TRANSCRIPTION FACTOR NFYB-1 REGULATES MITOCHONDRIAL FUNCTION AND PROMOTES LONGEVITY INDUCED BY MITOCHONDRIAL IMPAIRMENT

by

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TRANSCRIPTION FACTOR NFYB-1 REGULATES MITOCHONDRIAL FUNCTION AND PROMOTES LONGEVITY INDUCED BY MITOCHONDRIAL IMPAIRMENT

Inaugural – Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

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Cologne Feb 2019

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Tag der mündlichen Prüfung:	08.03.2018

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2 ABSTRACT

Mitochondrial integrity is critical for cellular function and organismal life span, yet the underlying mechanisms linking mitochondrial function to other intracellular organelles and animal longevity is unclear. In this study, we addressed these questions in nematode *C. elegans*. Upon shift from food to no food prior to sexual maturation, the nematode *C. elegans* can enter an adult reproductive diapause (ARD), during which they undergo metabolic remodelling and can live months without food. When reintroduced to food, ARD worms exit the ARD and recover. We found mitochondrial number, morphology and respiration rate to be regulated under ARD entry and exit. Additionally, ARD longevity is impacted by mitochondrial fusion/fission factors. We found several mitochondrial markers to be downregulated upon ARD entry and upregulated upon ARD exit, most prominently the nuclear encoded mitochondrial gene cytochrome C oxidase, *cco-1*.

In mammals, several factors are known for mitochondrial biogenesis and function. However, systematic screens for such factors in a whole animal model have not been performed. To identify novel loci involved in mitochondrial regulation, using ARD recovery as a tool we performed an RNAi screen monitoring expression of the mitochondrial marker *pcco-1::gfp* during ARD recovery. We identified presumed ancestral factors known to regulate mitochondrial function, including CREB, CREB binding protein, ATFS-1 and SKN-1/NFE2.

More importantly we also found several novel mitochondrial regulators such as NFYB-1, a subunit of the NF-Y transcriptional complex binding the CCAAT motif. Consistent with a role in mitochondrial physiology, we observed that loss of NFYB-1 leads to mitochondrial fragmentation, reduced mitochondrial reporter expression, lower oxygen consumption and

abolition of longevity triggered by reduced mitochondrial function by RNAi knockdown of *cco-1* RNAi and mitochondrial mutant *isp-1(qm150)*. Moreover, NFYB-1 loss also regulates mitochondrial to cytosolic stress response and expression of mitochondrial UPR factors ATFS-1 and DEV-1. Strikingly both proteomic and transcriptomics analysis indicates that NFYB-1 regulates a subset of endoplasmic reticulum associated genes. Taken together these findings suggest that NFYB-1 promotes mitochondrial gene expression, while suppressing ER stress response and functions a novel regulator of inter-organellar communication to maintain homeostasis and mitochondrial longevity.

3 ZUSAMMENFASSUNG

Für die Lebensdauer eines Organismus und die Funktionalität seiner Zellen ist ein integerer Zustand der Mitochondrien essentiell. Die zu Grunde liegenden Mechanismen, die es Mitochondrien ermöglichen mit anderen Zell-Organellen zu interagieren und so die Lebensdauer einer Zelle und des gesamten Organismus zu beeinflussen, sind jedoch weites gehend unbekannt. In dieser Doktorarbeit nutze ich den Modelorganismus *C. elegans* um Faktoren zu identifizieren, die Teil solcher Mechanismen sind.

Wird *C. elegans* Würmern vor der Geschlechtsreife die Nahrung entzogen, gehen sie in ein reproductive Diapause (adult reproductive diapause, ARD). Dieses Stadium ist durch erhebliche metabolische Veränderungen und Langlebigkeit gekennzeichnet. In ARD kann *C. elegans* mehrere Monate ohne Nahrung überleben. Sobald die Würmer wieder Zugang zu Nahrung haben, verlassen sie das ARD Stadium und reproduzieren sich. Interessanterweise werden durch ARD Eintritt und Austritt die Anzahl und Morphologie der Mitochondrien, sowie die Atmung beeinflusst. Darüber hinaus wird die ARD Langlebigkeit von Faktoren die die mitochondriale Struktur (Fragmentierung/Fusion) bestimmen, reguliert. Ich konnte bereits einige mitochondriale Marker identifiziert, die bei ARD Eintritt herunter- und bei ARD Austritt heraufreguliert sind. Am auffälligsten war hierbei das im Kern kodierte, mitochondriale Gen Cytochrom C Oxidase (*cco-1*).

Zwar sind in Säugetieren bereits einige wichtige Faktoren der mitochondrien Biogenese und ihrer Funktionalität identifiziert; es wurde jedoch noch kein systematischer Screen nach solchen Faktoren in einem Modelorganismus durchgeführt. Um neue Gene zu identifizieren haben ich daher in einem RNAi Screen nach Faktoren gesucht, die die Expression eines PCCO-1::GFP Markers während des ARD Austritts beeinflussen. In diesem Ansatz

wurden unter anderem konservierte Faktoren mit einer bekannten Rolle in Mitochondrien identifiziert, wie z.B. CREB, CREB binding protein, ATFS-1 und SKN-1/NFE2.

Jedoch wurden auch bis dato unbekannte Faktoren identifiziert, wie z.B. NFYB-1, eine Untereinheit des NF-Y Transkription-Komplexes, der an CCAAT Motive binden kann. Der Verlust von NFYB-1 führt zu einer Fragmentierung der Mitochondrien, verminderter Expression mitochondrialer Marker und niedrigerer Sauerstoffaufnahme. Diese Beobachtungen legen nahe, dass NFYB-1 eine wichtige Rolle für die gesamte Mitochondrien Physiologie spielt. Darüber hinaus führt NFYB-1 Verlust zur Reduktion von Langlebigkeit, die durch verminderter mitochondrien Funktionalität induziert wurde (z.B. durch *cco-1* RNAi oder in *isp-1(qm150)*). NFYB-1 Verlust beeinflusst außerdem die Kommunikation von der mitochondrialen zu der zytosolischen Stress Antwort und reguliert die Expression der mitochondrial UPR-Faktoren ATFS-1 und DEV-1. Interessanterweise zeigen sowohl Proteom- sowie Transkriptom-Analysen, dass NFYB-1 einige mit dem Endoplasmatischen Retikulum (ER) assoziierten Gene zu regulieren scheint. Zusammengefasst, lassen die Daten dieser Arbeit darauf schließen, dass NFYB-1 die expression mitochondrialer Gene fördert indem es die ER Stress Antwort inhibiert funktioniert es als ein neuer Regulator der für die mitochondriale Homeostase und Langlebigkeit erforderlich ist.

4 ABBREVATIONS

AMPK	adenosine monophosphate kinase
AMP	adenosine monophosphate
ANT	adenine nucleotide translocase
ATP	adenosine triphosphate
CGC	caenorhabditis genetics centre
CR	caloric restriction
CREB	cAMP response element
CRTC	CREB regulated transcriptional coactivator
DA	dafachronic acid
DNA	deoxyribonucleic acid
DR	dietary restriction
EMS	ethyl methane sulfonate
ETC	electron transport chain
ER	endoplasmic reticulum
ERR	estrogen related receptor
GFP	green fluorescent protein
HIF	hypoxia inducible factor
HSF	heat shock factor
IGF-1	insulin-like growth factor-1
IIS	insulin/IGF-1 like signaling
ILP	insulin like peptide
IMM	inner mitochondrial membrane
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LS	leigh syndrome
IRS-1	insulin receptor substrate-1
MFI	median fluorescence intensity
mtDNA	mitochondrial DNA
MTSS	mitochondrial single stranded binding protein
MUFA	mono unsaturated fatty acid

NAD	nicotinamide-adenine dinucleotide
NFYB	nuclear transcription factor Y subunit beta
NGM	nematode growth medium
NHR	nuclear hormone receptor
NS	not significant
nuDNA	nuclear DNA
OMM	outer mitochondrial membrane
OP50	*E. coli* strain OP50
PCR	polymerase chain reaction
PGC	PPAR coactivator
PI3K	phosphatidylinositol 3-kinase
PMT	photo multiplier tube
POLRMT	mitochondrial RNA polymerase
PPAR	peroxisome proliferator-activated receptors
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RNAi	RNA interference
rRNA	ribosomal RNA
ROS	reactive oxygen species
TFAM	transcription factor A
TGF-β	transforming growth factor -β
TIM	translocator of inner mitochondrial membrane
TOM	translocator of outer mitochondrial membrane
TOR	target of rapamycin
UPR	unfolded protein response
α	alpha
β	beta
°	degree
µ	micro
mm	millimetre

5 INTRODUCTION

5.1 Hallmarks of ageing

Aging is illustrated as the time dependent gradual deterioration of physiological functions; hence it is a key risk factor for many common pathological diseases such as cardiovascular disorders, neurodegenerative diseases, cancer and diabetes. Though the curiosity for understanding the process of ageing itself has always been prevailing, ageing research has gained its focus upland since the isolation of the first long-lived strains in *Caenorhabditis elegans (C. elegans)* (Klass 1983).

In 2013 (López-Otín et al. 2013) López-Otín and colleagues summarized the molecular hallmarks of ageing. Currently there are nine hallmarks of ageing grouped into three categories and interestingly the alteration of one of these factors could have subsequent impact on the others, as these factors are hypothesized to be highly interconnected – unraveling an underlying network connecting all hallmarks of ageing. The first category the primary hallmarks of ageing, which are suggested to be accumulative over time, include factors that cause damage to cellular functions such as genomic instability, telomere abrasion, epigenetic alterations and loss of proteostasis. The second category the antagonistic hallmarks, which are compensatory responses to damage include factors such as varied nutrient sensing, mitochondrial dysfunction and cellular senescence. The antagonistic responses follow a threshold effect: at low levels and intermittent activation they relieve the damage and thus these factors would be beneficial but become detrimental when chronically active. In the last category fall the integrative hallmarks that ultimately result in the functional decline of the organism, including altered intracellular communication and stem cell exhaustion, which appears to be the end result of the primary and antagonistic responses. A better understanding of the molecular pathways involved in the ageing process will help us to establish interventions that will

in turn diminish the threats of age related diseases. To study the basic mechanisms that influence the hallmarks of ageing many model organisms ranging from yeasts like *Saccharomyces cervisiae* to great apes have been used. One rather short lived multicellular organism has been proven to be particularly suitable for ageing research: *Caenorhabidtis elegans.*

5.2 *C. elegans* as a model organism for ageing

Caenorhabditis elegans (*C. elegans)* was introduced into the research field by Sydney Brenner in *1963.* Since 1974 (Brenner 1974) extensive studies have been conducted in the field of molecular genetics and developmental biology of this free-living nematode. While many model organisms such as *Saccharomyces cerevisiae, Drosophila melanogaster, Mus musculus* and *Nothobranchius furzeri* have been used to understand the process of ageing and age-related diseases, the worm is a preferred model for various reasons.

C. elegans is an ideal model for ageing research due to its short lifespan and well established genetic tools, which make it easier for epistasis studies to establish convergent genetic pathways. *C. elegans* genome has been well annotated (The C. elegans Sequencing Consortium 1998) and has 35 percentage homology with *Homo Sapiens* rendering it an optimal tool for studying conserved pathways. Moreover, *C. elegans* is optimal for chemical mutagenesis screens to identify candidate genes in an unbiased manner. One of the additional major advantage of C. *elegans* is that it has a well-established RNAi library by which a gene can be specifically knocked down by feeding double stranded RNA expressing bacteria (Boutros & Ahringer 2008). This tool makes it possible to conduct large scale RNAi screens to identify novel candidates. In addition, it is also a good model for drug screenings to identify new compounds that extend lifespan and for external interventions such as caloric restriction and stress resistance. Low cost of maintenance and small size allow for easy

handling and propagation of the organism on a large scale. Another advantage is that worms can be frozen in glycerol, this helps for large scale storage (Brenner 1974) and led to a enormous library of mutant strains (CGC, NBRP). Additionally, *C. elegans* is a transparent nematode with 959 somatic cells cells this give it the advantage to have extensive microscopic studies. In brief, providing a short lifespan combined with a highly conserved genome and a vast variety of genetic and external manipulations available C. *elegans* has become an indispensable organism for studying ageing and age-related diseases. Another almost unique feature of *C. elegans* is, that it is capable of ceasing ageing at different developmental stages.

5.3 Adult Reproductive Diapause (ARD)

Under favourable environmental conditions *C. elegans* develops from an egg through four larval stages L1, L2, L3 and L4 to an adult hermaphrodite. When conditions are unfavourable *C. elegans* possess excellent mechanisms for survival. For example, under nutritional deprivation *C. elegans* can enter diapauses at different larval stages including the L1 diapause or the L3 dauer diapause, characterized by increased longevity and alteration in metabolic activity (Cassada & Russell 1975). Most notably, when food is restored *C. elegans* shows remarkable rejuvenation mechanisms, animals exit the diapauses, resume larval development, and mature into adults with full reproductive capacity and a normal adult life (Fielenbach & Antebi 2008).

The latest arrest in the *C. elegans* lifecycle is particularly interesting, because it is entered after development, when larvae have matured into adult animals. Hence it is called Adult Reproductive Diapause (ARD). Marc Van Gilst identified that Adult Reproductive Diapause (ARD) is entered before *C. elegans* reaches sexual maturation (Angelo & Van Gilst 2009). Worms enter ARD when completely removed from their bacterial food

source late in larval development (L3/L4) (Figure 1). ARD worms arrest as adults harbouring no more than one live embryo per gonadal arm. Under ARD animals have a longer lifespan with up to 80 days at 20° C. They survive long times without food by undergoing metabolic remodelling. ARD worms cease growth, reproduction, decrease cellular volume and reduce the number of germ cells in the gonad (Angelo & Van Gilst 2009).

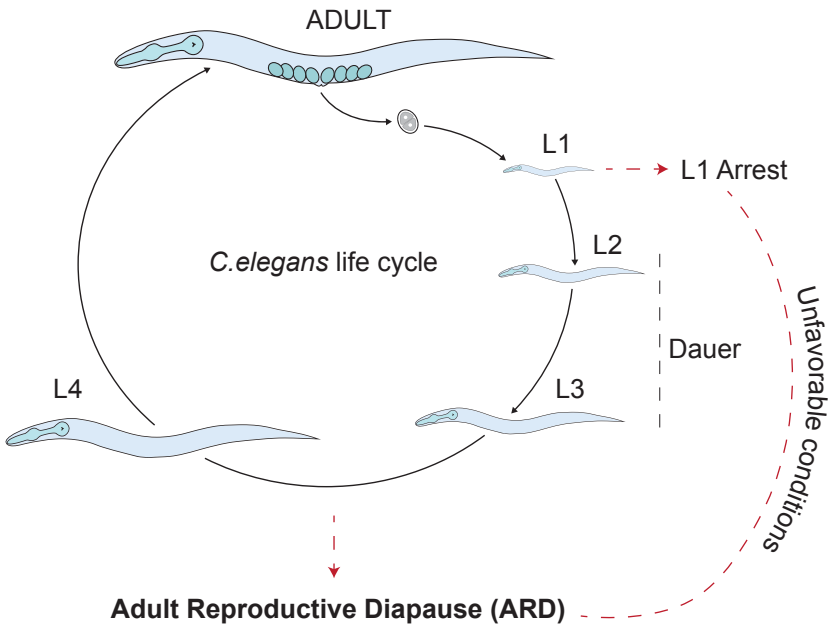


Figure 1: *C. elegans* life cycle under favorable and unfavorable conditions. Under favourable conditions, i.e. upon *ad libitum* food availability after embryonic development, the eggs hatch and pass through L1 to L4 larval stages, and further develop to sexually mature adults. Under unfavourable conditions L1 larvae can enter an L1 arrest stage. L2 to L3 larval stages can enter the dauer diapause where they live up to 4 months. Around mid L3 upon starvation, *C. elegans* enter adult reproductive diapause stage where they live up to 80 days in comparison to wildtype *ad libitum* fed animals, modified from (Altun & Hall 2009).

5.4 Ageing pathways

Extensive studies in well-established model organisms have identified highly conserved pathways that regulate ageing such as the nutrient sensing pathways: **Insulin/IGF signaling** and **TOR signaling**. In addition to these canonical pathways, reproduction and development of the **germ line** have also been shown to modulate longevity. Moreover, proteostasis and **mitochondrial dysfunction**s have implications on ageing. Modulation of these highly coordinated pathways and networks are essential for maintenance of a healthy lifespan. Deregulation of these pathways further leads to cellular dysfunction, accumulation of damage and in turn cell death.

5.4.1 Insulin / IGF-1 signaling

The highly conserved Insulin/ IGF signaling (IIS) was initially studied in the context of mammalian growth and metabolism as these have been shown to have implications in diabetes and neurodegenerative diseases (Trejo et al. 2004). Ageing research in *C. elegans* was initiated with the pioneering finding that mutations in *age-1*, the homologue gene of the mammalian phosphatidylinositol 3-kinase (PI3K) could extend longevity (Morris et al. 1996). Additional studies have given insights into the molecular mechanisms and pathways that regulate longevity by IIS. One milestone in ageing research was surely the discovery that different mutations in the *daf-2* insulin receptor (insR) extend *C. elegans* lifespan to about two fold (Kimura et al. 2011; C Kenyon et al. 1993). Excitingly subsequent studies in *Mus musculus* (Selman et al. 2007; Bartke 2008), and *Drosophila melanogaster* (Tatar 2001; Clancy 2001) could establish the conserved role of insulin signaling in regulation of longevity. Several studies implicate, that this conserved role might be further extended to humans. Polymorphism variants in the insulin/IGF signaling pathway have been linked to longevity in many human cohorts such as the Ashkenazi Jewish centenarians who have mutations known to impair the IGF-1 receptor (Suh et al. 2008) as well as a DNA variant in Japanese cohort (Kojima et al. 2004).

In *C. elegans* insulin like peptides (ILP) function as ligands for daf-2/insR. Binding of ligands to *daf-2/IRS* initiates the canonical insulin-signaling pathway where several kinases such as AGE-1/PI3K, 3-phosphoinositide-dependent kinase 1 (PDK)-1, AKT-1/2, and serine/threonine-protein kinase (SGK)-1 are activated by phosphorylation. This phosphorylation cascade ultimately leads to the phosphorylation and inactivation of the FOXO/*daf-16* transcription factor by AKT and SGK-1. This phosphorylation prevents the translocation of the transcription factor *daf-16* to the nucleus, and thus limits the regulation of target genes (Lee et al. 2001; Lin et al. 2001; Paradis et al. 1999; Hertweck et al. 2004; Henderson & Johnson 2001; Paradis & Ruvkun 1998). Upon reduction of insulin signaling such as in long lived *daf-2* mutants, the phosphorylation is prevented, *daf-16* can translocate to the nucleus and activates downstream target genes that not only promote longevity but also stress resistance (Murphy et al. 2003). Evidently, factors that were known to interact with IIS were found to also influence longevity. Among those are kinases such as JNK-1, which regulates longevity by modulating the translocation and localization of FOXO/*daf-16* by alternate phosphorylation (Oh et al. 2005). Moreover DAF-16 activity is further modulated by its co-regulatory factors like HSF-1, SMK-1 and inhibited by HCF-1 (Wolff et al. 2006; Li et al. 2008; Rizki et al. 2011). Upon reduced insulin signaling the Nrf homolog/transcription factor SKN-1 is localized in the nucleus independent of *daf-16* and is required for the expression of detoxification genes and for resistance to oxidative stress (Tullet et al. 2008). Along with the factors described above, the nuclear localization of *daf-16* upon reduced insulin signaling activates target genes such as heat shock proteins, cytochrome P40 enzymes, superoxide dismutases (e.g. *sod-3*). Thereby activating genes involved in various cellular process such as immunity, metabolism detoxification and oxidative stress. Several studies have identified factors that are altered in a *daf-16* dependent manner (Murphy et al. 2003; McElwee et al. 2003; Halaschek-Wiener et al. 2005). It has also been shown that *daf-16* can act cell non-autonomously similar to

HSF-1 and SKN-1. Expressing DAF-16 in the intestine or in neurons can extend lifespan of short-lived *daf-16; daf-2* mutant worms implicating the role of *daf-16* in modulating intercellular signals (Murphy et al. 2003). IIS has been the most intensively studied longevity pathway indicating the role of endocrine signaling for modulating independent processes such as metabolism and stress response thereby regulating longevity (Kenyon 2010).

5.4.2 TOR signaling and Dietary Restriction

TOR (Target of Rapamycin) signaling responds to amino acid availability and controls growth and reproduction. Inhibiting TOR signaling increases lifespan in many species from yeast to mice (Vellai et al. 2003; Sheaffer et al. 2008; Kapahi et al. 2004; Harrison et al. 2009; Kaeberlein 2005).TOR signaling is a highly conserved pathway that regulates longevity independent of IIS as it extends lifespan despite FOXO/*daf-16*. In *C. elegans* reduction of mTOR activity by (RNAi) of mTOR/*let-363* or the mTORC1 component *daf-15*/*raptor* leads to longevity (Jia 2004; Vellai et al. 2003). The Longevity effect of reduced TOR signaling requires regulation at both transcriptional and translational levels. Upon nutrient deprivation or low TOR signaling the translational levels are regulated via S6 kinase phosphorylation and the translational inhibitor 4E BP (Syntichaki et al. 2007). TOR signaling also upregulates autophagy by regulating the *pha-4*/FOXO transcription factor, likewise the lifespan extension by rapamycin, an inhibitor TOR, is also dependent on autophagy genes (Hansen et al. 2008; Gaudet 2002; Kaltenbach et al. 2005; Panowski et al. 2007).

The TOR signaling pathway is the one main nutrient-sensing pathway linked to dietary restriction. One form of dietary restriction, is Caloric restriction (CR), seemingly the most intensively studied longevity regime. CR is shown to extend lifespan in *C. elegans*, *Drosophila melanogaster*, *Mus Musculus* and in one study even in non-human primates (Lakowski & Hekimi 1998;

Anderson et al. 2009; Kennedy et al. 2007; Pletcher et al. 2002; McCay et al. 1935; Colman et al. 2009). TOR and dietary restriction mimic the same physiological effects and additionally, the lifespan extension induced by dietary restriction is not further increased by knocking down TOR-expression as observed in yeast, worms, and flies. Different modes of dietary restriction in *C. elegans* such as in *eat-2* mutants with lower pharyngeal pumping were shown to extend lifespan by inhibiting TOR signaling (Lakowski & Hekimi 1998). Furthermore, the *eat-2* mutant lifespan does not further increase further upon TOR inhibition. Accordingly, PHA-4 transcription, which is required for lifespan extension by TOR inhibition as described above, is also essential for longevity induced by *eat-2* mutation (Panowski et al. 2007). Additionally, upon chronic dietary restriction the NRF/SKN-1 transcription factor is essential for prolonging lifespan, clearly displaying a close connection of DR and the TOR pathway.

Strikingly, similarly to DAF-16 induced expression patterns, TOR and thus DR are also connected to stress responding pathways, for example to hypoxia inducible factor 1 (HIF-1) (Chen et al., 2009). HIF-1 in *C. elegans* is involved in the response to oxygen deprivation. During normoxic conditions HIF-1 is marked for degradation by the proline hydroxylase *egl-9*, hence HIF-1 is stabilized and increased upon *egl-9* loss. Additionally it was shown that *egl-9* is essential for DR induced lifespan extension of *hif-1* mutants (Epstein et al. 2001).

Additionally, two other factors that have been known to respond to changes in nutrient availability are AMP kinase and sirtuins. AMP kinase is activated when a cell's AMP/ATP ratio rises upon altered nutrient availability. Genetically mimicking this state of food scarcity by overexpressing AMPK-extends lifespan in *C. elegans* (Apfeld et al. 2004) and the same effect is observed when AMPK is activated by the drug metformin (Anisimov 2013; Chen et al. 2017; Martin-Montalvo et al. 2013; Slack et al. 2012; Onken &

Driscoll 2010). AMPK is shown to be required for longevity induced by both insulin/IGF-1 and in response to dietary restriction. Moreover, the AMPK/*aak-2* gene is essential for the longevity induced by the mutation of S6 kinase/*rsks-1* in *C. elegans* thereby further establishing similar mechanisms by TOR and dietary restriction (Curtis et al. 2006). Additionally it was also shown that AMPK longevity was also dependent on CRTC-1, the worm homologue of CREB regulated transcriptional co-activator (Mair, Morantte, Rodrigues, Manning, Montminy, Reuben J. Shaw, et al. 2011). Similarly, metabolic regulators NAD and NADH levels change upon nutrient deprivation and resveratrol leads to the activation of sirtuins. mTOR and sirtuins extend longevity by similar mechanisms. Overexpressing *sir-2.1* in *C. elegans* extends lifespan by activating DAF-16/FOXO. In response to dietary restriction sirtuins regulate a wide variety of stress pathways, which in turn leads to the longevity (Viswanathan & Guarente 2011; Hashimoto et al. 2010; Berdichevsky et al. 2006).

