	F35H10.4
unc-11	C32E8.10
vha-13	Y49A3A.2
cwn-2	W01B6.1
dyc-1	C33G3.1
chs-2	F48A11.1
syg-2	C26G2.1
hyl-2	K02G10.6
mlk-1	K11D12.10
nac-3	K08E5.2
pde-2	R08D7.6
frm-8	H09G03.2
sma-9	T05A10.1
pkc-1	F57F5.5
syd-2	F59F5.6
unc-18	F27D9.1
ncx-1	Y113G7A.4
opt-3	F56F4.5
kpc-1	F11A6.1
alh-4	T05H4.13

tth-1	F08F1.8
bar-1	C54D1.6
glt-6	R05G6.6
hpk-1	F20B6.8
vha-9	ZK970.4
daf-16	R13H8.1
abts-3	F57F10.1
sel-1	F45D3.5
vha-12	F20B6.2
grl-4	F42C5.7
nep-1	ZK20.6
sdn-1	F57C7.3
lin-26	F18A1.2
dhs-17	F29G9.6
ras-2	F17C8.4
vha-10	F46F11.5
zyx-1	F42G4.3
ncs-2	F10G8.5
igcm-3	T02C5.3

Table 11: List of up- or down-regulated genes and the respective logFC from proteomics analysis comparing *mir-1* mutants to wild-type. candidates significantly ($p<0.05$) upregulated in *mir-1* mutants compared to wild-type are highlighted in green. Candidates significantly ($p<0.05$) downregulated in *mir-1* mutants compared to wild-type are highlighted in green. Upregulated genes were tested in the proteomics-based selective RNAi suppressor screen for motility improvement.

Gene name	logFC	Gene name	logFC
cul-4	2,44	lsm-6	-2,75
ccdc-55	1,41	perm-4	-2,47
spt-4	1,39	tbc-14	-1,65
asns-2	1,38	txdc-9	-1,61
vha-19	1,36	vit-4	-1,50
jmjc-1	1,00	vit-6	-1,42
uri-1	0,93	nck-1	-1,41
snr-5	0,90	heh-1	-1,35
tni-1	0,78	ads-1	-1,30
sut-2	0,70	nuo-4	-1,20

gyg-1	0,52	vit-2	-1,09
ttr-26	0,45	col-106	-1,06
pinn-4	0,45	art-1	-1,01
hpo-18	0,42	dhs-9	-0,95
valv-1	0,42	alh-12	-0,91
zyx-1	0,40	prx-19	-0,88
sams-4	0,39	ivd-1	-0,87
dtmk-1	0,37	cysl-1	-0,81
dss-1	0,37	gst-7	-0,78
alp-1	0,36	hach-1	-0,76
wrt-2	0,35	usp-14	-0,75
aly-3	0,32	rack-1	-0,73
unc-23	0,32	fat-2	-0,72
klc-2	0,31	ttc-4	-0,62
npp-16	0,30	pyr-1	-0,58
mup-2	0,29	atn-1	-0,58
set-26	0,29	rps-22	-0,55
rnp-4	0,28	mlt-8	-0,55
hpo-34	0,28	sca-1	-0,54
nrfl-1	0,28	rps-9	-0,54
pfd-4	0,27	aco-1	-0,52
hsp-17	0,27	rps-7	-0,48
vha-10	0,27	pck-2	-0,47
hil-4	0,27	gta-1	-0,47
snx-6	0,26	pyc-1	-0,46
deb-1	0,25	fasn-1	-0,45
hsr-9	0,25	rps-8	-0,44
nex-1	0,25	cct-7	-0,42
lmn-1	0,25	got-2.2	-0,41
mrps-26	0,25	acdh-1	-0,41
unc-87	0,25	gcn-1	-0,39
pro-3	0,25	icl-1	-0,38
irg-7	0,25	phb-2	-0,37
afd-1	0,24	ogdh-1	-0,37
col-71	0,24	hphd-1	-0,37

fubl-1	0,23	asna-1	-0,37
frm-1	0,22	mrps-14	-0,33
amph-1	0,21	ipgm-1	-0,33
hmg-12	0,21	pccb-1	-0,33
lec-3	0,21	alh-8	-0,32
rbm-22	0,20	lap-2	-0,32
nono-1	0,20	rpl-9	-0,32
pfd-2	0,20	ctps-1	-0,30
mua-3	0,20	vbh-1	-0,29
rsp-3	0,17	rpl-33	-0,28
npp-10	0,17	eif-3.B	-0,28
		ttn-1	-0,28
		asp-1	-0,28
		gdi-1	-0,27
		rps-6	-0,27
		ketn-1	-0,26
		ech-6	-0,26
		hrp-2	-0,25
		mlp-1	-0,24
		spp-3	-0,24
		fah-1	-0,23
		unc-44	-0,23
		cth-2	-0,23
		fln-1	-0,22
		fbp-1	-0,22
		ttr-51	-0,21
		gln-3	-0,21
		lev-11	-0,20
		aco-2	-0,20
		rpl-10	-0,18

Appendix

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Work Contributions

I performed all experiments described in this work independently, except for:

Proteomics assay and analysis was performed by Dr. Ilian Atanassov at the proteomic core facility at Max-Plank for Biology of ageing.

Cloning of *ges-1p::GFP::3xmir-1-seed* construct was done by Dr Christoph Geisen.

Strains PHX506, PHX502, PHX586 and PHX587 were made by sunybiotech.

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Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Adam Antebi betreut worden.

Köln, September 2019 Isabelle Schiffer

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