5.4.3 Germ line signaling

Another signaling pathway shown to regulate longevity is germline signaling. Laser microsurgical ablation of the gonadal germ precursor cells could extend the lifespan of *C. elegans* by 60%. Interestingly this requires intact somatic gonad cells, suggesting involvement of two opposing signals (Hsin and Kenyon, 1999). Lifespan extension by germline signaling can also be achieved by genetic manipulation as in case of *glp-1*/Notch receptor knock-out mutants, that do not develop germ cells. Along the same lines knock-out of *gld-1* that leads to over-proliferation of the germline shortens lifespan (Arantes-Oliveira et al., 2002). Similarly, to the IIS/*daf-2* signaling pathway, *daf-16/*FOXO is required for the long lifespan of germline less animals – again connecting two pathways involved in longevity. DAF-16/FOXO translocates to the nucleus mainly in intestinal cells upon germline ablation (Hsin & Kenyon 1999). Germline longevity is also modulated by many other factors such as the oxidative stress response gene SKN-1/Nrf, the

autophagy regulator PHA-4/FOXA, heat shock factor HSF-1 or DAF-18/PTEN (Berman & Kenyon 2006; Hansen et al. 2005; Lapierre et al. 2011; Steinbaugh et al. 2015), as well as the steroid receptor DAF-12/FXR and its ligands (Gerisch et al. 2007; Gerisch et al. 2001; Hsin & Kenyon 1999). The germline signaling pathway affects downstream cellular processes namely lipid metabolism, autophagy and fatty acid metabolism. The triglyceride lipase *lipl-4* is required for longevity induced by germline loss (Wang et al. 2008). Fat metabolism also plays a significant role in the induction of longevity. Desaturases that regulate MUFA (mono unsaturated fatty acid) production play an essential role in germline signaling mostly through a complex network of nuclear receptors NHR-80 and NHR-49 (Goudeau et al. 2011; Folick et al. 2015). Further studies have identified longevity induced by germline signaling in higher organisms. In *Drosophila* the loss of adult germ cells has been linked to modulation of the IIS pathway and longevity (Flatt et al. 2008). Lifespan of old mice was increased when transplanted with ovaries from young mice, indicating a conserved link between germline signaling and ageing (Mason et al. 2009; Cargill et al. 2003) .

It is interesting to take into account the overlapping mechanisms and factors that affect these above-mentioned longevity pathways. Longevity induced by germline signaling such as in *glp-1*/NotchR worms is not further prolonged by TOR inhibition, indicating the evident overlap between germline signaling and TOR. On the other hand, germline removal in *daf-2*/InsR mutants leads to a further lifespan extension, proposing the possibility of parallel pathways regulating insulin/IGF signaling and germline signaling. The regulation of downstream cellular process such as autophagy, lipolysis and stress response overlaps amongst the conserved longevity pathways.

5.4.4 Convergent factors regulating longevity

As described above although many of the conserved molecular pathways that regulate longevity are independent of each other, recent studies have brought forward several factors that are involved in the crosstalk between these signaling pathways. These convergent mechanisms help us to better understand the complex modulation and regulation of processes that in turn lead to the lifespan extension. Hansen and Lapierre identified the *hlh-30* as one such factor which acts downstream of various signaling pathway to influence longevity. HLH-30 is the *C. elegans* homolog of the mammalian transcription factor EB (TFEB) that has been shown to regulate process such as lysosomal biogenesis, autophagy and fat metabolism. Localization of *hlh-30* has been shown to be pivotal for the longevity regulation, *hlh-30* shuttles to the nucleus to drive its downstream targets such as autophagy and liposomal genes. It has been shown that overexpression of *hlh-30* can induce longevity (O'Rourke & Ruvkun 2013; Lapierre et al. 2013). Interestingly in this contect was that our laboratory recently identified Myc family members MML-1 (Myc/mondo-like) and its partner MXL-2 (Max, Max-like) as another factor that is involved in the convergent mechanisms regulating lifespan in *C. elegans*. MML-1 and MXL-2, identified from and RNAi suppressor screen for suppression of germline-mediated longevity in worms, were also shown to be required for IIS and DR induced longevity, while they also co-regulate *hlh-30* and TOR activity (Nakamura et al. 2016).

Additional studies in our lab identified *ncl-1* as another convergent regulator from a RNAi suppressor screen for dietary restriction. NCL-1 was required for the longevity prompted by DR, TOR, reduced insulin/IGF-1, mitochondrial and gonadal longevity. In line with these findings *ncl-1* overexpression was sufficient to promote longevity (Tiku et al. 2016). NCL-1 is known to regulate nucleolar size and its loss leads to abnormally large nucleoli. Interestingly it was observed that the nucleolar size was smaller in longevity mutants in comparison to wild type. Alongside with the nucleolar

size, these long-lived mutants also showed reduced ribosome biogenesis and reduced levels of the nucleolar marker FIB-1. Astonishingly upon NCL-1 loss these effects were reversed, hence nucleolar size has been identified as a reliable predictor of the longevity. This change in nucleolar size is shown to be conserved in higher model organisms hence further validating the role primary convergent networks regulating lifespan across species. However, the complete molecular mechanisms and organellar communication regulating these pathways to modulate longevity and stress response need to be further examined.

5.5 Mitochondria

Mitochondria initially identified as an intracellular organelle by R. Altmann in 1886, is maternally inherited with eubacterial endosymbionts as ancestors**.** They were termed as the power house of the cell by Philip Siekevitz in 1957 as they are essential for cellular energy production (Ernster & Schatz 1981). Mitochondria are required for several functions including apoptosis, calcium signalling, heme synthesis, regulation of energy metabolism, heme synthesis, reactive oxygen species signalling and fatty acid oxidation. Mitochondria is also well known as one of the hallmarks of ageing (López-Otín et al. 2013).

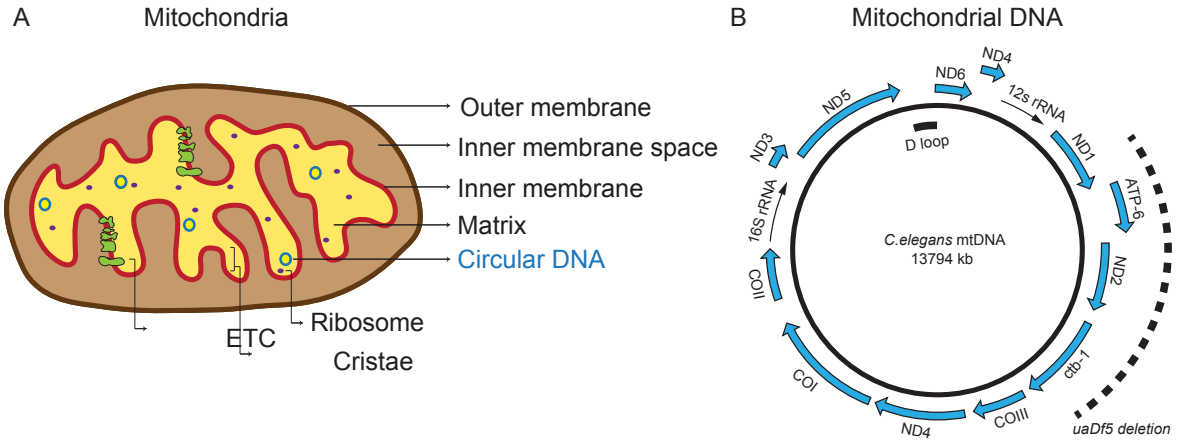


Figure 2: Mitochondrial structure and genome. A) Schematic representation of mitochondria**,** with lipid bilayers: inner (IMM) and outer

(OMM) mitochondrial membrane. These bilayers form compartments intermembrane space (IMS) and matrix. Protrusions of IMM into the matrix form cristae. mtDNA resides in the matrix area. B) *C. elegans* mtDNA encodes 36 genes, 12 subunits of ETC, 2 ribosomal RNA and 22 transfer RNAs. D. loop region contains the replication and transcription promoter sequence. Modified from (Palade 1952; William Y Tsang & Lemire 2002; Tsang & Lemire 2003; William Y. Tsang & Lemire 2002).

5.5.1 Mitochondrial structure genome and function

Mitochondria contain highly specialized phospholipid bilayer membrane structures, inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM). These membrane structures form separate compartments the intermembrane space (IMS) and internal matrix space (Frey et al. 2002). This specialized compartmentalization of mitochondria is essential for its function. Due to the presence of porins the OMM is permeable to low molecular weight small molecules. The less permeable IMM acts as a barrier for small molecules between matrix and cytosol due to its higher protein content, the composition of IMS is similar to the cytosol (Lodish et al. 2004; Cooper & Hausman 2000) (Figure 2A).

One of the particular features of mitochondria is that they possess their own genome (mtDNA), double stranded circular DNA similar to bacteria. The size of mtDNA varies between species with rather large mitochondrial genomes in plants (200 kb) and yeast (80 kb). The human mitochondrial genome encodes two ribosomal RNAs, 22 tRNAs and 13 electron transport chain (ETC) subunits. The *C. elegans* mitochondrial DNA (mtDNA) encodes 36 genes within its own genome 12 out of 80 subunits of ETC, two ribosomal RNA and 22 transfer RNAs (Okimoto R et al. 1991 1997) (Figure 2B). Mitochondria contain 5 mitochondrial respiratory complexes (MRC), except for complex II, the other complexes contain mtDNA encoded subunits. Unlike in nuDNA introns are absent in mtDNA. The remaining genes encoding approximately another 1500 proteins

needed for mitochondrial function are located on the nuclear genome (nuDNA).

Mitochondrial DNA is maternally inherited and follows a non-Mendelian pattern of inheritance. Another feature of mitochondria is its polyploidy, each mitochondrion can carry varying numbers of mtDNA and not every molecule is essentially replicated (Clayton 1982). The core components required for mtDNA replication are *polg-1* (DNA polymerase gamma), the helicase Twinkle and the mitochondrial single stranded binding protein (MTSS-1). mtDNA replication is independent of nuDNA replication and mtDNA replication and transcription happen as coupled events. The key components for mitochondrial transcription are POLRMT (RNA polymerase), TFAM (transcription factor A), RNA processing enzyme and topoisomerase (mtTOP1) (Falkenberg et al. 2007).

The nuclear encoded mitochondrial genes are translated in the cytosol and imported into the mitochondria (Larsson & Clayton 1995). Coordination of both the nuclear and the mitochondrial genome is necessary to have refined cellular machineries to replicate, transcribe, and translate the mitochondrial genome. For the transport/import of nuclear encoded mitochondrial genes, mitochondria possess specialised transport machinery which includes the protein complex translocator of OMM (TOM) and the translocator of IMM (TIM). The TOM complex recognizes mitochondrial-targeting sequences of the nuclear encoded protein, transports them to the IMS and incorporates to OMM. Some of these proteins are then further transported into the matrix by TIM (Lodish et al. 2004; Cooper & Hausman 2000).

As described above mitochondria are the energy factories of the body, one of the main functions of mitochondria is energy production from high energy substrates like glucose, amino acids and fatty acids to maintain optimal

cellular function. Adenine nucleotide translocase (ANT) exports ATP produced in the mitochondria to the cytosol for ADP. Mitochondrial ATP production depends on ETC, which contains 5 enzymes complexes attached to matrix surfaces. These complexes are namely complex I (nicotinamide adenine dinucleotide: ubiquinone oxidoreductase), complex II (succinate: ubiquinone- oxidoreductase), complex III (ubiquinone–cytochrome c oxidoreductase), complex IV (cytochrome c oxidase, COX). Electrons from NADH are transferred to respiratory complex I and then to ubiquinone (UQ), whereas electrons from succinate are transferred to complex II and subsequently to UQ. From UQ, electrons are consequently transferred to complex III, then to complex IV, which uses the electrons and hydrogen ions to reduce molecular oxygen to water. This electron transfer between complexes creates an electrochemical gradient across the IMS. This gradient helps for the transport of protons facilitating the reaction via the ATP synthase to make ATP from ADP (adenosine diphosphate) and Pi (inorganic phosphate (Wallace et al. 1988) .

5.5.2 Mitochondrial biogenesis

Cells increase their individual mitochondrial mass and copy number to meet greater energy requirement conditions, this process is known as mitochondrial biogenesis. Biogenesis of mitochondria is influenced by factors such as hypoxia, exercise, temperature, stress, nutrient availability and ageing (Annex et al. 1991; Freyssenet et al. 1996; Lee & Wei 2005; Lee et al. 2002; Nagino et al. 1989; Wu et al. 2007).

Mitochondrial biogenesis is a complex process, as it requires biogenesis of new organelle structures, along with synchronized synthesis and assembly of the dual origin proteins. The coordination of both nuclear and mitochondrial genomes is essential for regulation of mitochondrial biogenesis. 95% of mitochondrial proteins are encoded in the nuclear genome. These genes are required to be transcribed, translated and

imported into the mitochondria. Along with the nuclear encoded proteins, mitochondrial genomes that encode most hydrophobic proteins of the electron transport chain, tRNAs and rRNAs, need to be synthesized. In order to produce new mitochondria transcription affecting both nuclear and mitochondrial genomes must be coordinated (Lenka et al. 1998; Scarpulla 2002a; Roy et al. 2007).

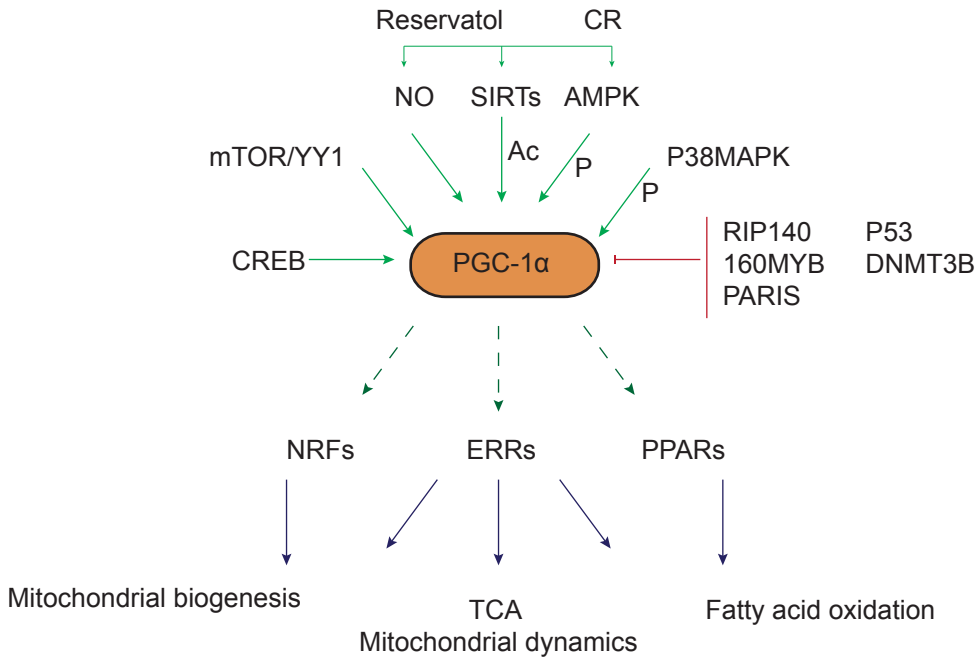


Figure 3: Mitochondrial biogenesis in mammals. PGC-1α functions as the master regulator of mitochondrial biogenesis that acts at the centre of a complex network of signaling pathways that are regulated at the level of post translational modification and gene expression in response to metabolic, nutrient and environmental changes. Modified from (López-Lluch et al. 2008; Wenz 2013).

In mammals, mitochondrial biogenesis and regulation is better understood than in *C. elegans*. Mitochondrial biogenesis is regulated by several cofactors such as nuclear respiratory factors (NRF-1, NRF-2), transcription factors (CREB, MEF-2/E-box, SP1, YY1,) and co-activators (PGC-1α, PRC). Nuclear respiratory factors govern the expression of nuclear encoded mitochondrial genes required for oxidative phosphorylation (OXPHOS) as well as the expression of nuclear encoded factors involved in mitochondrial transcription, protein import and protein assembly. PGC-

1α (PPAR coactivator 1α) is the master regulator of mitochondrial biogenesis. PGC-1α affecting factors can be modulated at different stages such as by posttranslational modifications or by directly regulating the gene expression. Along with factors NRF1 and NRF2, estrogen related receptor α (ERRα) and cAMP response element (CREB) are involved in the regulation of mitochondrial genes (Scarpulla 2002b). These transcription factors are also co-regulated by PGC-1α (Scarpulla et al. 2012) (Figure 3).

Similarly, to PPAR, CREB and YY1 can directly regulate the gene expression of PGC-1α. Under conditions such as cold temperature or upon altered cytosolic calcium homeostasis CREB induces PGC-1α. On the other hand, YY1 a common target of mammalian target of rapamycin (mTOR) and PGC-1α, physically interacts with the master regulator PGC-1α. This interaction is in turn influenced directly by mTOR (Cunningham et al. 2007). Thereby lower mTOR activity leads to deceased expression of mitochondrial genes (Scarpulla 2008). PGC-1α is also negatively regulated by factors such as PARIS, P53, RIP140, 160MYP and DNA methyl transferase 3b (DNMT3B). These factors act as repressors of PGC-1α by negatively regulating the gene expression and thereby supressing mitochondrial biogenesis (Fan et al. 2004; Powelka et al. 2006; Ho et al. 2009; Barrès et al. 2009; Shin et al. 2011).

Cellular stress, induced by e.g. caloric restriction (CR) leads to regulation of energy sensor modulators such as AMPK, mTOR and Sirtuins. AMPK and sirtuins regulate mitochondrial biogenesis by post- translational modification of PGC-1α. On the other hand, upon CR, the cellular AMP/ATP ratio is altered thus activating AMPK. AMPK modulates mitochondrial biogenesis upon varied nutrient availability by PGC-1α phosphorylation (Cantó & Auwerx 2009b). mTOR which senses nutrient availability is a major mediator of CR regulating mitochondrial biogenesis at different stages. Primarily, mTOR regulates the interaction of YY1 with PGC-1α as described

above. Secondly, mTOR activity is decreased by nutrient deprivation which in turn leads to lower translation levels, increased OXPHOS activity and fatty acid oxidation (Duvel et al., 2010). Moreover, decreased nutrient availability leads to tze modulation of mitochondrial biogenesis via sirtuins. NAD+ levels are altered in response to metabolic changes thereby activating Sirt1. Upon nutrient deprivation Sirt1 is activated which in turn de-acetylates and activates PGC-1α and thereby influences mitochondrial biogenesis (Cantó & Auwerx 2009b). PGC-1α in turn activates mitochondrial sirtuin, Sirt 3 a vital player in the antioxidant defence mechanism. The Sirt-1: PGC-1α: Sirt-3 axis deacetylates and activates mitochondrial superoxide dismutase upon nutrient deprivation (Qiu et al. 2010; Sebastian & Mostoslavsky 2010). Another known regulator in this context is ROS. As a compensatory mechanism ROS is known to increase biogenesis during mitochondrial respiratory chain dysfunction (Acin-Perez et al. 2009). An increase in the production of ROS also leads to the activation of Sirt3 via PGC-1α for the activation of antioxidant defence mechanism (Kim et al. 2011). Interestingly, PGC-1α levels are shown to decline with age, this decline is inhibited by CR. This in turn shows the interconnection of metabolic changes upon nutrient availability and its influence on longevity.

Even though, *C. elegans* lacks clear homologs of mammalian factors implicated in mitochondrial biogenesis such as ERRα, PGC1α, or NRF1, worm cells respond to increase in demand for energy by increasing the number of mitochondria. Such a demand can for example be observed during development when L4 stage and adulthood are entered. *C. elegans* embryos contain ~ 25,000 copies of mtDNA and this remains unchanged through the L1, L2, and L3 larval stages. However, when compared to L3 larvae the mtDNA content is increased five-fold and six-fold in L4 stage and in adults, respectively (Bratic et al. 2009).

Studies have also shown a possibility for an increase in mitochondrial copy number with a shift to higher temperature (Dillin et al. 2002). This reflects

an adaptation to higher metabolic demands in elevated environmental temperatures. Taken together this indicates that upon higher energy demand there is higher mitochondrial biogenesis. However, the molecular mechanisms regulating mitochondrial biogenesis and energy metabolism according to temperature, different developmental stages and during ageing are poorly understood in *C. elegans*.

5.5.3 Mitochondrial fission and fusion

Mitochondria undergo a continuous transformation through the regulation of mitochondrial fission and fusion. These processes function to maintain the integrity of mitochondria by eliminating mitochondria beyond damage and preserving mitochondrial function and are essential for replenishment of mitochondria. On one hand to dilute out the damage, mitochondria undergo fusion events to mix mtDNA, proteins, lipids and small-molecule metabolites. On the other hand, for an efficient removal of damaged mitochondria by cellular degradation via processes such as mitophagy, mitochondria undergo fission. As mitochondrial fission and fusion genes have been implicated in the maintenance of mitochondrial metabolism, maintenance of mtDNA, mitochondrial quality control mechanisms, mitophagy and reactive oxygen species (ROS) production, the regulation of mitochondrial dynamics is essential for modulating mitochondrial function and quality (Kujoth et al. 2005; Chan 2012; Bernhardt et al. 2015; Schiavi et al. 2015; Sebastián et al. 2017a).

Mitochondrial fusion events are regulated by members of the protein family of dynamin-related GTPases. FZO-1 the *C. elegans* homolog of Mfn1 in mammals and EAT-3 the *C. elegans* homolog of Opa1 in mammals are required for the fusion of the outer and inner mitochondrial membrane (Tan et al. 2008; Ichishita et al. 2008; Breckenridge et al. 2008). Whereas DRP-1, the *C. elegans* homolog of mammalian *Drp1*, is essential for mitochondrial fission. Hence the loss of *drp-1* leads to less fragmentation in comparison to wildtype whereas loss of *fzo-1* leads to more fragmented mitochondria

(Figure 4). As mitochondrial fission and fusion are essential for mitochondrial function, loss of *drp-1* has been shown to increase the mitochondrial respiration rate (Luz et al. 2015). EAT-3 is also essential for oxidative stress resistance caused by free radicals. Mitochondrial dynamics play an essential role in the apoptosis process. The role of mitochondrial fission genes in apoptosis and the mitochondrial fission process can be separated. *drp-1* has been shown to have pro- apoptotic function as over-expression of *drp-1* leads to increased apoptosis and loss of *drp-1* leads to a partial blocking of the apoptotic process. Although the role of *fzo-1* is not entirely clear in apoptosis, it is understood that decreased *opa-1* levels lead to increased apoptosis. In *C. elegans* the BCL-2 like protein *ced-9* has been shown to have implications in the mitochondrial fusion process of both the mitochondrial outer and inner membrane, *ced-9* regulates the mitochondrial fusion process by physically interacting with mitochondrial fusion protein FZO-l. *ced-9* also physically interact with *drp-1* and *egl-9* functions as a molecular switch for *ced-9* in regulating mitochondrial dynamics (Rolland & Conradt 2010; Martinou & Youle 2011; Autret & Martin 2010; Conradt & Horvitz 1998; Jagasia et al. 2005; Breckenridge et al. 2009; Tan et al. 2008).

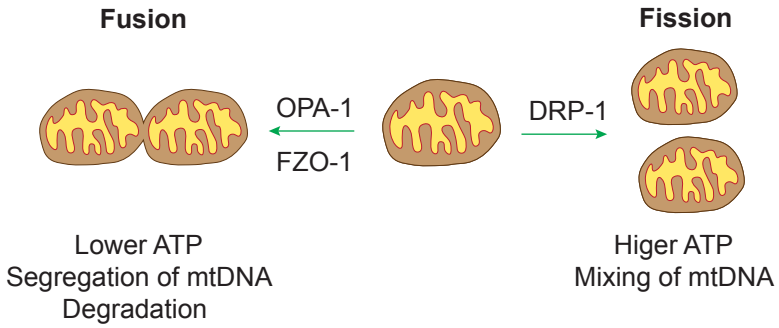


Figure 4: Mitochondrial fission and fusion process in *C. elegans* Mitochondrial dynamics are controlled by fission and fusion process. Mitochondrial fission is regulated by *drp-1*, whereas mitochondrial fusion is regulated by FZO-1 and OPA-1. Adapted from (Sebastián et al. 2017)

As described above mitochondrial fission and fusion are necessary for the maintenance of mitochondrial morphology and the mitochondrial network.

A change in mitochondrial morphology is observed with age: in body wall muscles of young worms mitochondria form tubular networks and with age the mitochondrial network becomes fragmented (Regmi et al. 2014), indicating an essential role of mitochondrial dynamics in maintaining mitochondrial function during the ageing process.

5.6 Mitochondria and their role in Ageing

Mitochondria are crucial organelles for metabolism, respiration, energy homeostasis, and development. Mitochondria are the major source for the production of reactive oxygen species (ROS). The Mitochondrial free radical theory of ageing (MFRTA) proposes that ageing is caused by the accumulation of damage caused by ROS production which further leads to severe oxidative stress and dysfunction (Harman 1956). A decline in mitochondrial function is observed with age, and therefore a robust correlation between ROS production and age. Accordingly, many age related diseases are linked to enhanced oxidative damage. MFRTA was further reformed by discovering the role of superoxide dismutase and its antioxidant activity in response to ROS levels (Harman 1992; Harman 1956; Harman 1972). Overexpression of *Sod1* and *Sod2* increases longevity in flies, in line with these observations *Sod1* and *Sod2* mutants of *C. elegans* are also short lived (Sun & Tower 1999; Phillips et al. 1989; Duttaroy et al. 2003). This theory has been highly debated in the field, however genetically engineered organisms with altered antioxidant activities failed to support the MFRTA. For instance, in *C. elegans* superoxide dismutases were shown to protect against oxidative damage but had no effect on lifespan and deletion of *sod-2* even extended lifespan (Van Raamsdonk & Hekimi 2012; Van Raamsdonk & Hekimi 2009; Yang & Hekimi 2010a).

This implies that the mitohormesis theory, suggesting that high levels of

ROS could be detrimental whereas the lower dosage of ROS contributes to beneficial effects on health and longevity, might be more suitable to explain the effects of mitochondrial functions on longevity. ROS could function as a probable signalling molecule in response to stress. When the level is optimal it regulates stress response pathways which in turn brings favourable effects to longevity. In line with this is the finding that with progression of age ROS levels increase and become damaging (Hekimi et al. 2011; Ristow & Zarse 2010; Schulz et al. 2007; Lee et al. 2010).

Alteration of mitochondrial activity affects life span of diverse organisms, from yeast and *C. elegans* to mammals (Copeland et al. 2009; Dell'Agnello et al. 2007; Liu et al. 2005; Lee et al. 2003; Feng et al. 2001a; Kirchman et al. 1999; Braeckman et al. 1999; Lakowski & Hekimi 1996). Partial deficiency of Mclk1 in mice leads to increases in lifespan (Lapointe & Hekimi 2008; Liu et al. 2005) and a knock down of Surf1(Complex V1) in mice leads to lower complex 1V activity and increased lifespan (Dell'Agnello et al. 2007). RNAi knockdown of mitochondrial subunits have been shown to increase lifespan in flies. Knockdown in just the nervous system is sufficient for this lifespan extension suggesting a tissue-non-autonomous mechanism (Copeland et al. 2009).

In *C. elegans* knockdown of many ETC genes using RNAi increases longevity, for instances *nuo-2* (NADH/ ubiquinone oxidoreductase: Complex I), *cyc-1* (cytochrome C reductase: Complex III) and *cco-1* (cytochrome c oxidase: complex IV). The lifespan extension is also accompanied by a slow growth rate, low oxygen consumption and lower ATP levels, again showing a close linkage between longevity, mitochondria and food intake (ATP levels) (Dillin et al. 2002). Interestingly, to induce this lifespan extension, it is absolutely necessary to feed the RNAi during the larval stages especially during L3/L4 transition (Dillin et al. 2002; Rea et al. 2007). As mentioned above mitochondria undergo a dramatic proliferation between the L3-L4 transition stage (William Y. Tsang & Lemire 2002), an

increase in mtDNA levels is also observed during these stages (Bratic et al. 2009). Mitochondrial longevity by reduction of ETC subunits is not achieved upon knockdown starting only in the adult life stage, even though ATP levels are reduced (Durieux & Dillin 2007). This emphasizes the connection of mitochondrial function, development and longevity. Mitochondrial longevity benefits are also achieved in a cell-non-autonomous manner. As knockdown of *cco-1* just in the intestine and neurons increases lifespan, but knockdown in muscles only is not sufficient. Interestingly knockdown of *cco-1* just in the intestine results in an cell-non-autonomous upregulation of UPR^{mt} induced heat shock factor *hsp-6* in the intestine (Durieux & Dillin 2010).

Moreover, mutants for individual ETC subunits not only show long-lived phenotypes, but also short lifespan such as in *mev-1* (complex II) and *gas-1* (complex I) (Senoo-Matsuda et al. 2001; Kayser et al. 2001), as well as larval arrest *atp-2 1* (complex V) and *nuo-1* (complex I) (Tsang et al. 2001). Additionally, a knockout of *clk-1* reduced mitochondrial metabolic activity, complex I activity, whereas *clk-1* overexpression leads to increased mitochondrial activity and a reduced lifespan (Miyadera et al. 2001; Kayser, Sedensky, Morgan, et al. 2004; Bratic & Trifunovic 2010). Mutation of *isp-1(qm150)*, affects the iron sulfur protein of complex III and leads to a significantly longer lifespan, slower growth rate, a lower respiration rate and higher ROS levels (Feng et al. 2001b). Similarly, mutation in nuo-6 (complex I) also leads to lower respiration and longer lifespan (Yang & Hekimi 2010b). Paraquat (PQ) treatment which leads to higher ROS levels, extends lifespan similarly to mitochondrial mutants and for instance lifespan of *isp-1* is not further extended by PQ treatment ((Yang & Hekimi 2010a). Short lived *mev-1* and *gas-1* also show lower oxygen consumption and increased ROS levels but they also have increased oxidative damage (Kayser, Sedensky & Morgan 2004; Hartman et al. 2001).

Reduction of electron chain activity which is involved in lifespan extension

of mitochondrial mutants leads to activation of mitochondrial stress response pathways such as mitochondrial unfolded protein response UPR^{mt} and ROS signalling-induced mitohormesis (Schulz et al. 2007; Durieux et al. 2011; Jensen & Jasper 2014; Lee et al. 2010). The antioxidant defence response is essential for mitochondrial longevity as knockout of *sod-2* abolishes mitochondrial longevity in *isp-1* mutants (Van Raamsdonk & Hekimi 2009). Accordingly, UPR^{mt} induction has been shown to prevail upon knockdown by RNAi or by knockout of ETC components, while the absence of the UPR^{mt} gene DVE-1 has been shown to abolish the longevity of mitochondrial mutants (Haynes et al. 2010).

The apoptotic signalling pathway has also been associated with an increased lifespan of mitochondrial mutants *isp-1 and nuo-6* as well as lifespan extension induced by PQ treatment. Whereas reduction of many essential factors of the apoptotic signalling pathway such as *ced-9*, *ced-4* and *ced-3* abrogated mitochondrial longevity. mtROS production is essential for the activation of the apoptotic signalling pathway upon mitochondrial dysfunction (Yee et al. 2014) .

Additionally, HIF signalling is also shown to be essential to mitochondrial impairment induced longevity. Higher ROS levels in mitochondrial ETC subunits leads to the stabilisation and increased activity of HIF-1. Mitochondrial mutants *clk-1* and *isp-1*, as well as PQ treatment induced several HIF-1 target genes and these mitochondrial mutants required HIF-1 transcription factor for the induction of longevity (Hwang et al. 2014). CEP-1 the mammalian homolog of p53 is necessary for the mitochondrial longevity of *isp-1* and *nuo-6* and interestingly rescued the short lifespan of *mev-1* and *gas-1.* Indicating that *cep-1* is essential for physiological changes in both short lived and long lived ETC mutants which might function through the *cep-1* regulated frataxin gene *ftn-1* (Baruah et al. 2014). Stabilised HIF-1 has also been shown to be functioning upstream of *ftn-1*.

Interestingly manipulation of mitochondrial ribosomal proteins such as *mrps-5* have been shown to increase lifespan in different organisms by instigating an imbalance between mitochondrial and nuclear encoded mitochondrial proteins known as mito-nuclear protein imbalance. Mito-nuclear imbalance leads to the activation of UPR^{mt} stress signalling and thereby longevity. Additionally, upregulation of the metabolic regulator sirtuin by altered NAD levels also leads to mito-nuclear protein imbalance, UPR^{mt} activation as well as ROS mediated antioxidant response, which in turn leads to lifespan extension as observed in *C. elegans* and well as higher model organisms (Mouchiroud et al. 2013; Houtkooper et al. 2013).

Interestingly epigenetic factors such as histone lysine demethylase family of Jumanji proteins *jmjd1.2* and *jmjd 3.1* have been described to be essential for UPR^{mt} and mitochondrial longevity in *C. elegans,* as well as the mammalian homologs of these factors PHF8 and JMJD3 showed similar corresponding association to UPR^{mt} (Merkwirth et al. 2016). Upon mitochondrial stress the chromatin structure in the intestinal nuclei seem smaller and condensed, this appears to be disrupted in the absence of chromatin modifier *lin-65.* Additionally, upon mitochondrial stress *lin-65* is nuclear localised in the intestine, this nuclear localisation is mediated by H3K9me1/2 methytransferase *met-2*. *lin*-65 is also essential for *dve-1* nuclear localisation and UPR^{mt}. Theses chromatin modifiers *met-2* and *lin-65* function in synchronisation with the UPR^{mt} transcription factor *atfs-1* to modulate longevity induced by mitochondrial stress (Tian et al. 2016a) (Figure 5)

Many other factors such as TAF-4, CEP-23, AHA-1, CEH-18 have been shown to regulate mitochondrial longevity (Khan et al. 2013). It is also suggested that upon mitochondrial stress *gcn-2* phosphorylates eIF2α which function as a complementary arm and has also been shown to be essential for mitochondrial longevity such as in *clk-1* and *isp-1* (Baker et al. 2012).

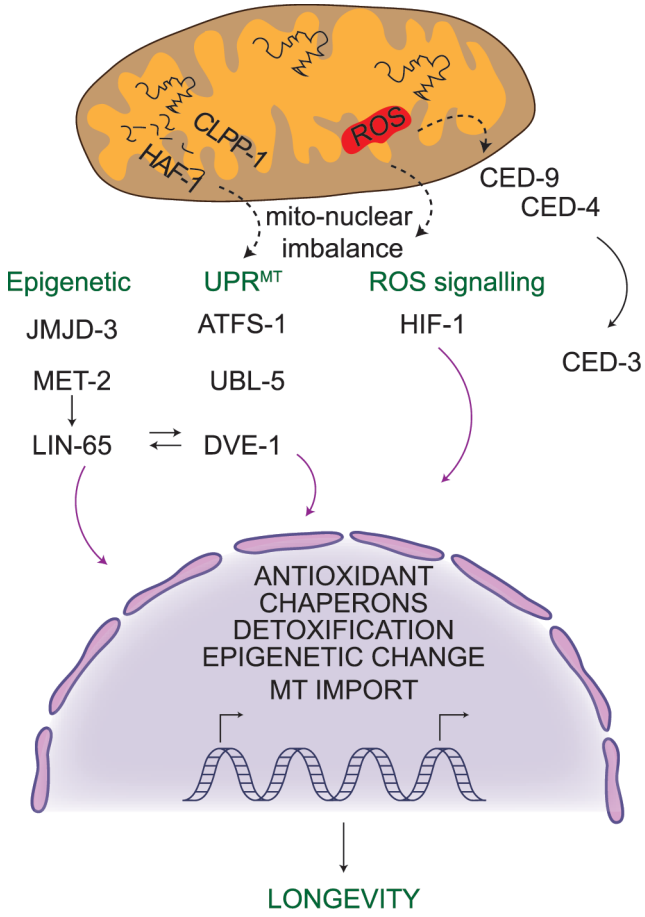


Figure 5: **Mitochondria and ageing:** Longevity induced by mitochondrial impairment is regulated by epigenetic factors, UPR^{mt}, ROS signaling, etc, which leads to activation and nuclear localization of specific factors. These factors trigger downstream target genes and process which inturn leads to longevity (refer text for more details). modified from (Pellegrino et al. 2013; Yee et al. 2014; Tatar & Sedivy 2016; D'Amico et al. 2017).

Mitochondrial malleability is compromised with age. In the body wall muscle of young worms the mitochondria form tubular networks and with age this network is disrupted leading to fragmented mitochondria (Regmi et al. 2014). The age dependent fragmentation and loss of mitochondrial volume occurs sooner in animals at higher temperature. Interestingly this phenomenon is slower in the long lived mitochondrial mutant *clk-1.* As well as the mitochondrial fragmentation has been shown to be delayed in long lived mutants*, age-1, glp-1, daf-2* whereas in *daf-16* the rate of fragmentation is faster (Regmi et al. 2014)**.**

Though various well conserved longevity pathways function parallel to each other, there are definite overlap in factors and processes that modulate longevity. In *C. elegans* a lower mitochondrial membrane potential has also been linked to lifespan extension in long lived mutants of diverse longevity pathways insulin/IGF signalling mutant *daf-2*, DR/TOR signalling mutant eat-2 and mitochondrial mutants *isp-1* and *clk-1* (Lemire et al. 2009). Mito-flash which is described as a read out for free radical production, is shown to be delayed in long-lived *daf-2*, *age-1*, *glp-1*, *eat-2*, *pha-4* strains. Whereas Paraquat and H_20_2 which increase ROS show increased mito-flash and defective ETC subunit strains have lower mito-flash (Shen et al. 2014). The *skn-1* transcription factor, which was shown to be pivotal for diverse longevity pathways such as of low-insulin/IGF-1 signaling or mitochondrial impairment induced longevity, also regulates mitochondrial biogenesis and mitophagy via the mitophagy gene *dct-1* in *C. elegans* (Palikaras et al. 2015). Mitochondrial signaling and function may mediate the cross-talk between the insulin/IGF-1 signaling and TOR pathways to regulate longevity. Caloric restriction was shown to delay the decline of mitochondrial biogenesis during ageing (Baker et al. 2006; Hepple et al. 2006; Lopez-Lluch et al. 2006). Nutrient availably regulates the activation of metabolic factors such as AMPK and sirtuins thereby modulation longevity. Nutrient deprivation regulates mitochondrial function specifically through sirtuins that regulate the PGC-1α mediated mitochondrial biogenesis, the sod/foxo axis mediated antioxidant defense response and the mito-nuclear protein imbalance (Cantó & Auwerx 2009a; Houtkooper et al. 2013; Mouchiroud et al. 2013; Baker & Haynes 2011).

As described above changes in mitochondrial metabolism, morphology and function are observed with the progression of age, this disrupts mitochondrial turnover by worsening mitophagy and biogenesis, leading to the accumulation of damaged mitochondria in aged cells, ultimately causing age related diseases. However further studies need to be

conducted to better understand the regulation of mitochondrial function and cross talk between mitochondria and other organelles in response to stress and longevity regulation.

5.7 Unfolded protein response (UPR)

Organisms have evolved to adapt and respond to external stress stimuli at a cellular level. Interestingly, most of the molecular pathways such as insulin/IGF signaling, TOR signaling or mitochondrial impairment, that regulate longevity, activate downstream stress defense mechanisms and responses, thereby underlining the phenomenon of ageing processes being closely linked to stress signaling(Kourtis & Tavernarakis 2011) (Tavernarakis et al. 2011).

Along the same lines another cell-stressor, accumulation of misfolded proteins, has also been described as one of the hallmarks of ageing (López-Otín et al. 2013). For optimal cellular function and safeguarding of protein homeostasis efficient protein quality control mechanisms have been adapted by subcellular compartments. Several age-related diseases such as the neurodegenerative Alzheimer disease have also been linked to dysfunctions of proteostasis. Such a dysfunction can occur when misfolded or unfolded proteins accumulate (Balch et al. 2008; Gidalevitz 2006; Kikis et al. 2010). Recent studies have shed light on mechanisms regulating the unfolded stress response (UPR) in specific organelles. Exclusive cellular stress response pathways come into play for each organelle compartment to limit the damage and restore homeostasis. Most proteins are folded in the cytosol after translation. Membrane and secreted proteins on the other hand are folded in the endoplasmic reticulum (ER). Even though it is well understood that the subcellular organelles - the endoplasmic reticulum, Golgi network, mitochondria and also the cytosol - have independent UPR pathways to restore protein homeostasis the required crosstalk between

these different UPRs remains largely unclear (Pellegrino et al. 2013; Cohen et al. 2006; Haynes et al. 2007; Malhotra & Kaufman 2007). It is essential for these UPR response pathways to detect and transduce the stress signal to the nucleus as well as to induce the defense mechanisms to restore the cellular homeostasis.

5.7.1 Mitochondrial unfolded protein response (UPR^{mt})

The mitochondrial environment is sensitive to protein folding stress that is generated by the complexity of mitochondrial structure, radical oxygen species (ROS) generated by the ETC as well as environmental factors like temperature and toxins which damage DNA and proteins. As mitochondrial proteins are encoded in both nuclear and mitochondrial genomes, tightly orchestrated translation and import is essential to maintain protein homeostasis. Nuclear encoded mitochondrial genes are translated in the cytosol and well establish import machineries are responsible for the delivery of these proteins to their desired sites in the mitochondria (Hoogenraad & Ryan 2001). Once translocated in to the mitochondria the mitochondria targeting sequence (MTS) is cleaved off and the remaining protein undergoes protein assisted folding. The retrograde response pathway is the best described mitochondrial to nucleus signal transduction pathway. That suggests the adaptation of nuclear transcription in response to mitochondrial activity. Strikingly, the same direction of interaction is observed under the scenario of metabolic adaptation (Liu and Butow 2006; Butow & Avadhani 2004). Already in 1996, Martinus *et al.* first described UPR^{mt} in mammals, but only recent studies in *C. elegans* by Haynes and Ron have shed light on the molecular mechanism that regulate UPR^{mt} (Haynes et al. 2007; Haynes et al. 2010)

Interestingly UPR^{mt} is not only induced by unfolded proteins but also occurs in response to other mitochondrial stressors. Among those being treatments with ethidium bromide and doxycycline, that both interfere with DNA

translation, or paraquat that leads to increased ROS levels and thus mimicks oxidative stress (Yoneda 2004; Rauthan et al. 2013; Yang & Hekimi 2010a). Moreover, genetic manipulations induce UPR^{mt}. Knockdown of genes involved in the mitochondrial protein handling machinery namely PQC protease/spg-7 will induce the UPR as well as knockdown of mitochondrial ribosomal proteins (Houtkooper et al. 2013) or downregulation of nuclear-encoded ETC genes *cco-1* (complex IV), *isp-1* (complex III) or *clk-1* (ubiquinone synthesis) (Dillin et al. 2002; Haynes et al. 2010; Baker et al. 2012; Durieux et al. 2011; Durieux & Dillin 2007; Haynes et al. 2007). Additionally, mtDNA depletion and the stoichiometric imbalance of OXPHOS components encoded in both mitochondria and the nucleus induce UPR. Hence, UPR can be regarded as an essential line of defense against a vast variety of mitochondrial stresses.

RNAi screens by Haynes and Ron *et al.* using *C. elegans* mitochondrial heat shock factor HSP-60 identified a network of factors that regulate the UPR^{mt} response in *C. elegans.* Upon UPR^{mt} induction ClpP the quality control protease chops the unfolded and misfolded proteins down to short peptides of 6-30 amino acids (aa) in length in the mitochondrial matrix. Theses peptides are effluxed through HAF-1 into the cytoplasm. The main mitochondrial UPR leucine zipper transcription factor ATFS-1 containing both nuclear localization signal (NLS) and mitochondrial localization signal (MLS) is translocated to the mitochondria under basal condition and degraded by LON (mitochondrial protease). Upon UPR^{mt} the effluxes of peptides by HAF-1 leads to the nuclear localization of ATFS-1 and thereby activation of chaperon and protease genes. Additionally, ATFS-1 induces genes that are required for the modulation of mitochondrial import, detoxification and function thereby shifting the energy production from respiration to glycolysis. A RNAi screen performed by Haynes et al. additionally identified two factors that play a significant role in the regulation of UPR^{mt}, namely UBL-5 the ubiquitin-like protein and DVE-1

homeodomain-containing transcription factor. Upon UPR^{mt} induction UBL-5 and DVE-1 translocate into the nucleus and along with ATFS-1 induce the expression of genes which are required for restoring protein homeostasis, including mitochondrial chaperone genes (Haynes et al. 2007; Haynes & Ron 2010; Nargund et al. 2012; Nargund et al. 2015; Pellegrino et al. 2013; Baker & Haynes 2011) (Figure 6).

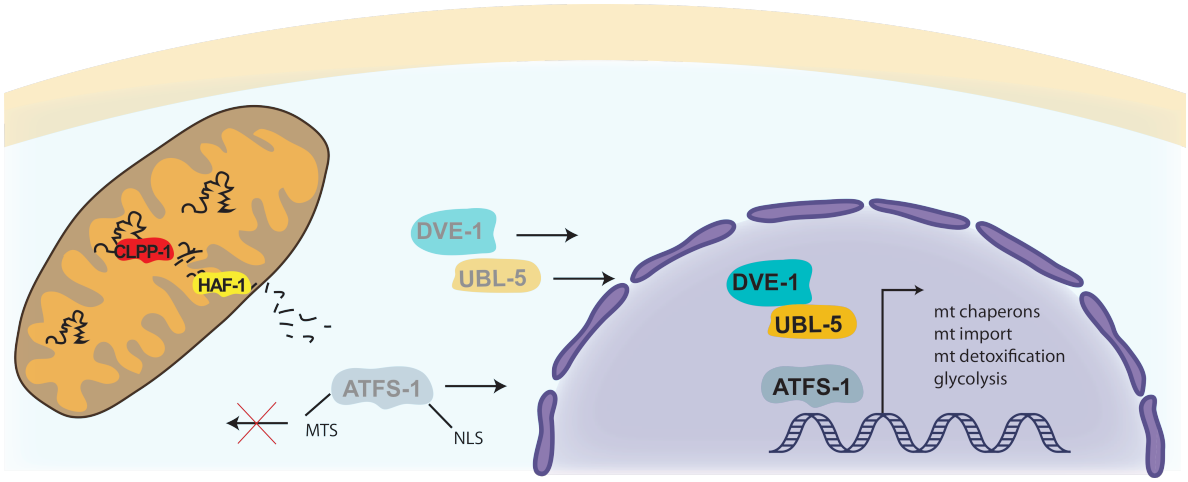


Figure 6: Scheme of the UPR^{mt} signaling pathway in *C. elegans*. Unfolded proteins are degraded to peptides by ClpP-1 and the HAF-1 mediated efflux activates the transcription factor ATFS-1 to translocate into the nucleus. UBL-5 builds a complex with DVE-1 and localizes to the nucleus along with ATFS-1. Modified from (Haynes & Ron 2010).

Accordingly, in mammals upon UPR^{mt} induction, mitochondrial chaperones HSP60, HSP10 mtDnaJ and protease ClpP expression is stimulated similar to *C. elegans*. Moreover, the proteases YME1L1 and PMPCB and the import component TIMM17A, enzymes NDUFB2 endonuclease G and thioredoxin 2 are induced. This UPR^{mt} induction in mammalian cell culture can be achieved by ethidium bromide treatment. Transcription factor Chop and C/EBPβ form a heterodimer and binds to the conserved regulatory elements of the UPR^{mt} responsive genes (Martinus et al. 1996; Zhao et al. 2002; Horibe & Hoogenraad 2007; Aldridge et al. 2007). Another pathway that comes into play for the UPR^{mt} response is the JNK pathway. The transcription factor c-Jun binds to the AP-1 site in Chop and C/EBPβ

(Jaeschke et al. 2006). Additionally, similar to the situation in *C. elegans,* in the mammalian system protein translation in the cytosol is attenuated during UPR^{mt} by eukaryotic translation initiation factor 2-α kinase 2 (EIF2AK2)-mediated phosphorylation of eIF2α (Balzola et al. 2012). Further studies are essential to better understand the mechanisms regulating UPR^{mt} in mammals.

5.7.2 Endoplasmic reticulum unfolded protein response (UPR^{ER})

Endoplasmic reticulum is essential for many processes such as calcium storage, lipid and carbohydrate metabolism, protein folding and glycosylation. The membrane and secreted proteins are folded in the endoplasmic reticulum. Disruption of calcium homeostasis, redox imbalance, altered protein glycosylation or protein folding defects lead to the accumulation of unfolded proteins and in turn manifests to endoplasmic reticulum stress. To restore homeostasis cells have developed signaling systems which include the activation of endoplasmic reticulum unfolded protein response (UPR^{ER}), ER associated degradation pathway, hypoxia response, autophagy and mitochondrial biogenesis.

UPR^{ER} is differentiated into three arms, that are mediated by (inositol-requiring protein IRE-1, activating transcription factor 6 (ATF6), and protein kinase RNA (PKR)-like ER kinase (PERK) (Hetz 2012; Rutkowski & Hegde 2010; Frakes & Dillin 2017a; Senft & Ronai 2015; Malhotra & Kaufman 2007; Kaufman et al. 2002) (Figure 7). These factors sense the protein folding environment and promote the transcription of downstream target genes to enhance the capacity of the ER and resolve the stress. The *ire-1* and the transcription factor *xbp-1* (X-binding protein 1) signify the most conserved branch of the UPR^{ER}. Upon stress *ire-1* activates its cytosolic kinase domain which initiates unconventional splicing of mRNA encoding the transcription factor X box-binding protein 1 (XBP1)/HAC-1 in yeast to create an active spliced transcription factor (sXBP1), the active transcription

factor translocates into the nucleus and induces the expression of chaperones, components of the endoplasmic reticulum associated degradation machinery (ERAD), and proteins involved in lipogenesis (Calfon et al. 2002; Yoshida et al. 2001; Li et al. 2010; Senft & Ronai 2015; Frakes & Dillin 2017b; Ron & Walter 2007) .

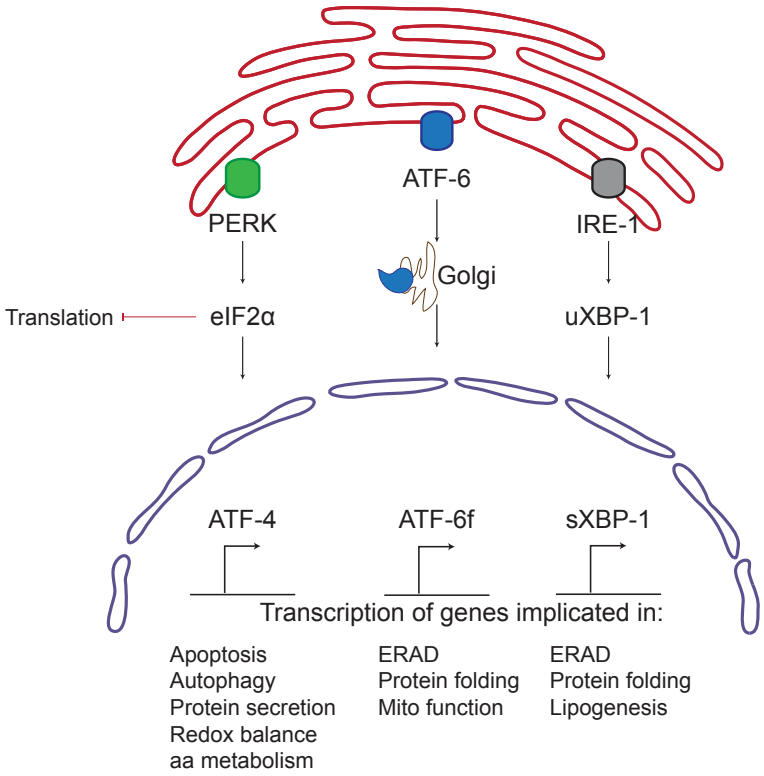


Figure 7: Scheme of the UPR^{ER} signaling pathway. IRE1, ATF6 and PERK are the three arms of UPR^{ER} which sense unfolded proteins and initiate the transcription of downstream target genes: genes encoding ER chaperones and regulating autophagy and ERAD degradation thereby increasing the folding capacity of the cell. Modified from (Senft & Ronai 2015).

ATF-6 which forms the next branch of the UPR^{ER} was first identified by analyzing promoter regions of stress induced genes. ATF-6 moves from the ER membrane to the Golgi apparatus upon protein folding stress where it is cleaved by proteases to form the cytosolic ATF-6 fragment, ATF-6f translocates to the nucleus and activates genes encoding for ERAD

components, protein folding and mitochondrial biogenesis via PGC-1α. The activity and specificity of ATF-6 cytosolic fragment to regulate target genes is controlled by its physical interaction with factors such as nuclear factor y (NF-Y) and YY1 (Haze et al. 1999; Yoshida et al. 2001; Yoshida et al. 2000; Chen et al. 2002).

Yet another branch of UPR^{ER} is the PERK, which decreases the overall translation of new proteins by the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) and reduces the ER stress. Though the overall translation is reduced the transcription factor ATF-4 is favorably translated under these conditions. ATF-4 translocates into the nucleus and activates the downstream target genes involved in autophagy, protein secretion, redox balance, amino acid metabolism and even apoptosis if the stress is too high (Vattem & Wek 2004; Harding et al. 1999; Bertolotti et al. 2000). PERK signaling also facilitates the synthesis of ATF-6 and its trafficking from ER to Golgi apparatus. This mechanism is conserved in higher model organisms.

All arms of UPR^{ER} described above individually and collectively regulate expression of ER chaperones and other stress response genes to resolve ER stress. Endoplasmic-reticulum-associated protein degradation (ERAD) marks unfolded or misfolded proteins, translocates them across the ER membrane to the cytosol, where they are ubiquitylated and degraded by the proteasome (Travers et al. 2000).

Additionally, the UPR^{ER} genes have also been shown to be required to prevent larval lethality and intestinal degeneration in *C. elegans.* As mentioned above the *ire-1/xbp-1* branch functions as the major arm for the UPR^{ER} response. Interestingly a double knockout of *atf-6* or *pek-1* with *ire*-1 or xbp-1 shows a synthetic phenotype such as lethality, UPR deficiency and L2/L4 larval arrest. The most conserved arm of UPR^{ER} the *xbp-1/ire-1* controls the vast majority of the transcription control upon induced UPR^{ER} in

C. elegans (Sakaki et al. 2012; Shen et al. 2005).

ER proteostasis declines with age thus playing an essential role in regulating longevity, for example XBP-1 is essential for stress resistance and longevity which function in a cell non-autonomous manner (Taylor & Dillin 2013). ER stress response factors are also associated with known longevity pathways as in insulin/IGF mutants, where *xbp-1* improves ER stress resistance and enhances genes that regulate longevity by associating with the activated FOXO transcription factor *daf-16* (Henis-Korenblit et al. 2010). Our lab also showed that activation of the hexosamine pathway by stimulation of GFAT-1, the key enzyme of the pathway or by supplementation of N-acetylglucosamine leads to the activation of downstream processes such as ER-associated protein degradation, proteasome activity, and autophagy which in turn results in improved protein quality control, reduced protein aggregation and lifespan extension (Denzel & Antebi 2015).

5.7.3 Cytosolic unfolded protein response (UPR^{cyt})

Mostly cellular proteins are folded after translation in the cytosol. Cytosolic UPR (UPR^{cyt}) is considered as a part of the eukaryotic heat-shock response (HSR). The family of heat shock proteins is key in regulating many functions such as folding and translocation of newly synthesized proteins as well as the refolding of aggregated proteins (Bukau & Horwich 1998). HSR regulates the misfolded or denatured protein accumulation in the cytosol. The key player in UPR^{cyt} is the heat shock factor 1 (HSF-1), which under normal conditions is bound to cytosolic chaperones like Hsp70 and Hsp90. Upon protein-misfolding-stress these chaperons are released from HSF1 and bind to misfolded proteins. HSF1 itself trimerizes and translocates into the nucleus, where it is modified post-translationally. This leads to the activation of downstream HSPs and other genes which enhance the ability of cytosolic protein-folding (Ruan et al. 2017; Labbadia et al. 2017; D'Amico et al. 2017; Geiler-Samerotte et al. 2011; Ben-Zvi et al. 2009). HSF-1 is also

regulated in response to metabolic changes as in insulin/IGF signaling where HSF-1 regulates many downstream target genes. Additionally, upon changes in nutrient availability HSF-1 is directly activated by post-translational modifications via metabolic sensors such as AMPK-1 and Sirt1. Moreover, mitochondrial ROS levels have also been shown to regulate HSF-1 activity (Anisimov 2013; Annex et al. 1991; Anderson et al. 2009; Aldridge et al. 2007; Ackerman & Gems 2012; Ackema et al. 2014). One of the main cytosolic heat shock factors in *C. elegans* is *hsp-16.2* which is altered in response to temperature and mitochondrial dysfunction (Kim et al. 2016).

5.7.4 Cross talk between organelles for unfolded protein response

Though each organelle has unique unfolded protein response mechanisms, loss of homeostasis in one organelle can lead to deleterious effects on other cell compartments, thereby affecting the whole cell. Hence for cell survival it is essential to have efficient cross communications between the different compartments to maintain cellular homeostasis (Veatch et al. 2009; Hughes & Gottschling 2012; Sasaki & Yoshida 2015). Communication between organelles also plays a role in neurodegenerative and cardiovascular diseases. Organellar communication is not only necessary to maintain proper protein folding but also for lipid metabolism particularly regulated in the ER and mitochondria (Kikis et al. 2010; Balch et al. 2008; Kirstein-Miles & Morimoto 2010; Szymański et al. 2017).

Additionally, under protein stress GCN-2 mediated phosphorylation of eIF2α, which is regulated by ROS levels, is essential to reduce the protein translation in the cytosol. That in turn diminishes the load on mitochondrial chaperons (Baker et al. 2012). A RNAi screen for *ire-1* synthetic lethality also identified a subset of nuclear encoded mitochondrial genes such as *nuo-2* and *mrps-5* suggesting a functional co-regulation between the organelles (Shen et al. 2005).

Moreover, in *C. elegans* few ER components have also been shown to be essential for the survival of short lived *mev-1* mutants, e.g. a reduction of *xbp-1* leads to reduced survival under heat stress and hormesis response in *mev-1* mutant is mediated by PEK-1 (Eisermann et al. 2016). Nuclear encoded mitochondrial genes are translated in the cytosol, recent studies by Kim *et al.* show cross talk between the mitochondrial and the cytosolic stress responses (Kim et al. 2016). Upon knockdown of mitochondrial heat shock factor hsp-6 the cytosolic heat shock factor *hsp-16.2* is upregulated and this is described as the mitochondrial to cytosolic stress response (MCSR). Induction of MCSR is mediated by *dve-1, hsf-1* and fatty acid synthesis. Lipid signaling is essential for the induction on MCSR mediated by cardiolipins and ceramides. Indicating that regulator of protein quality control and other metabolic process which affects many subcellular compartments coordinate with each other for maintenance of homeostasis and cell survival.

5.8 Mitochondrial disease

Mitochondria play a pivotal role in the regulation of metabolism and energy consumption, thatfor mitochondrial integrity is critical for cellular function and organismal life span. Mitochondrial dysfunction mostly arises from dysfunction of the mitochondrial oxidative phosphorylation (OXPHOS) system, which includes proteins encoded by both mitochondrial and nuclear genes, or by defects in enzymes that control DNA replication. Some of the most common metabolic disorders are caused by mitochondrial dysfunction. Some can onset in children as well as in adults (Leonard & Schapira 2000) and appear in various organs such as the brain, liver, the pancreatic β-cells or in the auditory system (Gorman et al. 2016). Moreover, mitochondrial disease can have many phenotypes caused by defects in various genes. The mitochondrial genome encounters high mutation rates due to OXPHOS originated ROS and resulting a high oxidative damage of mtDNA.

The first mitochondrial disease was identified by Luft *et al.* in 1962 (Dimauro 2011) since then vast clinical studies on mitochondrial disease have been conducted and are still ongoing. Pathogenic mtDNA mutations are heteroplasmic, with a mixture of mutated and wild-type mtDNA inside each single cell. Thus, mtDNA mutations follow a threshold effect where it should exceed a critical threshold of about 60 – 80% to be detected (Holt et al. 1988). A subcategory of mitochondrial diseases is mitochondrial myopathy, a neuro-muscular disease caused by damage to mitochondria. Muscles and nerve cells require higher energy levels and therefor they are more vulnerable to mitochondrial dysfunction. Many mitochondrial diseases are accompanied by immense proliferation and expansion of mitochondria in myo-fibers (Craven et al. 2017). Few of the common mitochondrial diseases are e.g. Kearns-sayre syndrome (KSS), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELS), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) (Dimauro 2011; Gorman et al. 2016; Suomalainen & Battersby 2017). Leigh syndrome (LS) which mainly affects the brainstem is one of the many mitochondrial disease that is heavily studied. LS is caused due to mutations in almost 75 genes that are encoded by mtDNA and nuDNA. ATP levels are depleted and ROS levels are shown to be high in patients with LS (Ferlin et al. 1997; Johnson et al. 2013; Lake et al. 2016; Leshinsky-Silver et al. 2003; Zhu et al. 1998) .

Nutrient availability impacts the process of mitochondrial biogenesis. Recent studies have initiated many clinical interventions that lead to enriched mitochondrial biogenesis, increased lipid oxidation subsequently leading to improvement of muscle metabolism in mitochondrial myopathy models (Yatsuga & Suomalainen 2012). Mitochondrial disease related myopathy symptoms were reduced also by improving mitochondrial biogenesis by

induction of sirtuins and PGC-1α using Vitamin B3 that alter NAD levels (Khan et al. 2014; Cerutti et al. 2014). Johnson *et al.* additionally reported that inhibition of the major nutrient sensor mTOR by rapamycin reduced neurological defects, delayed disease progression and improved survival in LS mouse models (Johnson et al. 2013).

Hindering mitochondrial protein quality control can also lead to neurological disorders Mitochondrial quality control mechanisms as well as stress responses such as mitophagy play an important role in mtDNA maintenance and translational regulation. Nuclear and mitochondrial genome has to be efficiently coordinated ad disruption of machinery that regulate mitochondrial genes at nuclear, mitochondrial or cytoplasmic levels can disrupt the homeostasis (Gorman et al. 2016; Suomalainen & Battersby 2017). Taking together it is imperative to better comprehend molecular mechanisms and pathways that regulates nutrient sensing and stress responses to better understand pathogenesis of mitochondrial diseases.

6 AIM OF STUDY

6.1 AIM 1: Characterize morphological and physiological regulation of mitochondria under ARD and upon recovery.

Despite the central role of mitochondria in regulating respiration, metabolism, energy homeostasis and longevity, the relationship between nutrient sensing, longevity, mitochondrial function and biogenesis is unclear. As mitochondrial biogenesis appears induced upon ARD exit, we hypothesize that mitochondrial physiology, gene expression, morphology, and turnover are highly regulated upon ARD entry and exit. ARD leads to an increase in longevity in wildtype worms in comparison to worms grown at ad libitum. We further analysed the role of factors regulating mitochondrial dynamics in ARD longevity and its implications in modulating mitochondrial physiology under ARD.

6.2 AIM 2: Identify novel factors regulating mitochondrial function during *C. elegans* ARD recovery.

ARD recovery is a higher energy demanding condition hence modulating mitochondrial physiology. Therefore, we used ARD recovery as a tool to study mitochondrial regulation and biogenesis in *C. elegans* and to potentially discover novel regulators. To establish a read-out for mitochondrial biogenesis we tested several mitochondrial markers and found the nuclear encoded mitochondrial gene cytochrome C oxidase, *cco-1* to be downregulated upon ARD entry and upregulated upon ARD exit most prominently. Thus, we used a transcriptional reporter marker *pcco-1::gfp* to screen for novel factors. We performed an RNAi screening for transcription factors, nuclear hormone receptors, chromatin regulators, phosphatases and kinases with a total of 2244 clones. Notably we identified 38 novel factors regulating mitochondrial function under nutrient shifts. To unravel the underlying mechanisms, we further characterized the

expression levels of 24 mitochondrial genes and the mitochondrial copy number upon knockdown of these candidates. We further performed lifespan analysis with wildtype (WT/N2) and mitochondrial mutant *isp-1* on knockdown of the candidates.

6.3 AIM 3: Decipher the role of transcriptional factor NFYB-1 in regulating mitochondrial physiology and function.

The RNAi screen using *pcco-1::gfp* expression upon ARD recovery yielded factors known to regulate mitochondrial function, as well as several novel factors. Among them we focused on NFYB-1, a subunit of the NF-Y transcriptional complex binding the CCAAT motif. RNAi against NFYB-1 downregulated *pcco-1::gfp* expression and abolished longevity induced by mitochondrial impairment in *isp-1(qm150)*. We further examined the role of NFYB-1 in regulation of mitochondrial function using a null mutant *nfyb-1(cu13).* We investigated how NFYB-1 modulates expression of mitochondrial markers under higher energy demand conditions, mitochondrial morphology, oxygen consumption and mtDNA levels. We further explored the role of NFYB-1 in known longevity pathways such as insulin/IGF signaling pathway*,* DR induced longevity, germline signalling and specifically mitochondrial longevity induced by RNAi knockdown of *cco-1* and mitochondrial mutant *isp-1(qm150*). To better comprehend the regulation of NFYB-1 in mitochondrial stress response pathways we examined function of NFYB-1 in UPR^{mt} as well as the mitochondrial to cytosolic stress response. Further deciphering mitochondrial longevity, we performed transcriptomic and proteomic analyses. These studies will help us understand how a highly conserved transcription factor regulates mitochondrial function and longevity.

7 RESULTS

7.1 Chapter 1: Mitochondrial regulation under ARD

7.1.1 Morphological and physiological regulation of mitochondria under ARD.

To decipher how mitochondria are regulated upon fasting and refeeding, I examined mitochondrial copy number (mito copy no/ mtDNA levels), respiration rate, and mitochondrial morphology, of wildtype worms under ARD and upon ARD recovery.

In *C. elegans* reared under ad libitum food conditions, an increase in mtDNA levels coincides with sexual maturation and germline expansion (Bratic et al. 2009). Under ARD conditions, germline proliferation is largely arrested (Angelo & Van Gilst 2009). We therefore wondered what happens to mtDNA in these circumstances. I observed that mtDNA levels under ARD were lower than ad libitum controls, but increased significantly upon recovery within 24 hours (Figure 8A). To specifically measure mtDNA levels in somatic tissue, I compared the copy number of germline deficient *glp-4(bn-2)* under ARD and upon recovery. Strikingly I also observed a significant increase in mitochondrial copy number in *glp-4(bn-2)* animals upon recovery, suggesting a contribution of mitochondrial biogenesis in somatic tissues as well (Figure 8B). To further examine mitochondrial physiology under ARD and upon recovery, I measured respiration rate using Oroboro, an apparatus that allows the measurement of oxygen consumption in live cultures. I found that under ARD oxygen consumption was significantly lower than day 1, whereas on recovery, oxygen consumption increased within 24 hours to a level comparable to ad libitum grown day 1 adults. This increase was not due to a difference in worm size since I observed a similar increase upon normalisation to protein levels (Figure. 8C and 8D). These observations reveal that mitochondrial

biogenesis and respiration are repressed under ARD, and restored during recovery in both soma and germline.

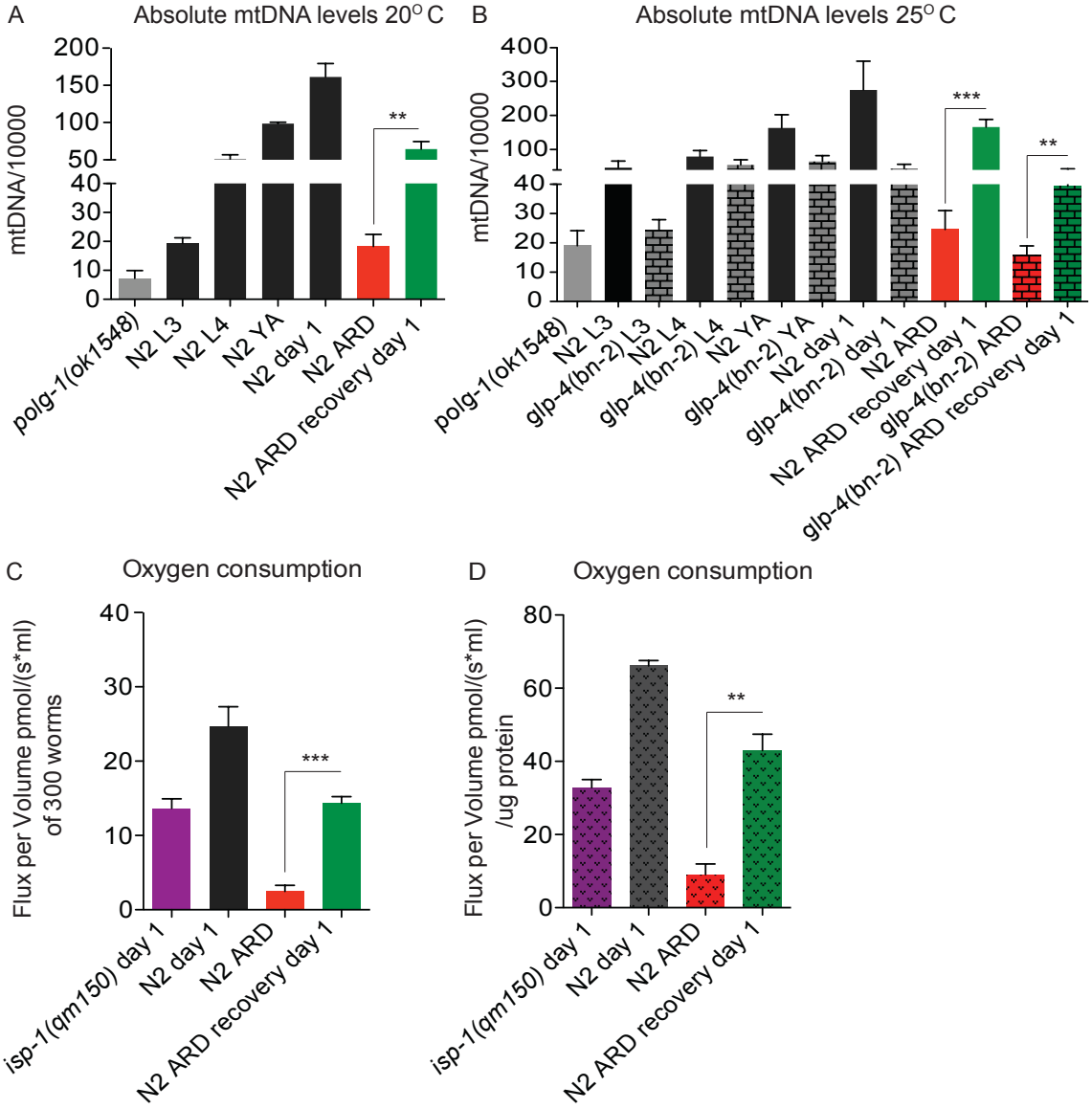


Figure 8: Mitochondrial regulation under ARD and upon recovery. A) Mitochondrial copy number measurement of N2 under ad libitum from L3 larval stage to day 1 adult shows an increase and day 1 *polg-1(ok1548)* shows a reduction in mtDNA levels (Bratic et al. 2009). mtDNA levels increase upon recovery from day 10 ARD at 20° C (n=3, error bar indicating SEM, t. test, ** p<0.01). B) mtDNA levels of germline-less *glp-4(bn-2)* worms show an increase in mtDNA levels upon recovery from day 10 ARD 25° C (n=3, error bar SEM, t. test, * p<0.5, ** p<0.01, ***p<0.001). C) Oxygen consumption measurement using Oroboro of 300 N2 wildtype shows an increase in respiration rate from ARD to recovery, day 1 *isp-1(qm150)* was used as a control, n=3. D) Oxygen consumption normalised to protein levels also shows an increase in respiration rate from ARD to recovery (n=3, error bar indicating SEM, t. test, * p<0.5, ** p<0.01, ***p<0.001). E) Mitochondrial morphology of *pmyo-3::gfp(mt)* expressed in the body wall muscle measured using confocal microscopy, day 10 ARD worms form fused mitochondrial structures while day 10 ad libitum fed controls show fragmented mitochondria. ARD day 1 recovered worms showed tubular mitochondrial structures (n=3, >20 worms per condition, scale bar 5 μm).

We next wondered whether ARD affected mitochondrial dynamics. To examine mitochondrial morphology, I used the strain *pmyo-3::gfp(mt)* in which a mitochondrial targeted protein is expressed in body wall muscles. Interestingly in ARD day 10 worms I noticed that mitochondria formed a more fused network, while mitochondria were more fragmented in day 10 reproductive adults (Figure 8E). Upon recovery from ARD, mitochondria re-established a tubular network similar to young adults. Altogether these data indicate that mitochondria undergo remarkable metabolic and physiological remodelling under ARD and upon recovery.

7.1.2 Role of mitochondrial fission and fusion in ARD longevity.

Wildtype ARD worms have a maximum lifespan of 80 days and thus live significantly longer than ad libitum fed wildtype worms (Gerisch et al unpublished). As I observed a highly fused mitochondrial network under ARD, I speculated that mitochondrial dynamics regulated by fission and

fusion processes might be essential for ARD longevity. Thus, Birgit Gerisch performed lifespan analysis for mutants of fusion gene *fzo-1* and fission gene *drp-1* under ARD and under ad libitum in comparison to wildtype. Under ad libitum *fzo-1(tm1133)* and *drp-1(tm1108)* lifespans were not significantly altered compared to wildtype. Interestingly under ARD, we observed a partial increase in longevity upon loss of fission gene *drp-1* (Figure 9A), whereas upon loss of fusion gene *fzo-1* ARD longevity was partially abolished (Figure 9B). These findings suggest that mitochondrial fusion promotes and fission impedes ARD longevity.

To discern how mutants of fission and fusion regulate mitochondrial dynamics and morphology under ARD I performed confocal microscopy. Whereas wildtype animals exhibit a fused mitochondrial network at day 10 ARD, *drp-1(tm1108)* animals displayed hyper fused globular mitochondria (Figure 9C), while, *fzo-1(tm1133)* produced fragmented mitochondria (Figure 9.D). Thus, these mutants exacerbate fission and fusion events under ARD.

To further decipher the physiological role of mitochondrial fission and fusion in ARD longevity, I measured oxygen consumption and mitochondrial copy number. Under reproductive day 1 conditions, *drp-1(tm1108)* oxygen consumption resembled that of wildtype, whereas *fzo-1(tm1133)* showed a partial reduction. By contrast under ARD conditions, *drp-1(tm1108)* mutant showed a partial reduction in oxygen consumption, while *fzo-1(tm1133)* showed little difference from wildtype (Figure 9E). Under reproductive conditions mtDNA levels were higher for *drp-1(tm1108)* but not significantly different for *fzo-1(tm1133)* in comparison to wildtype. Similarly, loss of *drp-1(tm1108)* also led to an increase in mitochondrial copy number under ARD, whereas for *fzo-1(tm1133)* mtDNA levels remained unchanged (Figure 9F).

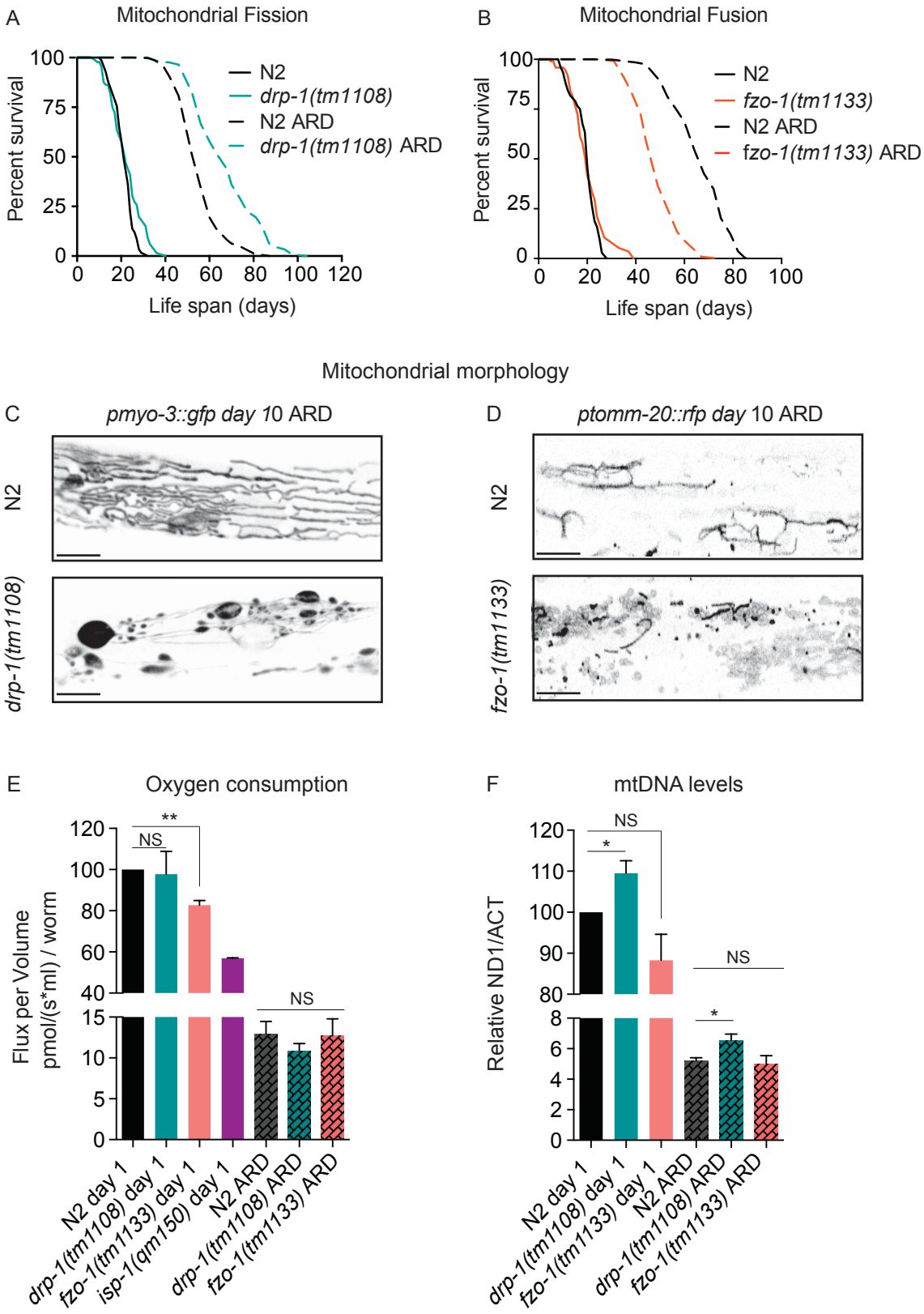


Figure 9: Mitochondrial fission and fusion genes partially regulate ARD longevity. A) *drp-1(tm1108)* partially increases ARD but not ad

libitum longevity in comparison to wildtype. (Mantel-Cox Log Rank test, n=3, refer appendix table 5 for statistics). B) *fzo-1(tm1133)* decreases ARD but not ad libitum longevity in comparison to wildtype (Mantel-Cox Log Rank test, n=3, refer appendix table 5 for statistics). C) Mitochondrial morphology of *pmyo-3::gfp(mt)* expressed in the body wall muscle measured using confocal microscopy. *drp-1(tm1108)* animals show hyper fused mitochondrial structures in comparison to N2 under day 10 ARD (n=2, >15 worms per condition, scale bar 5μm). D) *fzo-1(tm1133)* animals show fragmented mitochondrial structures in comparison to N2 under day 10 ARD (n=2, >15 worms per condition, scale bar 5μm). E) Oxygen consumption measurement per worm of day 1 *drp-1(tm1108) and fzo-1(tm1133)* in comparison to N2 at ad libitum (300 worms per measurement) and under day 10 ARD (1000 worms per measurement) relative to N2 day 1. *drp-1(tm1108)* show a partial decrease (n=3, error bar indicating SEM, t. test, * $p<0.5$, ** $p<0.01$, ***$p<0.001$). F) Measurement of mtDNA levels of *drp-1(tm1108) and fzo-1(tm1133)* in comparison to N2 at ad libitum L4 and under day 10 ARD (n=3, error bar indicating SEM, t. test, * $p<0.5$, ** $p<0.01$, ***$p<0.001$).

Taken together these data suggest that under ARD, worms establish a low metabolic rate and form a fused mitochondrial network to maintain the integrity of mitochondria and enhance survival. Altering mitochondrial dynamics in turn leads to a change in mitochondrial physiology, thereby affecting lifespan.

7.2 Chapter 2: RNAi screen and characterization

7.2.1 Identification of factors for mitochondrial regulation and biogenesis.

I took advantage of the remarkable changes in metabolic and physiological function of mitochondria upon ARD recovery, to identify novel factors regulating mitochondrial function. First, I tested different mitochondrial markers such as (*pvdac::gfp, pmtss-1::gfp* and *pcco-1::gfp)* to examine how ARD affects mitochondrial gene expression upon ARD entry and exit. Mitochondrial marker *pcco-1::gfp (*nuclear encoded ETC subunit complex IV) was the most striking, and gave significant downregulation of *gfp* expression upon ARD entry and upregulation upon recovery. Additionally, I also observed an increase in expression in intestinal tissues (Figure 10A & B). Hence, we decided to use alterations in *pcco-1::gfp* intensity upon ARD recovery as a readout for altered mitochondrial function and biogenesis.

I performed an RNAi screen upon ARD recovery using RNAi libraries for transcription factors, nuclear hormone receptors, chromatin regulators, phosphatases and kinases, totalling 2244 clones. I measured *pcco1::gfp* expression upon ARD exit using the copas biosorter- an apparatus that allows a fluorescent analysis of whole worm populations, thereby strengthening the statistical significance of the results (Figure 10C). The *pcco1::gfp* expression on RNAi was quantitated relative to the *pcco1::gfp* expression on empty vector L4440 control. RNAi samples with reduction in relative expression <0.5 or an increase in expression >1.8 were considered as candidates. I identified a total of 38 candidates, of which 32 showed downregulation and 6 candidates showed upregulation of *pcco-1::gfp* expression, presumably identifying positive and negative regulators of mitochondrial function upon ARD exit (Figure 10D).

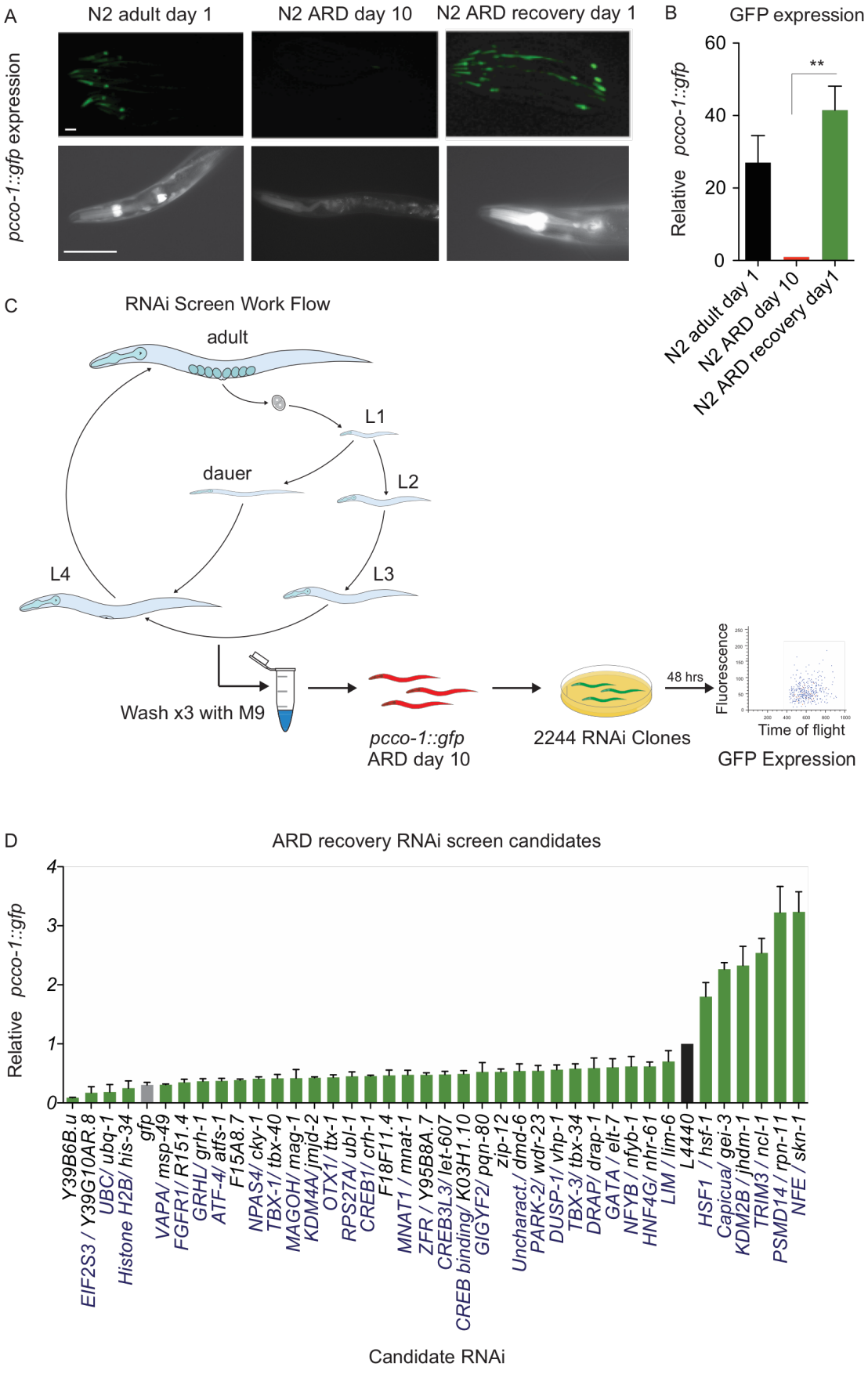


Figure 10: ARD recovery as a tool for identifying novel mitochondrial

regulators. A) microscopic images of *pcco-1::gfp* N2 day 1 adult, N2 day 10 ARD, N2 ARD recovery (scale bar 75 μm). B) *pcco-1::gfp* expression as measured by biosorter shows a decrease in expression under ARD and an increase upon ARD recovery (n=3, error bar indicating SEM, t. test ** p<0.01). C) RNAi screen workflow with a total of 2244 RNAi clones, *pcco1::gfp* expression upon ARD exit measured using the biosorter. D) Relative *pcco1::gfp* expression on RNAi with respect to empty vector L4440 gives a total of 38 candidates, of which 32 showed < 0.5 and 6 showed > 1.8-fold change, n=minimum 3. Optimisation for the screen and the transcription factor RNAi library screen was performed during my Master's thesis (ref master thesis: Mitochondrial Biogenesis in *C. elegans* ARD, R. Tharyan 2013).

Notably, among the 38 candidates we identified a subset of factors already implicated in regulating mitochondrial function. These included transcriptional regulators such as *crh-1*, *let-607* and *K03H1.10,* homologs to CREB, CREB3L3, and CREB-binding protein, respectively. Interestingly we also identified mitochondrial unfolded protein response transcription factor *atfs-1* (Haynes et al. 2007; Haynes & Ron 2010; Nargund et al. 2012; Nargund et al. 2015; Pellegrino et al. 2013; Baker & Haynes 2011), *jmjd-2* an epigenetic factor whose paralogs *jmjd-1* and *jmjd-3.1* are linked to mitochondrial function (Merkwirth et al. 2016) and *wdr-23,* a negative regulator of *skn-1*/Nrf-2. Among the upregulated candidates we identified *skn-1*/Nrf-2 and heat shock factor *hsf-1* both known to be linked to mitochondrial regulation and function (Palikaras et al. 2015; Kim et al. 2016)) (Figure 11A). In general, most candidates are evolutionarily conserved and have mammalian/human homologs (Figure 11B). The identification of known factors validates the approach and implies that novel molecules identified from the screen could play a significant role in mitochondrial regulation.

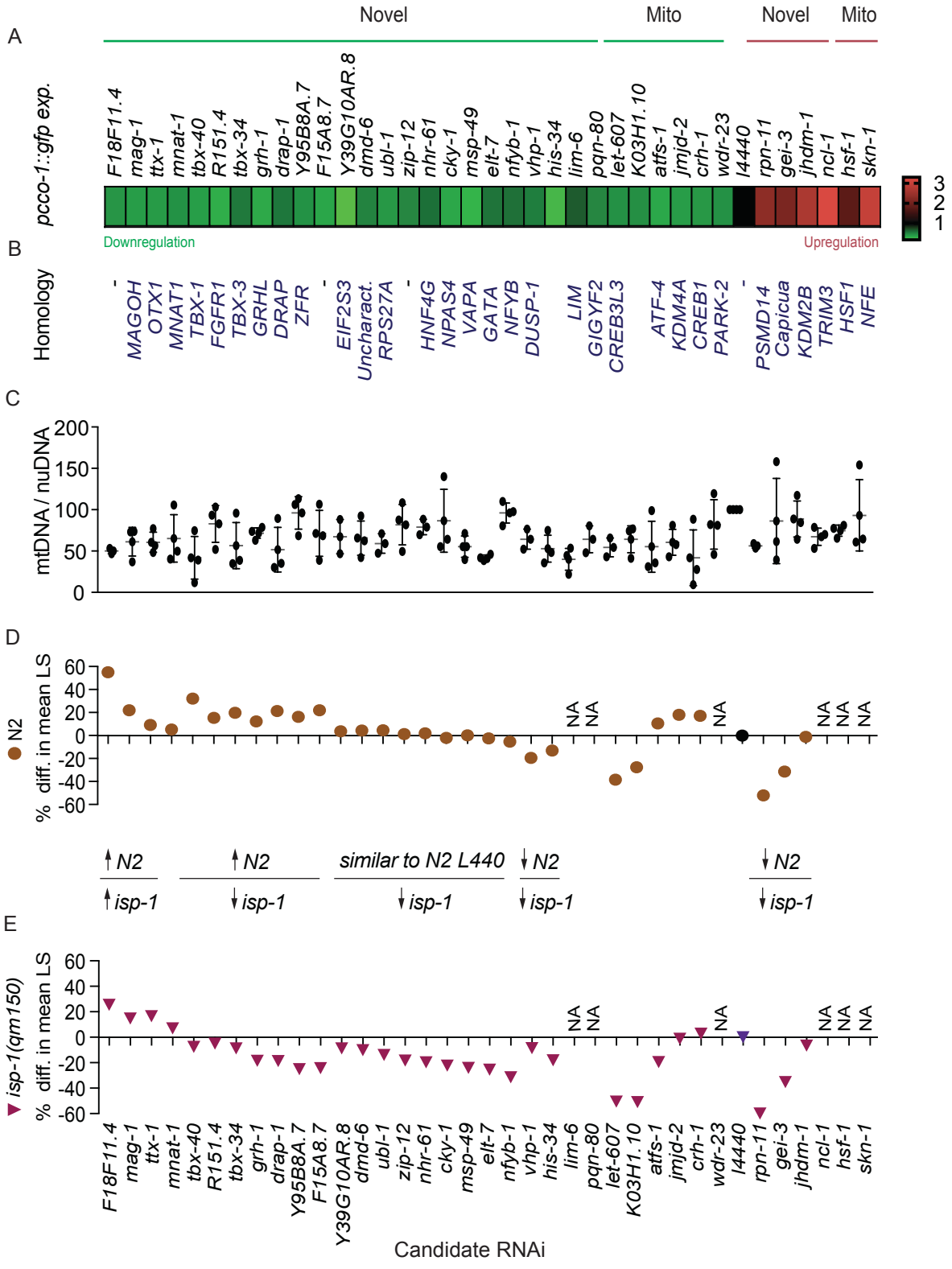


Figure 11: Characterization of RNAi screen candidates. A) Heat map representation of 38 candidate genes identified from the RNAI screen for modulators of *pcco-1::gfp* expression upon ARD recovery. Candidates with lower (green) and higher (red) relative *pcco-1::gfp* expression manually clustered as novel and known mitochondrial factors. B) Respective

mammalian/human homologs of RNAi candidates. C) Relative mtDNA levels of wildtype worms on RNAi knockdown of candidates. RNAi treatment from egg on to late L4 stage (n=3, t. test: refer appendix table 4 for statistics). D) N2 and E) *isp-1(qm150)* percentage change in relative mean lifespan upon RNAi knockdown of candidate genes from egg on in comparison to RNAi control bacteria (n=1, Mantel-Cox Log Rank test: refer appendix table 5 for statistics).

7.2.2 Mitochondrial DNA content upon candidate knockdown.

To examine if the knockdown of the candidates affects mtDNA copy number, I measured the mitochondrial DNA (mtDNA) levels upon knockdown by candidate RNAi from egg on to late L4 of wildtype worms. To do so I compared the amount of mtDNA to the amount of nuclear DNA (nuDNA). I observed a reduction in the mtDNA/nuDNA ratio upon knockdown by RNAi of several candidates: namely *let-607, ubl-1, msp-49, his-34, elt-7, lim-6, F18F11.4, drap-1,* CREB binding protein *crh-1,* T-box transcription factors *tbx-40 and tbx-34* showed ca. 40% reduction in mtDNA/nuDNA ratio relative to L4440 empty vector control. *pqn-80, F15A8.7, mag-1, vhp-1, KO3H1.10, dmd-6, jmjd-2, mnat-1, grh-1* and *Y39G10AR.8* showed about 30% reduction in the relative levels of mtDNA/nuDNA levels (Figure 11C). These changes may reflect either an imbalance of nuclear and mitochondrial DNA copy number, or changes in the proportion of somatic and germline cells.

7.2.3 Effect on longevity upon knockdown of candidates.

Mitochondrial impairment has been shown to regulate longevity as seen with mitochondrial mutants *nuo-6, clk-1* and *isp-1* (Yang & Hekimi 2010b; Miyadera et al. 2001; Feng et al. 2001b). We speculated that our candidates might influence the lifespan extension of mitochondrial mutant *isp-1(qm150)* or wildtype lifespan itself. Indeed, a handful of the candidates identified as positive regulators of *pcco-1::gfp* expression, namely, *crh-1, jmjd-2, mag-1, wdr-23,* are also already known to be involved in the determination of lifespan (Ni et al. 2012; Mair, Morantte, Rodrigues, Manning, Montminy, Reuben J. Shaw, et al. 2011). Similarly, among the

group of potential negative regulators, we also found candidates known to extend lifespan, namely *skn-1, hsf-1* (Robida-Stubbs et al. 2012; Onken & Driscoll 2010; Tullet et al. 2008; Salminen & Kaarniranta 2012; Palikaras et al. 2015; Nishizawa et al. 1999; Steinkraus et al. 2008; Ackerman & Gems 2012)*, ncl-1* (Tiku et al. 2016) and *gei-3 (*Oezlem Karalay unpublished).

I performed lifespan analysis of wildtype (Figure 11D) and *isp-1(qm150)* strains (Figure 11E) treated with RNAi knockdown of candidates from egg on. Note that RNAi knockdown during the L3-L4 transition has shown to be essential for attaining lifespan benefits induced by mitochondrial impairment (Dillin et al. 2002; Rea et al. 2007). The percentage difference in relative mean lifespan of wildtype and *isp-1(qm150)* upon knockdown candidates was quantitated in comparison to empty vector L4440. As shown previously, I observed an increase in the lifespan of wildtype upon knockdown of *crh-1* and *jmjd-2.* Additionally knockdown of UPR^{mt} transcription factor *atfs-1* partially reduced the lifespan extension of mitochondrial mutant *isp-1(qm150)* (Haynes et al. 2010).

The other candidates can be clustered according to their effect on longevity in wildtype and mitochondrial mutant *isp-1(qm150).* The first cluster includes candidates whose knockdown, increased longevity of both wildtype and *isp-1(qm150)*, namely *F18F11.4, mag-1* and *ttx-1.* These factors probably regulate longevity independent of the known mitochondrial longevity pathway. The second cluster includes only one candidate, *mnat-1,* whose knockdown had neither an effect on wildtype nor on *isp-1* lifespan. The third cluster consists of all candidates that enhance wildtype longevity but reduce mitochondrial longevity, namely *R151.4, tbx-40, tbx-34, grh-1, drap-1, Y95B8A.7,* and *F15A8.7*. These factors might cause partial mitochondrial impairment and activation of stress responses leading to lifespan extension in wildtype; knockdown of these factors in the mitochondrial mutant background might disrupt the threshold effect

essential for modulating mitochondrial longevity. A fourth cluster consists of factors such as *Y39G10AR.8/eIF2gamma, dmd-6, ubl-1, zip-12, nhr-61, cky-1, msp-49, elt-7* and *nfyb-1*. Knockdown of these candidates led to a partial reduction in mitochondrial longevity without affecting wildtype lifespan. Conceivably, they might mediate processes essential for life extension induced upon mitochondrial impairment, and were of greatest interest. Knockdown of candidates in a fifth cluster led to lifespan reduction in both wildtype and *isp-1(qm150)* background. Among candidates in this cluster are *vhp-1* and *his-34* and the CREB factors *let-607/CREB3L3 and KO3H1.10/CBP.* Knockdown of the latter two reduced lifespan extension of *isp-1(qm150*) by around 40% and wildtype lifespan by 30-40%. Moreover, we found the negative regulators of *pcco-1::gfp* expression, *gei-3* and *rpn-11,* to be in this group. Note that these lifespan data are preliminary and further need to be validated (refer appendix table 5 for statistics).

7.2.4 Transcriptional alteration of mitochondrial genes.

To understand the effect of screen candidates on the transcription level of different mitochondrial genes I surveyed changes in mRNA levels of 24 representative mitochondrial genes upon candidate RNAi knockdown. The genes surveyed included those encoding mitochondrial functions in OXPHOS, replication, transcription, translation, UPR^{mt}, antioxidant defence, oxidative stress response and fission/fusion (refer appendix table 2) (Figure 12A).

For ease of analysis, I clustered transcript levels into a heat map (refer methods section). Candidates grouped into three main clusters: the first cluster showed lower, the second similar, and the third higher transcript levels in comparison to L4440 control. The candidates further clustered to sub-groups, with an initial group including *pqn-80, msp-49, ubl-1* and *let-607* showing a lower level of *ant1.4,* which mediates the exchange between mitochondrial ATP with ADP in cytosol. This sub-group also

showed increased mRNA levels of genes for stress response and fission.

skn-1, Y95B8A.7 and *zip-12* knockdown gave higher transcript levels for mitochondrial encoded OXPHOS genes, *ant1.4* translocase and genes necessary for mitochondrial replication. These genes also showed a reduction in mRNA levels for fission genes. *skn-1* and *Y95B8A* knockdown also exhibited a decrease in UPR^{mt} associated genes, in particular *dnj-10*. Knockdown of *gei-3* led to increased mRNA levels for *ant1.4* translocase*,* mitochondrial single strand binding protein *mtss-1* and antioxidant defence genes. This shows a possibility that knockdown of *gei-3* disrupts the protein-folding environment and internally activates mitochondrial defence mechanisms such as UPR^{mt} and antioxidant defence. *nfyb-1* knockdown also upregulated mRNA levels of *ant1.4* translocase, UPR^{mt} associated genes, in particular *dnj-10* and antioxidant defence genes. Knockdown of *his-34* similarly induced activation of UPR^{mt} and antioxidant defence.

Knockdown *elt-7* and *lim-6* decreased *ant1.4* translocase mRNA levels, although a few changes were observed for other tested genes none of the regulation reached significance. A mild increase in mRNA levels of mitochondrial single strand binding protein *mtss-1* and UPR^{mt} associated genes *dnj-10* was seen on knockdown of *R151.4* and *hsf-1*. F18F11.4 knockdown increased UPR^{mt} genes along with activation of the antioxidant gene *sod-3.* Knockdown of the nuclear hormone receptor *nhr-61* shows a rather broad effect: *ant1.4* translocase mRNA levels were down, higher levels of mitochondrial DNA polymerase and RNA polymerase, and additionally levels of stress response genes, mainly of those involved in antioxidant defence and oxidative stress response, were increased. Knockdown of *mag-1* lowered *ant1.4* mRNA levels, increased levels of mitochondrial DNA polymerase, RNA polymerase and oxidative stress response. *ncl-1* knockdown had lower *ant1.4* levels and slightly higher levels of the UPR^{mt} gene *hsp-6.* Knockdown of *drap-1, vhp-1* and *jhdm-1* had similar expression patterns as the empty vector control *L4440.*

Knockdown of *drap-1 and vhp-1* resulted in slightly lowered *ant1.4* mRNA levels. *jhdm-1* knockdown induced mild reduction in mRNA levels for mitochondrial single stranded binding protein, DNA polymerase and RNA polymerase.

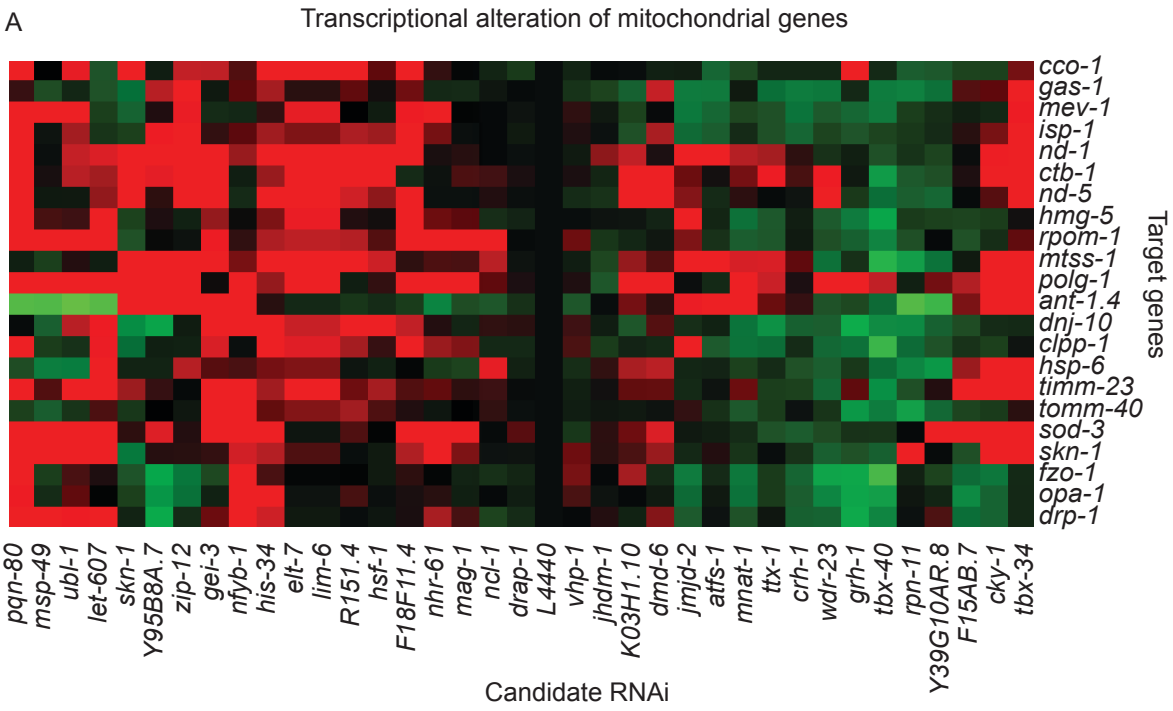


Figure 12: Transcriptional alteration of mitochondrial genes. A) Heat map representing the transcript levels of mitochondrial gene upon RNAi knockdown of candidates of N2 worms from egg on for 48 hours (late L4 stage) n=3. The transcript levels of samples are normalized to empty vector L4440 and *act-1*. For clustering we used average linkage with Manhattan distance technique. Red representing higher and green representing lower level of mRNA relative to L4440 (refer methods).

Knockdown of *dmd-6* and CREB binding protein *KO3H1.10* led to higher levels of mitochondrial DNA polymerase, rather lower levels of UPR^{mt} associated genes *clpp-1* and *dnj-10* but slightly higher level of UPR^{mt} gene *hsp-6*, antioxidant defence and stress response genes. Knockdown of histone demethylase *jmjd-2* showed a higher level of mitochondrial DNA encoded OXPHOS genes but a slightly lower level of nuclear encoded OXPHOS genes. Moreover, knockdown of *jmjd-2* and the UPR^{mt} transcription factor *atfs-1* showed a similar expression pattern including

higher mRNA level for mitochondrial single strand binding protein *mtss-1* and *ant1.4* translocase. Knockdown of *mnat-1, ttx-1,* CREB binding protein *crh-1* showed a slightly higher level of mitochondrial DNA polymerase and single strand binding protein *mtss-1,* but the mRNA level for multiple mitochondrial genes were rather low. Similarly, knockdown of *wdr-23*, *grh-1* and T-box transcription factor *tbx-40* resulted in lower mRNA levels for multiple mitochondrial genes with a slightly higher expression of mitochondrial DNA polymerase, which could be compensatory mechanism. *rpn-11, Y3G10AR.8 and F15A8.7* also showed comparable pattern but had a higher anti-oxidant defence mechanism. Knockdown of *cky-1* and T-box transcription factor *tbx-34* demonstrated a higher mRNA level of mitochondrial DNA polymerase, single strand binding protein *mtss-1* and *ant1.4* translocase. *cky-1* and *tbx-34* knockdown activated stress response pathways such a UPR^{mt} seen by higher *hsp-6* mRNA level, also higher antioxidant defence and oxidative stress response. Taken together the RNAi characterization of the candidates obtained from ARD recovery screen suggest that I have identified some potential modulators of mitochondrial regulation and function. I plan to perform further analysis with mutants of promising candidates to investigate the role of in mitochondrial function.

7.3 Chapter 3: NFYB-1 a novel mitochondrial regulator

7.3.1 NFYB-1 regulated mitochondrial function.

I identified *nfyb-1* as a potential candidate from the RNAi screen and focused on this factor after a more in-depth analysis revealed it to be a potent regulator of mitochondrial function. As observed in the original screen, RNAi knockdown of *nfyb-1* resulted in a decrease in mitochondrial marker *pcco-1::gfp* expression. I further confirmed this phenotype using an existent null deletion mutant of *nfyb-1(cu13)*. *nfyb-1* also affected the expression of another mitochondrial gene, *mtss-1 (ppar2.1::gfp)*, which encodes the mitochondrial single strand binding protein in *C. elegans* (ref master thesis: Mitochondrial Biogenesis in *C. elegans* ARD, R. Tharyan 2013).

I next wondered whether changes in mitochondrial marker gene expression were specific to ARD recovery, or altered under other conditions of high energy demand such as higher temperature. I observed that *nfyb-1(cu13*) regulated *pcoo-1::gfp* and *pmtss-1::gfp* at 25° C (Figure 13A-D), as well as normative temperature of 20° C under ad libitum conditions (Figure 13E and F), indicating a general role of NFYB-1 in regulating mitochondrial function even at a basal level.

Given the observed changes in mitochondrial marker expression, I next asked whether *nfyb-1(cu13)* impacts mitochondrial respiration. To address this, I measured oxygen consumption under ad libitum conditions and upon ARD recovery in *nfyb-1(cu13)* mutants. Consistent with an important role, I observed that under both conditions *nfyb-1* loss reduced oxygen consumption (Figure 14A). Furthermore *nfyb-1* mutation reduced oxygen consumption in the germline deficient *glp-4(bn-2)* background as well (Figure 14B), indicating a function of *nfyb-1(cu13)* in promoting respiration in both germline and soma.

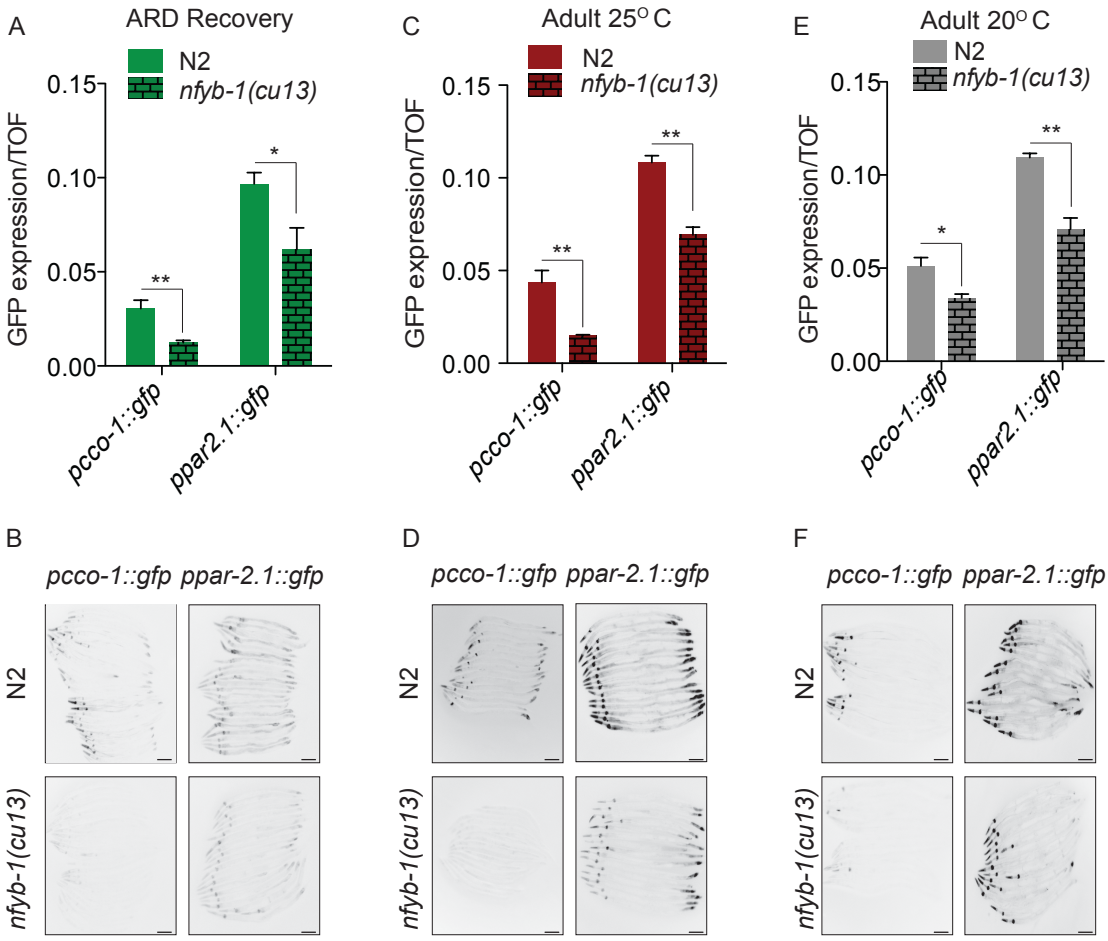


Figure 13: NFYB-1 regulates mitochondrial marker expression. *nfyb-1(cu13)* regulates expression of mitochondrial markers *pcco-1::gfp and ppar2.1::gfp (pmtss-1::gfp)* under higher energy demand conditions such as ARD day 1 recovery (A and B), adult day 1 higher temperature 25° C (C and D) and day 1 ad libitum (E and F). Biosorter measurement (A, C and E) (n=3, error bar indicates SEM, anova test ** p<0.01, * p<0.05). Microscopic images (B, D and F) (scale bar 75μm).

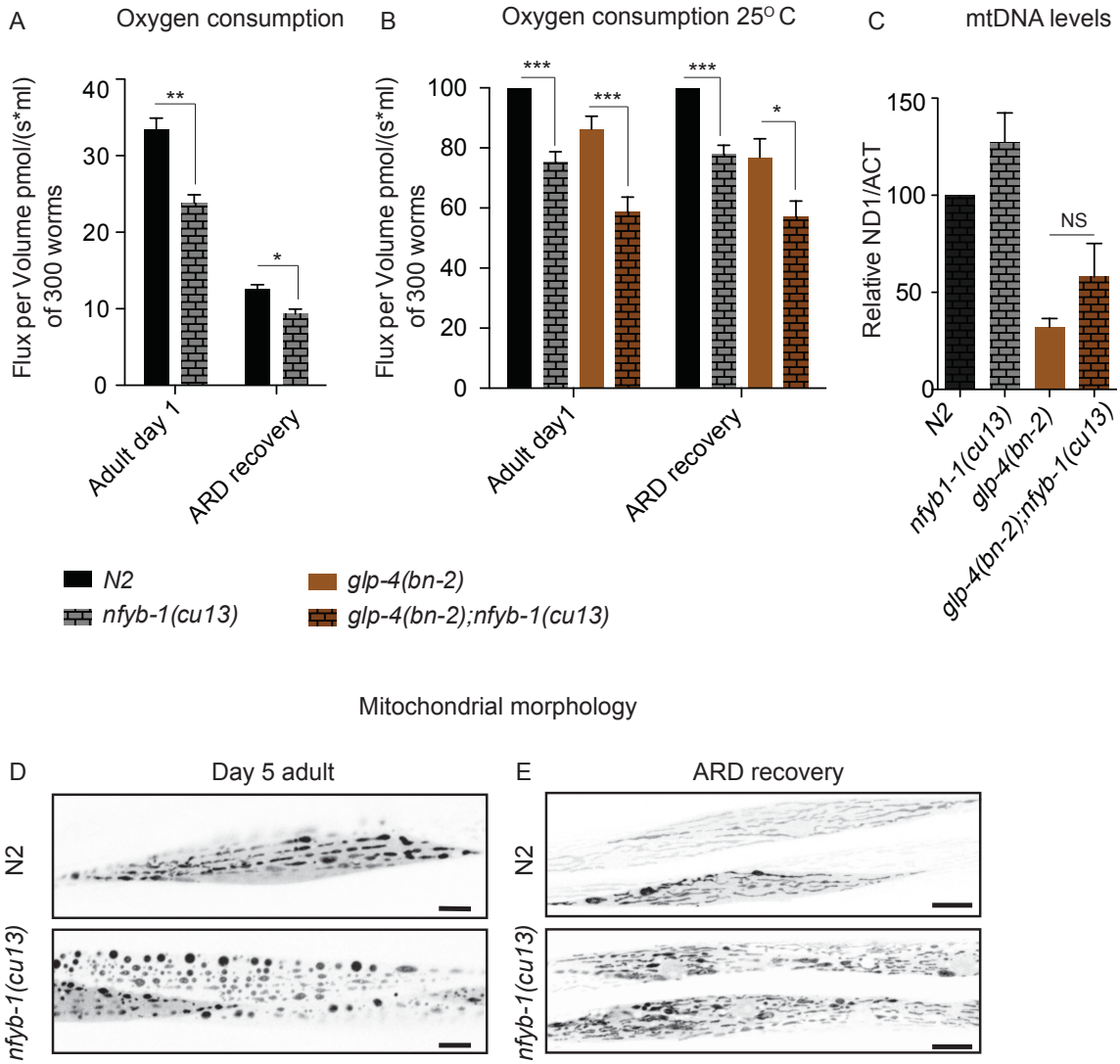


Figure 14: NFYB-1 regulates oxygen consumption and mitochondrial morphology. A) *nfyb-1(cu13)* downregulates oxygen consumption under of ad libitum day 1 and upon ARD recovery day 1 in comparison to N2 (n=3, error bar indicates SEM, anova test, * p<0.5, ** p<0.01, ***p<0.001). B) *nfyb-1(cu13)* downregulates oxygen consumption under ad libitum day 1 and upon ARD recovery also in germline less *glp-4(bn-2)* background (n=3, error bar indicates SEM, anova test, * p<0.5, ** p<0.01, ***p<0.001). C) relative mtDNA measurement shows an upregulation in mtDNA levels in *nfyb-1(cu13)* upon ARD recovery (n=3, t. test, not significant). Mitochondrial morphology of *pmyo-3::gfp(mt)* expressed in the body wall muscle measured using confocal microscopy, loss of NFYB-1 leads to fragmented mitochondria in D) day 5 reproductive adult (n=2, >15 worms per condition), E) upon ARD recovery day 1 in comparison to wildtype under the same conditions (n=3, >15 worms per condition, scale bar 5μm).

I further examined the regulation of mitochondrial copy number in *nfyb-1(cu13)* and specifically in somatic tissues using germline deficient *glp-4(bn-2)* (Figure 14C*).* Unexpectedly, I found that mtDNA levels were increased in *nfyb-1(cu13)* in comparison to wildtype, perhaps reflecting a compensatory mechanism in response to mitochondrial dysfunction upon NFYB-1 loss. To better understand the role in mitochondrial biology, I next asked if *nfyb-1* influenced mitochondrial morphology and mtDNA levels. Whereas wildtype *C. elegans* day 1 adults formed healthy tubular mitochondrial structures, older adults show mitochondrial fragmentation with age under ad libitum conditions (Regmi et al. 2014). Under ARD, worms formed highly fused mitochondria, which, upon recovery were remodeled into more tubular structures similar to young adults, as visualized with *myo-3::gfp(mt)* expressed in body wall muscle. Consistent with a perturbation of mitochondrial dynamics, loss of *nfyb-1*, resulted in more fragmented mitochondria structures at ad libitum (Figure 14D) and upon ARD recovery (Figure 14E) in comparison to wild type.

As I observe that mitochondrial regulation upon loss of *nfyb-1* is not condition specific, I went ahead and performed transcriptome RNA sequencing analysis for *nfyb-1(cu13)* in comparison to wildtype. RNA sequencing analysis was performed under conditions of ARD recovery, ad libitum day 1, ad libitum late L4. I was particularly interested in the overlap of factors under above mentioned conditions, as those factors might shed light on the mechanism of how *nfyb-1* modulates mitochondrial regulation and function. There are 541 factors common to conditions of ARD recovery, ad libitum day 1, ad libitum late L4 (Figure 15A). Interestingly the common factors showed an enrichment particularly for ER associated genes and immune response genes. Also, a slight enrichment for metabolic processes such as organic cyclic, nitrogen and nucleic acid metabolic process (Figure 15B). This sheds light on downstream factors regulated upon NFYB-1. As our data so far clearly showed pivotal role of

NFYB-1 in regulating mitochondrial function. Additionally, transcriptomic analysis showed enrichment for ER associated differentially expressed genes. This points at a potentially role of NFYB-1 in regulating gene expression to maintain homeostasis of different intra cellular organelles.

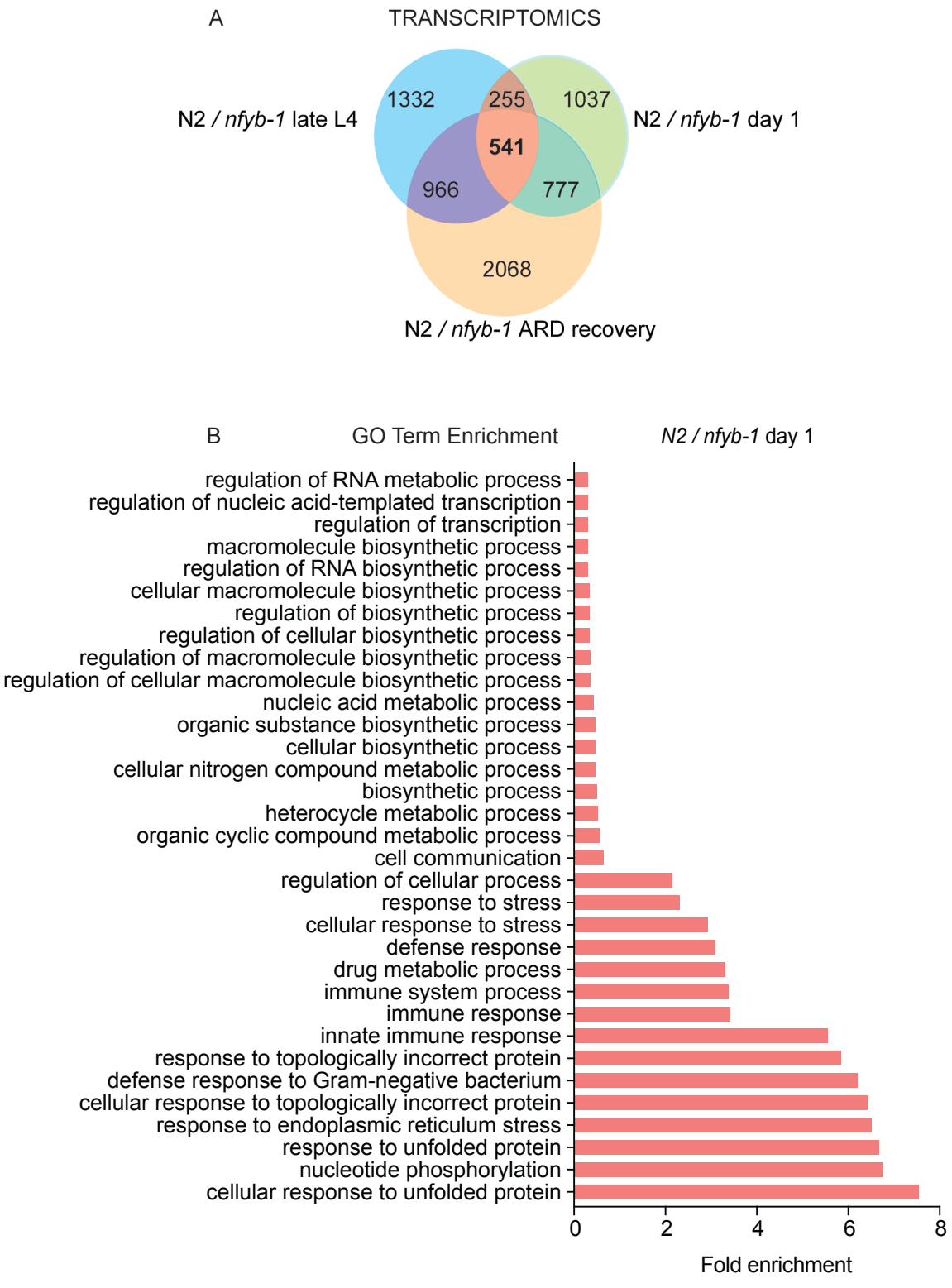


Figure 15. NFYB-1 regulates transcripts of metabolic genes. RNA seq analysis of ARD recovery, ad libitum day 1, ad libitum late L4 of *nfyb-1(cu13)* in comparison to N2 (n=3, significance $p<0.05$). A) Venn diagram representation shows an overlap of 541 common factors (significance $p<0.05$). B) GO term analysis of common factors shows an enrichment for metabolic genes and ER associated genes. RNA seq analysis was performed at Max-Plank Genomic Center, Koln and the bioinformatics analysis on RNA seq data obtained was performed by the bioinformatics core faculty at Max-Plank for Biology of ageing.

7.3.2 NFYB-1 regulates longevity induced by mitochondrial impairment.

Next, I addressed the question whether NFYB-1 regulates longevity via the major known longevity pathways including reduced insulin/IGF signaling, dietary restriction mediated longevity, germline signaling and longevity prompted by mitochondrial impairment.

Reduced insulin signaling (IIS pathway) (Cynthia Kenyon et al. 1993) is known to extend lifespan across taxa. To analyze the role of NFYB-1 in longevity modulated by insulin signaling I crossed null mutant *nfyb-1(cu13)* to *C. elegans* insulin/IGF receptor *daf-2(e1370).* I observed that upon NFYB-1 loss *daf-2(e1370)* longevity was somewhat reduced but not brought back to wildtype (Figure 16A), indicating that NFYB-1 might partially regulate longevity induced by insulin/IGF signaling. Another well characterized longevity model is dietary restriction (DR). I examined the role of NFYB-1 in longevity induced by DR using the genetic DR model *eat-2(ad465)* (Panowski et al. 2007). Upon NFYB-1 loss, DR induced longevity of *eat-2(ad465)* was abolished. In fact, *eat-2(ad465); nfyb-1(cu13)* double mutants were short lived and sick in comparison to wildtype, suggesting a synthetic phenotype (Figure 16B).

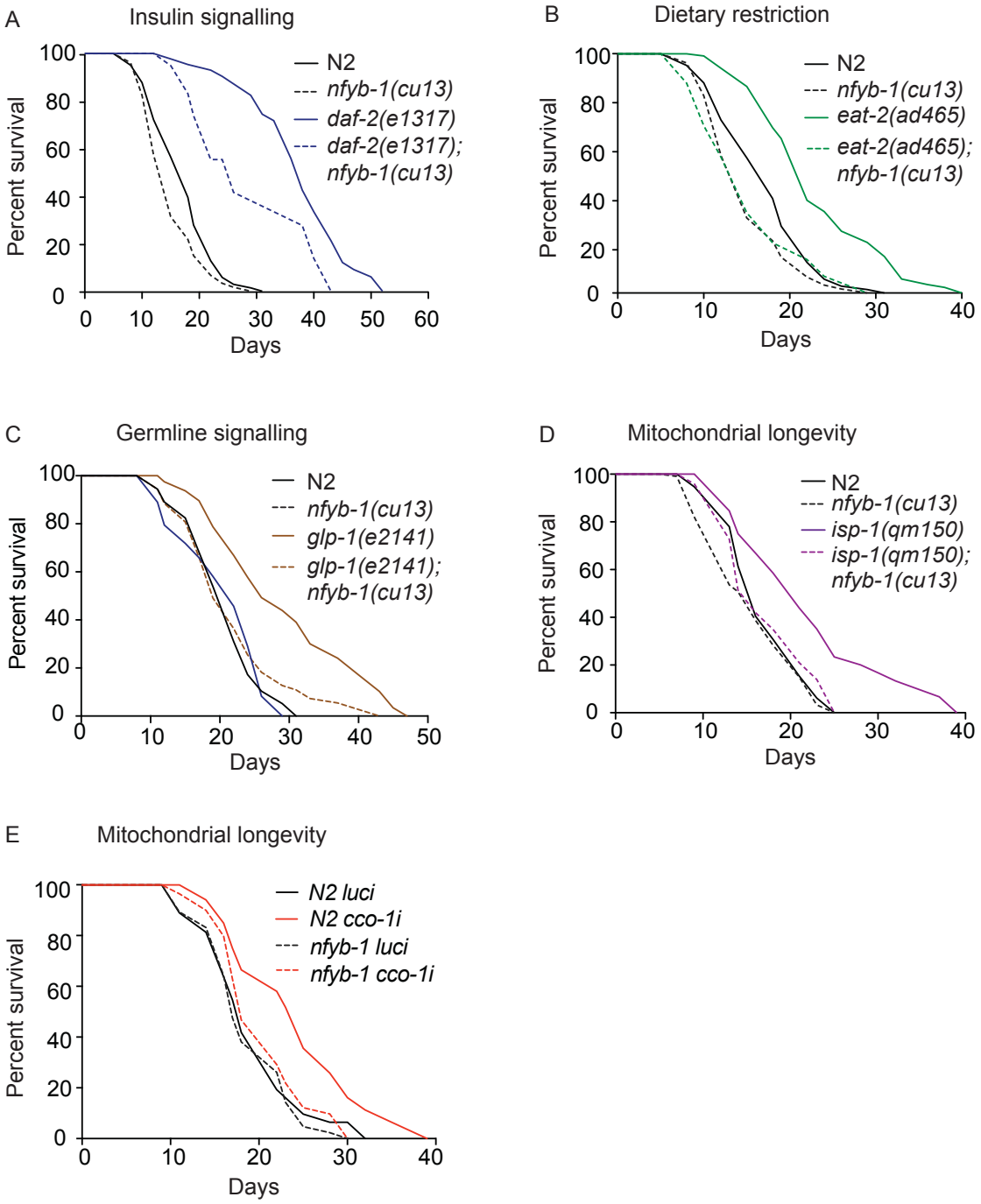


Figure 16: NFYB-1 regulates mitochondrial longevity. A) *nfyb-1(cu13)* partially regulates longevity induced by loss of insulin/IGF receptor *daf-2(e1317)* n=3. B) *nfyb-1(cu13)* abolishes longevity and show synthetic phenotype with DR model *eat-2(ad465)* n=3. C) *nfyb-1(cu13)* reduces longevity induced by germline signaling induced longevity of *glp-1(e2141)* n=2. D) *nfyb-1(cu13)* abolishes longevity induced by mitochondrial impairment in *isp-1(qm150)*, n=3 and E) RNAi knockdown of *cco-1*, n=2, (Mantel-Cox Log Rank test: refer appendix table 5 for statistics).

Germline signaling is another well-established longevity pathway (Hsin & Kenyon 1999). Germline removal by laser microsurgery or by genetic ablation as seen in *glp-1(e2141)* worms, results in a robust extension of life. *glp-1* encodes the notch transmembrane receptor, and growth at 25° C prevents the development of germline. I crossed the *nfyb-1(cu13)* to *glp-1(e2141)* and performed lifespan analysis. I saw that *nfyb-1* mutation largely reduced *glp-1(e2141)* longevity (Figure 16C).

Mitochondrial impairment leads to lifespan extension by activating mitochondrial stress response pathways. Longevity induced by mitochondrial impairment can be achieved by genetic mutations such as in case of *isp-1(qm150),* an iron sulfur protein in mitochondrial complex III and loss of *isp-1* leads to impaired electron transport function, activation of stress response and longevity. Mitochondrial impairment induced longevity can also be achieved by RNAi mediated knock down of *cco-1,* cytochrome c oxidase in complex IV (Schulz et al. 2007; Durieux et al. 2011; Jensen & Jasper 2014; Lee et al. 2010; Feng et al. 2001b).

Because of the observed changes in mitochondrial physiology upon loss of NFYB-1, I was particularly interested in the role of NFYB-1 in regulating longevity induced by disruption of mitochondrial function. To examine this, I first performed lifespan analysis using *nfyb-1(cu13); isp-1(qm10)* double mutant. Interestingly I found that upon loss of NFYB-1, longevity of *isp-1(qm10)* was abolished and brought back to that of wildtype (Figure 16D) Similarly *nfyb-1(cu13)* abolished lifespan extension induced by RNAi knockdown of *cco-1* (Figure 16E). Collectively these data indicate that NFYB-1 promotes longevity in several pathways, and most prominently in mitochondrial pathways.

7.3.3 NFYB-1 regulates mitochondrial unfolded protein response (UPR^{mt}) factors.

Reduction of electron chain activity by mutation of *isp-1(qm150)* or RNAi knockdown of *cco-1* leads to lifespan extension by activation of mitochondrial stress response pathways, including the mitochondrial unfolded protein response UPR^{mt} and ROS signalling-induced mitohormesis (Hekimi et al. 2011; Pellegrino et al. 2013). Upon mitochondrial stress, it has been shown that the ubiquitin like molecule UBL-5 and the compass/homeodomain transcription factor DVE-1 translocate into the nucleus along with bZIP transcription factor ATFS-1, and induce the expression of genes which are required for restoring protein homeostasis including mitochondrial heat shock factors HSP-60 and HSP-6. Notably, DVE-1 has also been shown to be essential for longevity in multiple models of mitochondrial impairment genes (Haynes et al. 2007; Haynes & Ron 2010; Nargund et al. 2012; Nargund et al. 2015; Pellegrino et al. 2013; Baker & Haynes 2011)

Because NFYB-1 plays an essential role in longevity induced by *isp-1(qm150)* and RNAi knockdown of *cco-1,* I wondered whether NFYB-1 regulates the mitochondrial stress response and their mediators. I crossed *nfyb-1(cu13)* to the translational reporter *pdve-1::dve-1::gfp,* and saw as expected, that DVE-1 was nuclear localized in the wildtype background upon mitochondrial stress induced by *cco-1* RNAi. In contrast, the nuclear localization of DVE-1 was dramatically reduced in the *nfyb-1* mutant background under basal conditions as well as mitochondrial stress. These observations were further confirmed, upon measuring the *pdve-1::dve-1::gfp* expression using the biosorter (Figure 17A and B) and western blot analysis (Figure 17C and D).

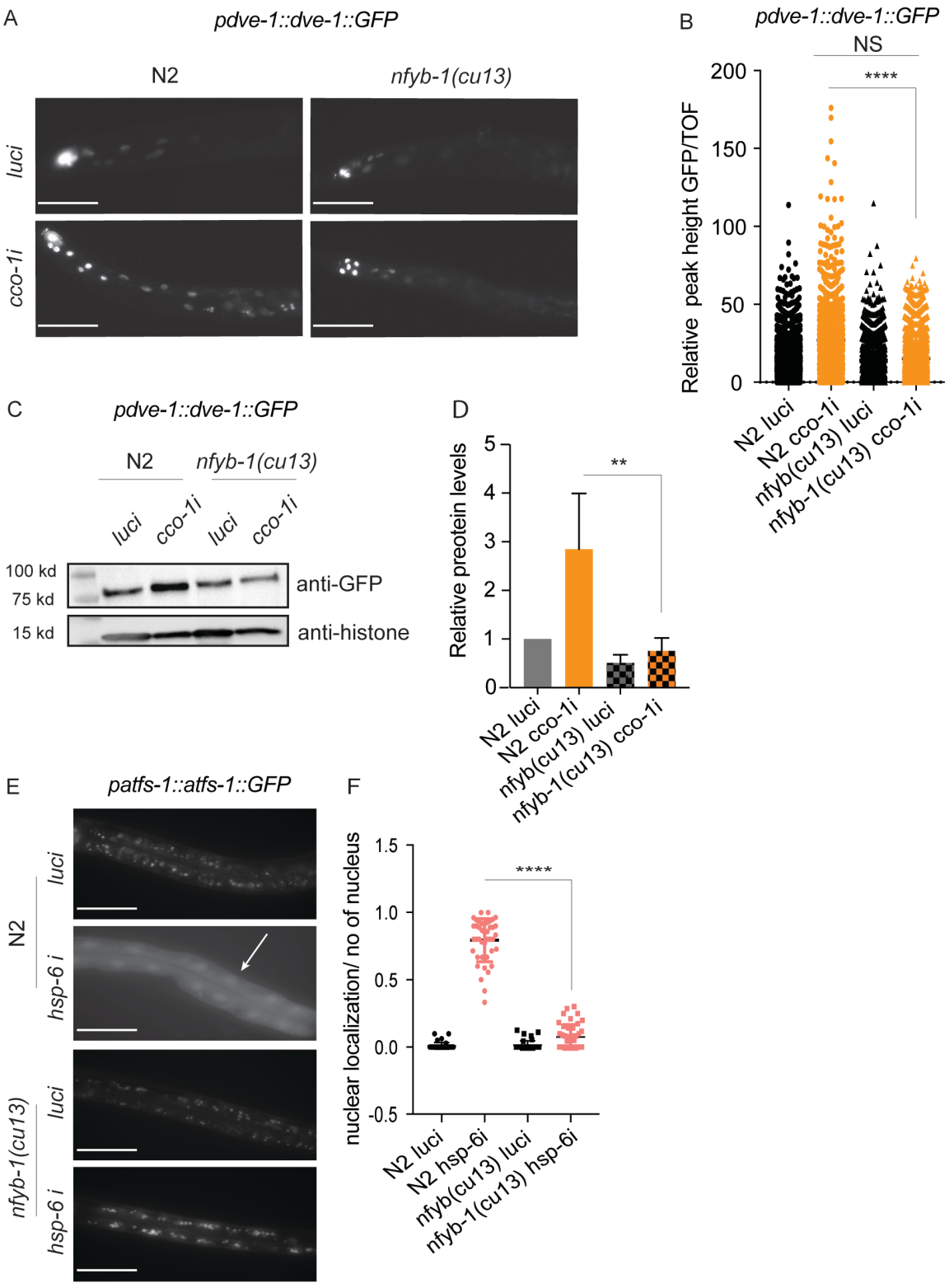


Figure 17: NFYB-1 regulates UPR^{mt} factors. *nfyb-1(cu13)* abolishes the nuclear localization of day 1 *pdve-1::dve-1::gfp* induced upon mitochondrial stress by RNAi knockdown of *cco-1* from egg on A) microscopic images, B)

relative gfp expression measured using biosorter, C) and D) western blot analysis of *pdve-1::dve-1::gfp* (n=3, scale bar 10μm, error bar indicates SEM, anova test, ***p<0.001, ** p<0.01, * p<0.05). E) and F) *nfyb-1(cu13)* abolishes the nuclear localization of *patfs-1::atfs-1::gfp* induced upon mitochondrial stress by RNAi knockdown of *hsp-6i (*L3-L4*)* (n=3, n>20 worms per condition, scale bar 10μm, error bar indicates SEM, anova test, ***p<0.001, ** p<0.01, * p<0.05)

Next, I examined if loss of NFYB-1 regulates the nuclear localisation of UPR^{mt} transcription factor ATFS-1. Upon induction of mitochondrial stress by RNAi knockdown of *hsp-6*, I observed *patfs-1::atfs-1::gfp* nuclear localisation in the intestine cells as seen previously (Haynes & Ron 2010; Nargund et al. 2012; Nargund et al. 2015). However, localisation of ATFS-1 under mitochondrial stress was diminished upon *nfyb-1* mutation (Figure 17E and F).

Since NFYB-1 regulated the nuclear localisation of both DVE-1 and ATFS-1, we further examined if NFYB-1 also regulates the expression of UBL-5 and mitochondrial heat shock factors HSP-6 and HSP-60. As expected, a transcriptional reporter of *ubl-5* showed an increase in expression upon induction of mitochondrial stress by *cco-1* RNAi in the wildtype background; *nfyb-1* mutation did not affect this expression significantly (Figure 18A). I further measured the expression of transcriptional reporters of mitochondrial heat shock factors HSP-6 and HSP-60. Similar to *ubl-5* I saw an increase in expression upon induction of mitochondrial stress by *cco-1* RNAi in the wildtype background, and mutation of *nfyb-1* did not significantly affect induction of these factors (Figure 18B and C). I further examined the protein levels of HSP-60, and confirmed that *nfyb-1* had no effect on induction either under *cco-1* RNAi or *isp-1* mutation (Figure 18D and E). Nor were mRNA transcript levels of these factors affected by *nfyb-1* mutation under mitochondrial stress (Figure 18F).

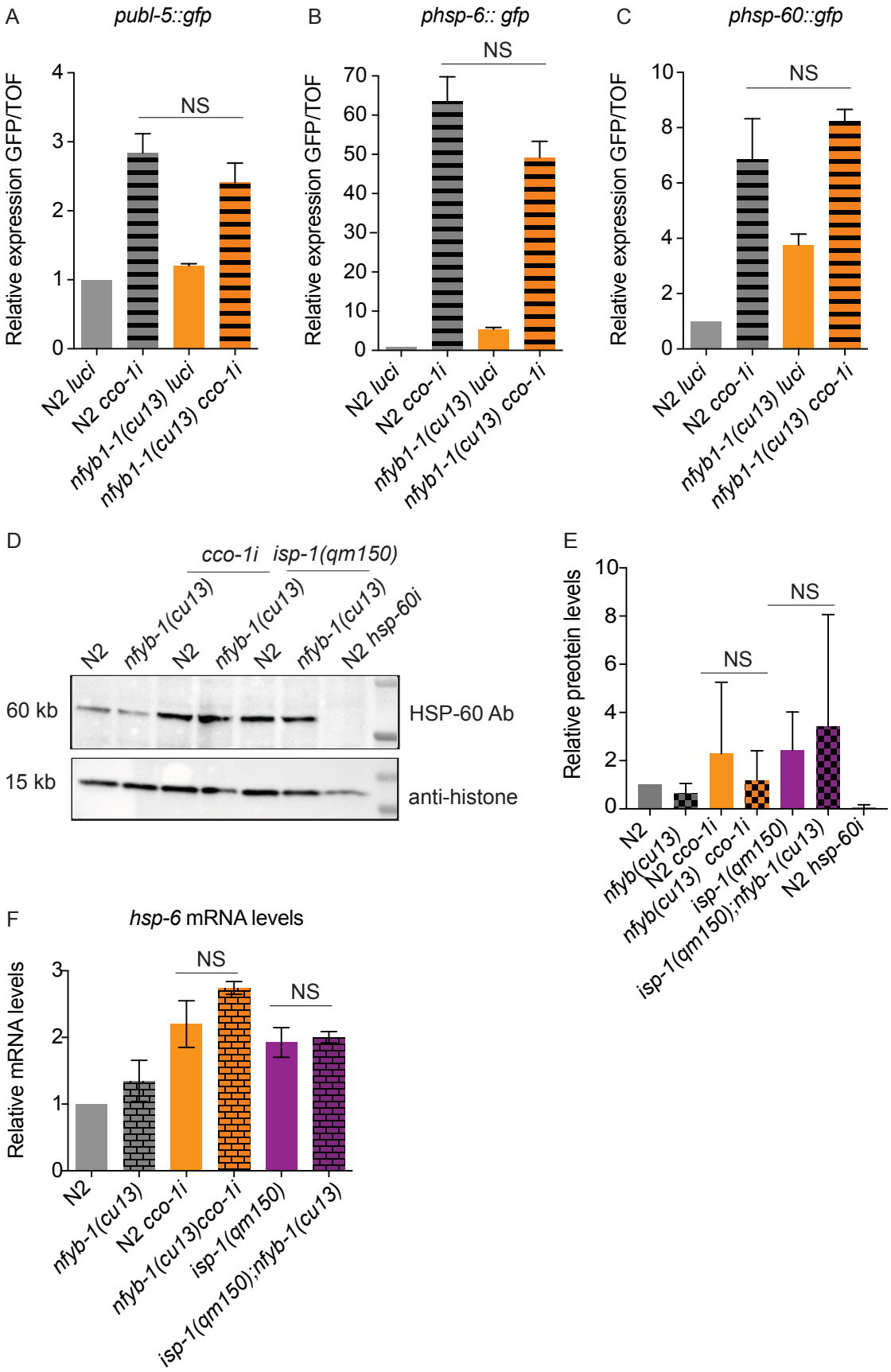


Figure 18: NFYB-1 does not regulate mitochondrial heat shock factors. *nfyb-1 (cu13)* does not affect the induction of mitochondrial stress response

by knockdown of *cco-1* RNAi from egg on to day 1 in transcriptional reporter of A) mitochondrial factor *publ-5::gfp,* mitochondrial heat shock factor B) *hsp-6::gfp,* n=2 C) *hsp-60::gfp,* n=3. D) and E) protein levels of mitochondrial heat shock factor HSP-60, n=5 F) transcript levels of *hsp-6* upon mitochondrial impairment in day 1 *isp-1(qm150)* and RNAi knockdown by *coo-1* (n=3, error bar indicates SEM, anova test).

These data indicate that NFYB-1 significantly regulates the nuclear localisation of DVE-1 and ATFS-1, but not the expression of *ubl-5* or mitochondrial heat shock factors, indicating a role of NFYB-1 in modulating UPR^{mt} factors but not of UPR^{mt} itself.

7.3.4 NFYB-1 regulates mitochondrial to cytosolic stress response.

Recent studies by Kim et al reveal a cross talk between the mitochondrial and the cytosolic stress responses, termed the mitochondrial to cytosolic stress response (MCSR). Typically, during MCSR, induction of mitochondrial stress as induced by RNAi knockdown of the mitochondrial heat shock protein, *hsp-6*, triggers the upregulation of the cytosolic heat shock factor HSP-16*.2*. Induction of MCSR has shown to be mediated by DVE-1, HSF-1 and genes involved in fatty acid synthesis, since their loss dampened the response (Kim et al. 2016). Since NFYB-1 regulates UPR^{mt} factors I hypothesized that NFYB-1 could modulate the MCSR. As previously described I observed that *hsp-6* RNAi led to upregulation of an *hsp-16.2* transcriptional reporter in the wild type background. Interestingly mutation of *nfyb-1* abolished the MCSR, both when measured with a *hsp-16.2* reporter construct (Figure 19A and B) and at the transcript level by qRT-PCR (Figure 19C).

MCSR has been shown to depend on various functions involved in fat synthesis and storage. Furthermore, RNAi knockdown of *hsp-6i* boosts the fat content in the wildtype background as measured by bodiPY staining. I observed that, in contrast to wild type, *nfyb-1* deletion diminished the fat content induced by mitochondrial stress (Figure 19D and E). Taken

together, these observations suggest a broad role of NFYB-1 in regulating MCSR by modulating DVE-1 and fatty acid synthesis.

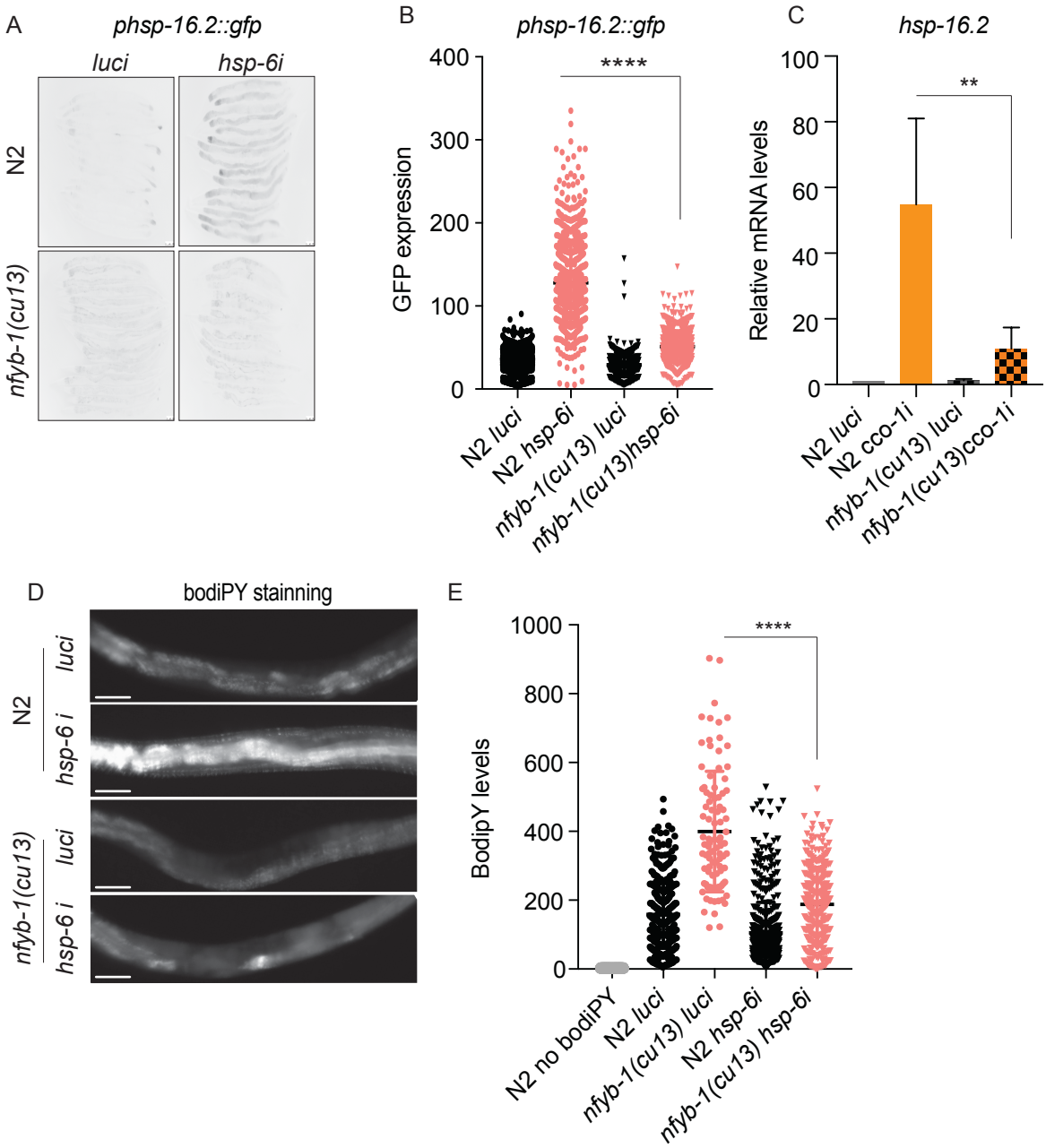


Figure 19: NFYB-1 regulates MCSR. *nfyb-1(cu13)* downregulates the expression of transcriptional reporter of cytosolic heat shock factor *phsp-16.2::gfp* upon mitochondrial stress induced by RNAi knockdown from day to day 3 of *hsp-6* A) microscopic images (scale bar 75μm), B) gfp expression quantitated using biosorter. Or upon mitochondrial stress induced by RNAi knockdown of *cco-1* from egg on to day 1 (n=3, error bar indicates SEM, anova test, ****p<0.0001, ***p<0.001, ** p<0.01, * p<0.05). *nfyb-1(cu13)* downregulates fat content necessary for cytosolic stress response upon mitochondrial stress induced by RNAi knockdown of *hsp-6*

from day 1 to day 3 measured by bodiPY staining D) microscopic images (scale bar 10μm) and E) expression quantitated using biosorter (n=3, error bar indicates SEM, anova test, ****p<0.0001, ***p<0.001, ** p<0.01, * p<0.05).

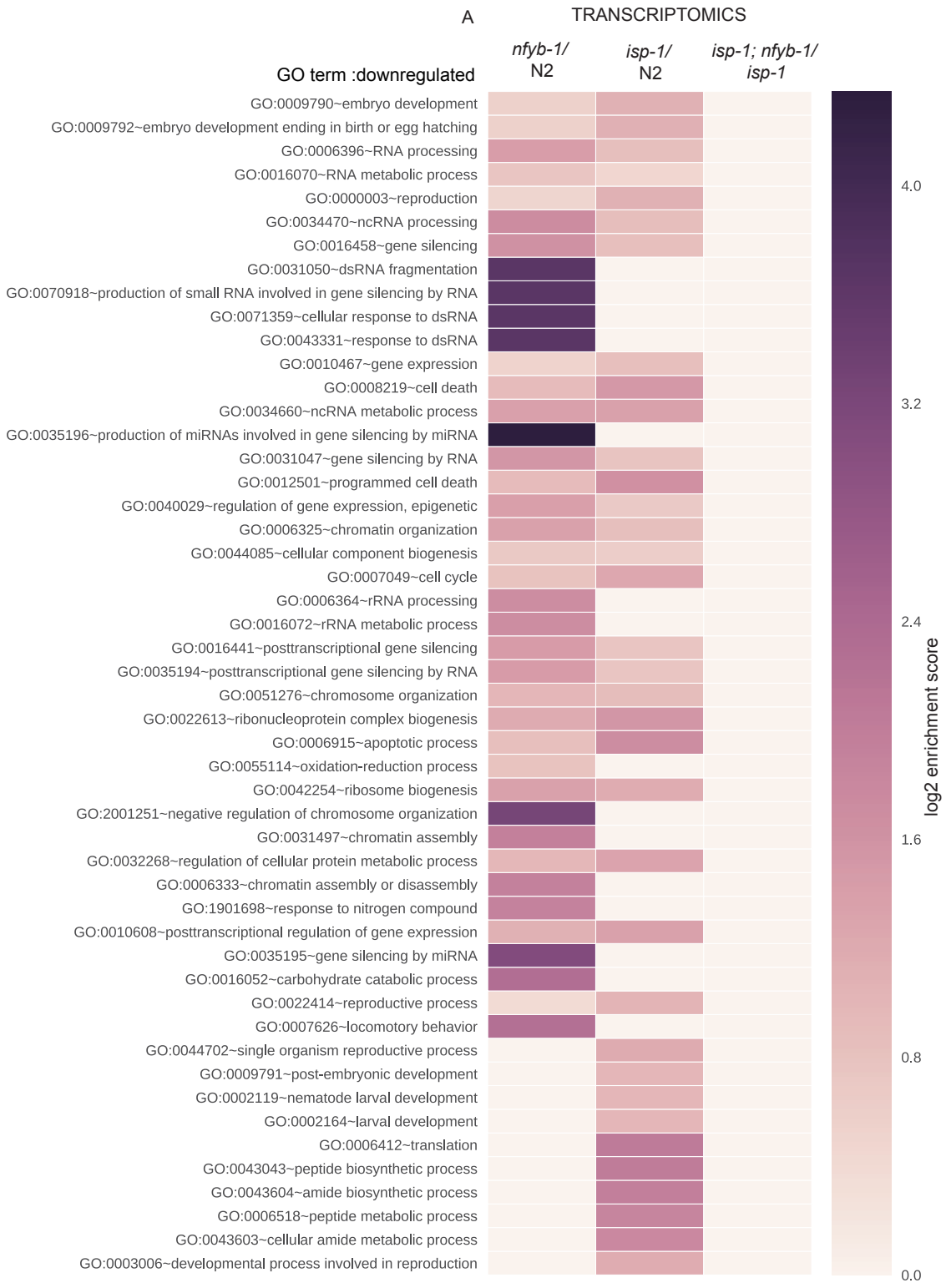
7.3.5 NFYB-1 regulates mitochondrial longevity by modulating ER genes.

To shed light on the downstream process and factors regulated by NFYB-1, I took an unbiased global approach. I performed transcriptomics and proteomics analysis, comparing *N2, nfyb-1(cu13),* as well as the long lived mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* double mutant, which reduces the mitochondrial longevity.

Transcriptomics analyses identified 1027 differentially regulated transcripts between *nfyb-1(cu13)/N2,* 2265 between *N2*/*isp-1(qm150)* and 395 between *isp-1(qm150)/ isp-1(qm150); nfyb-1(cu13*) (significance p<0.05). To gain an overview of enriched genes, I performed GO term enrichment analysis. Among the significantly downregulated genes, no obvious enrichment of GO terms for the *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13)* comparison was apparent. For the *nfyb-1(cu13)/N2* comparison, there was an enrichment for the dsRNA response, microRNA biogenesis, carbohydrate catabolic process and chromosome organisation (Figure 20A). However, transcriptomics analysis clearly showed that NFYB-1 loss regulates a different subset of genes in comparison to ISP-1.

Among the significantly upregulated genes, *nfyb-1*/N2 comparison also revealed an enrichment of axon regeneration and wound healing GO terms. However most strikingly both *nfyb-1(cu13)/N2,* and *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13)* comparisons, revealed a significant enrichment for immune response genes and ER associated genes (Figure 20B). Interestingly theses GO enrichments are similar to the initial transcriptomics analysis performed between *N2/nfyb-1(cu13)* (refer section above Figure

15B) to examine the overlap under conditions of ARD recovery, ad libitum day 1, ad libitum late L4. Again, supporting the idea that NFYB-1 is essential for regulation of intracellular gene expression.



(Figure 20: refer page 88 for figure legend)

(Figure 20: refer page 88 for figure legend)

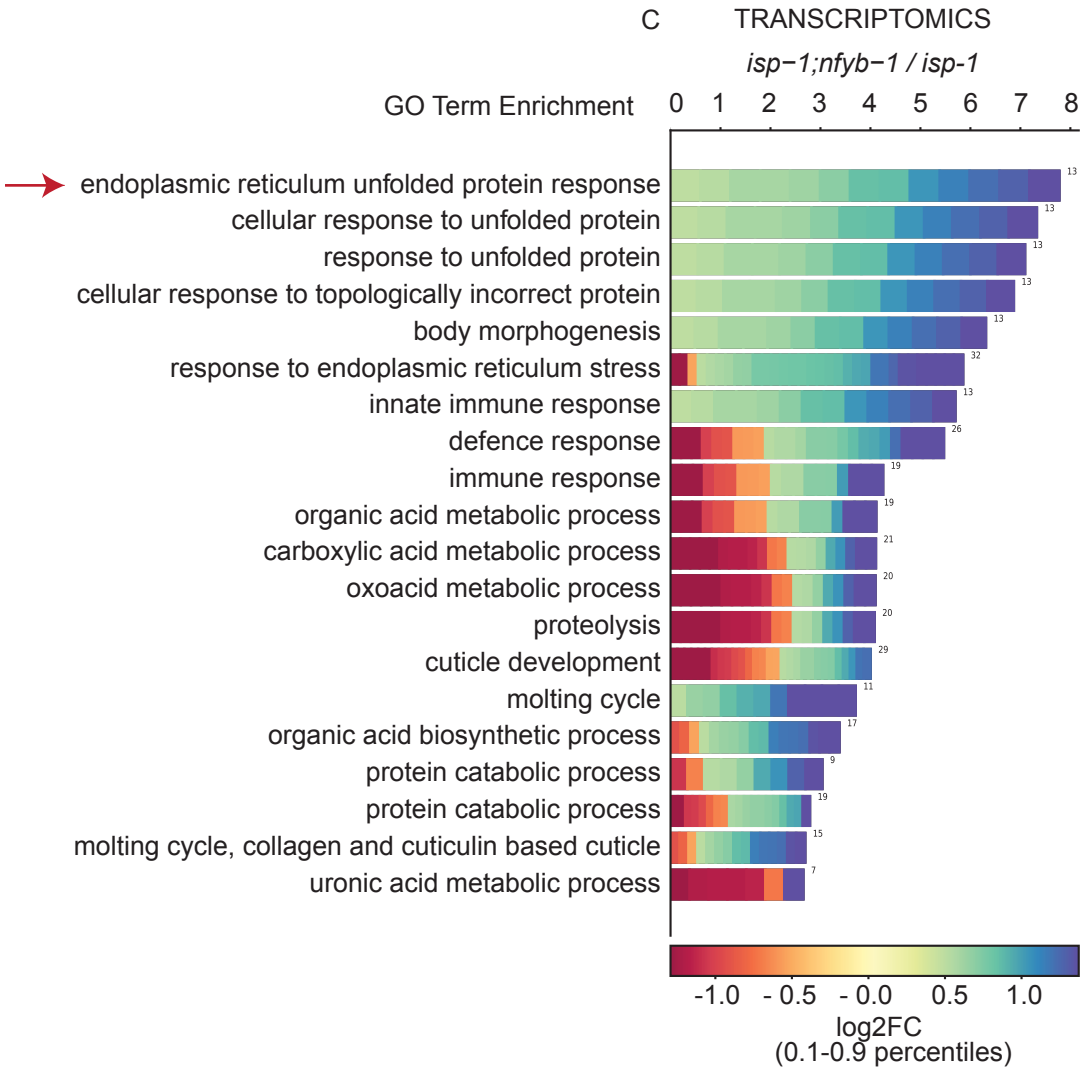
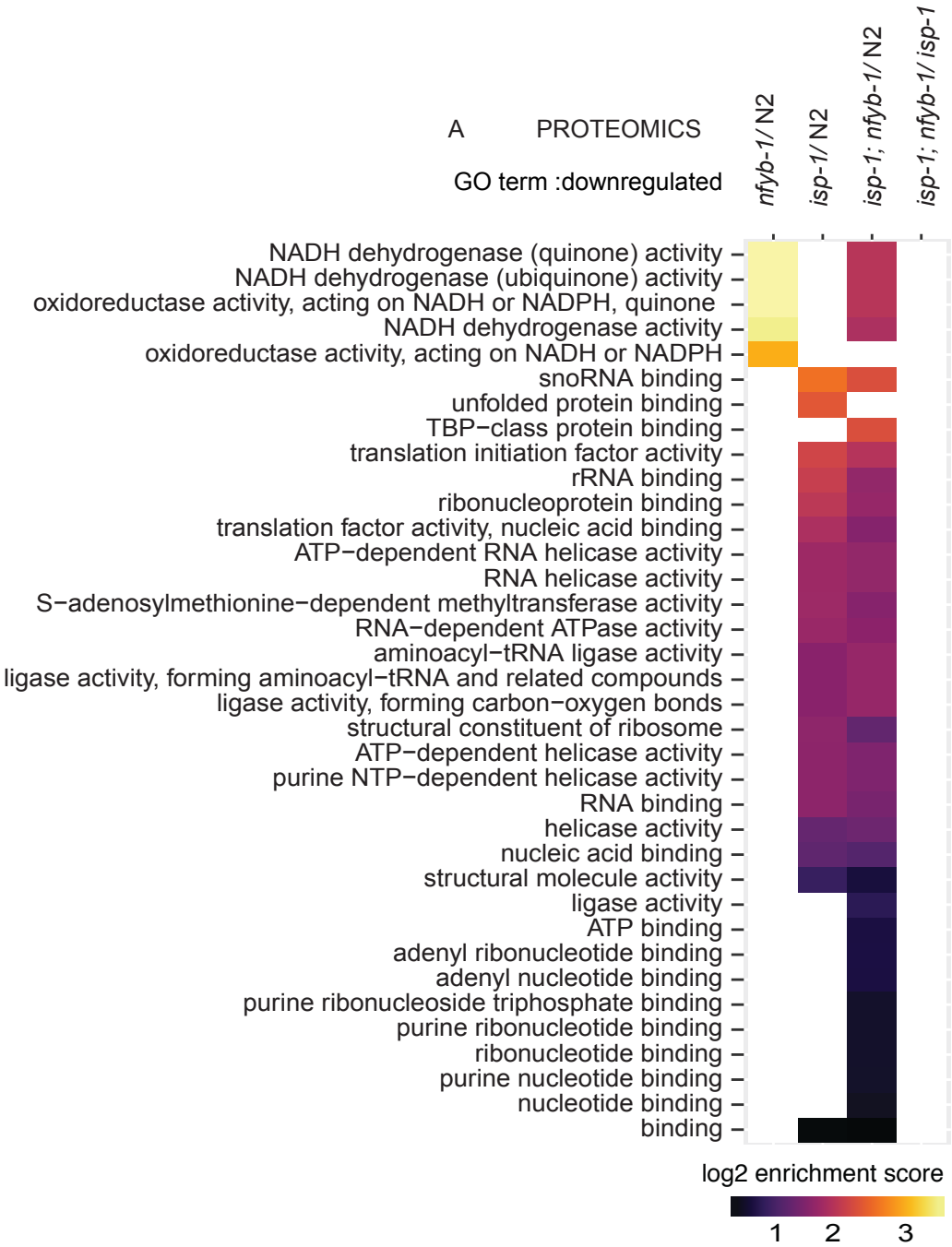


Figure 20: Transcriptomic analysis indicate regulation of ER genes upon loss of NFYB-1. GO term enrichment for *N2, nfyb-1(cu13*), long lived mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13),* n=3 (significance $p<0.05$). A) GO term enrichment significantly changed downregulated genes. B) GO term enrichment significantly changed upregulated genes (ER genes marked with red). C) GO term enrichment for ER genes upon comparing long lived mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13*). RNA seq analysis was performed at Max-Plank Genomic Center, Koln and the bioinformatics analysis on transcriptomics data was performed by the bioinformatics core faculty at Max-Plank for Biology of ageing (refer methods).



(Figure 21: refer page 90 for figure legend)

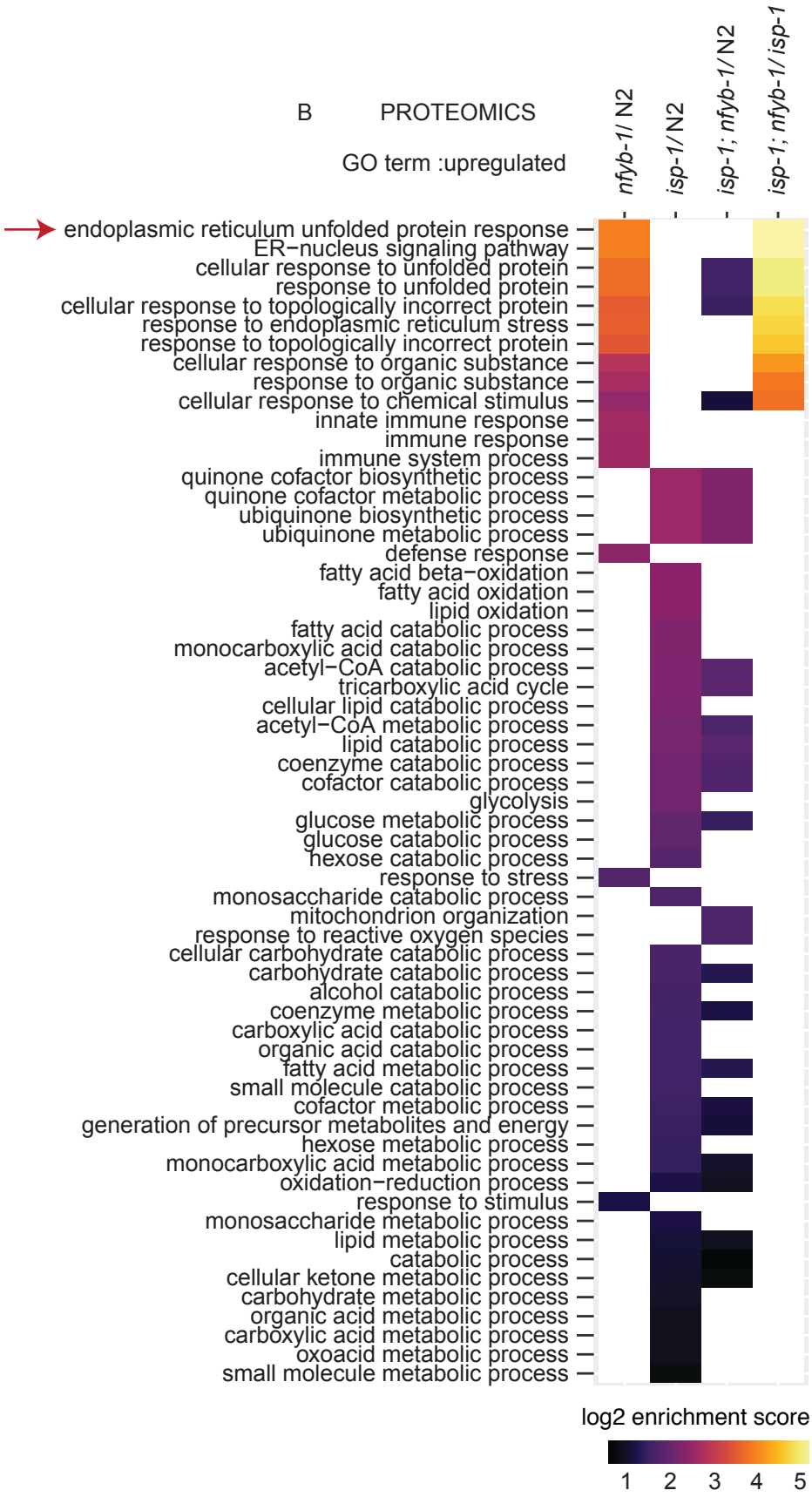


Figure 21: Proteomics analysis indicate regulation of ER genes upon loss of NFYB-1. GO term enrichment for *N2, nfyb-1(cu13),* long lived

mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13),* (significance p<0.05, n=5). A) GO term enrichment for significantly changed downregulated. B) GO term enrichment significantly changed upregulated genes (ER genes marked in red). Proteomics analysis was performed at the proteomic core faculty at Max-Plank for Biology of ageing (refer methods).

To decipher the genes regulated under mitochondrial longevity upon NFYB-1 loss I was particularly interested in the *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13)* comparison. Like mentioned above strikingly I observed an enrichment for ER associated genes and immune response genes in particular (Figure 20C). This could indicate that NFYB-1 modulates mitochondrial longevity by regulating these processes.

We next performed shotgun proteomic analysis of these genotypes. Proteomic analysis identified overall a total of ca. 3724 proteins. Differentially regulated proteins included 270 between *nfyb-1(cu13)/ N2,* 1178 between *N2/ isp-1(qm150)* and 49 between *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13).* Among the downregulated proteins for *N2/nfyb-1(cu13)* were GO terms enriched for complex 1 NADH dehydrogenase activity (Figure 21A). This could indicate a role for NFYB-1 in promoting mitochondrial respiration, and would be consistent with changes in mitochondrial physiology seen in the *nfyb-1(cu13)* mutant. Among the significantly downregulated genes, similar to the transcriptomic analysis for the *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13)* comparison no obvious enrichment of GO terms was apparent. Remarkably among the upregulated differentially expressed proteins, for both *nfyb-1(cu13)/N2* and *isp-1(qm150)/isp-1(qm150); nfyb-1(cu13)* were GO terms enriched for immune response and ER associated proteins (Figure 21B), similar to transcriptomic analysis. Notably *isp(cu13)/N2* shows enrichment for mitochondrial genes such as quinone genes, tricarboxylic acid cycle genes which absent in *nfyb-1* comparison

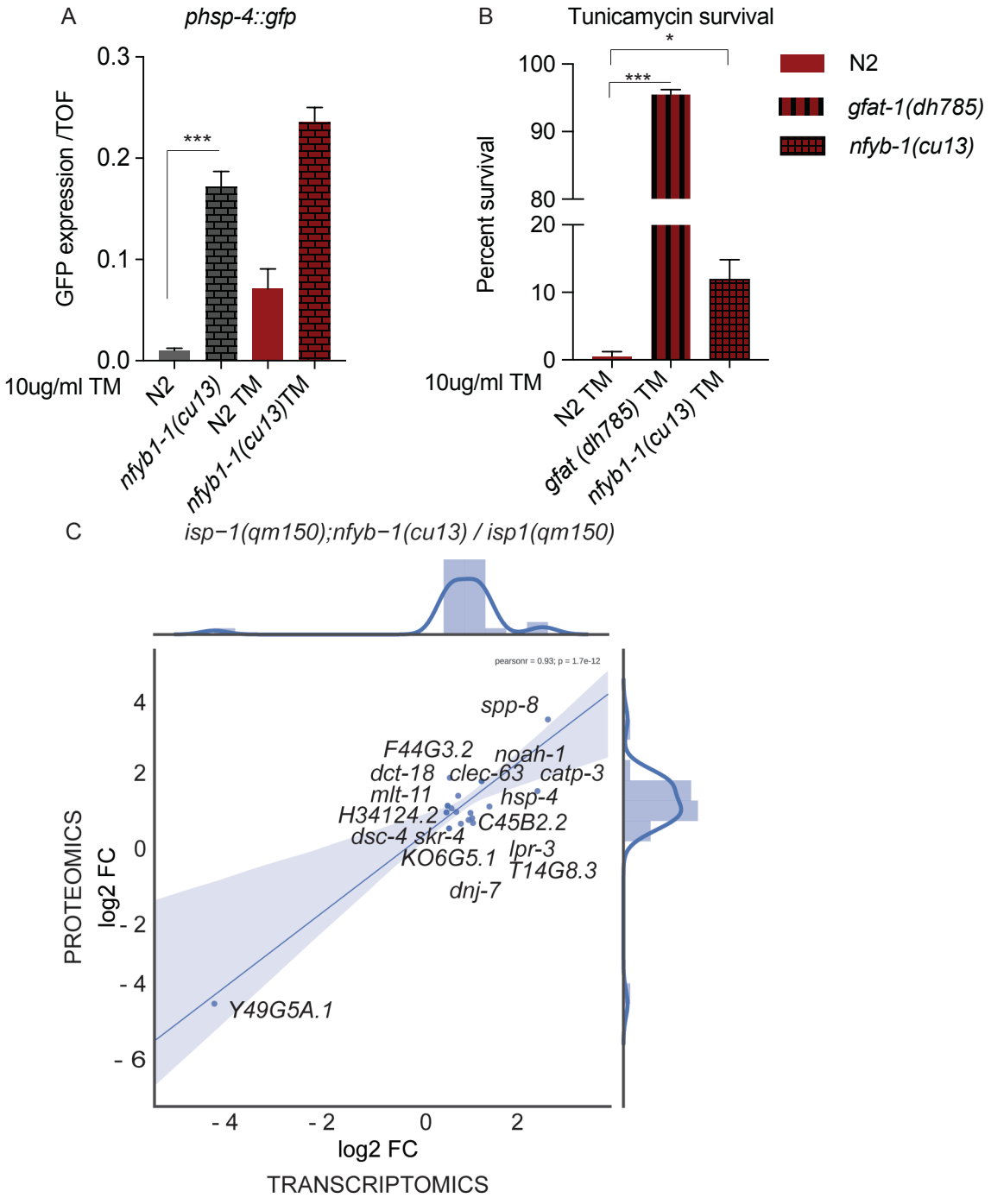


Figure 22: Regulation of ER genes upon loss of NFYB-1. A) loss of NFYB-1 leads to upregulation of UPR^{ER} reporter day 1 *phsp-4::gfp* expression levels relative to TOF measured using biosorter. induction of ER stress response by feeding tunicamycin (TM) (10ug/ml) for 6 hours (n=3, error bar indicates SEM, anova test, ****p<0.0001, ***p<0.001, ** p<0.01, * p<0.05). B) *nfyb-1(cu13)* show resistance to tunicamycin (10ug/ml) feeding in comparison to wildtype, n=2. anova test, ****p<0.0001, ***p<0.001, ** p<0.01, * p<0.05. C) Overlap of transcriptomics and proteomics analysis (significantly changed genes

p<0.05) upon comparing long lived mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)*. Correlation of transcriptomics and proteomics data (performed by bioinformatics core facility at the MPI for Biology of Ageing).

The omics data clearly indicated a significant regulation of ER associated genes upon loss of NFYB-1. To assess the functional significance of these changes, I tested whether aspects of the UPR^{ER} were altered by *nfyb-1* mutation in vivo. Under normal growth conditions, a transcriptional reporter for UPR^{ER} heat shock factor *hsp-4/BIP* is basally expressed. Exposure to tunicamycin (10ug/ml) induces ER stress by blocking N-glycan synthesis, leading to an accumulation of misfolded glycoproteins in the ER, induction of UPR^{ER} and massive upregulation of HSP-4/BIP. In accord with proteomic data, I observed that *nfyb-1* mutation caused *phsp-4::gfp* expression levels to be constitutively upregulated even in the absence of tunicamycin exposure (Figure 22A).

Consistent with a constitutive response, *nfyb-1(cu13)* conferred modest resistance to tunicamycin challenge (10ug/ml). As a control for the tunicamycin resistance assay, I used *gfat-1(dh785)* gain-of-function mutant, which activates the hexosamine (Denzel & Antebi 2015) pathway and confers resistance to ER stress induced by tunicamycin (Figure 22B). These findings suggest that NFYB-1 functions as a repressor for ER stress genes under normal growth conditions, and that loss of NFYB-1 leads to the upregulation of ER stress response pathways or that NFYB-1 loss induces ER stress by perturbing the balance of ER enzymes.

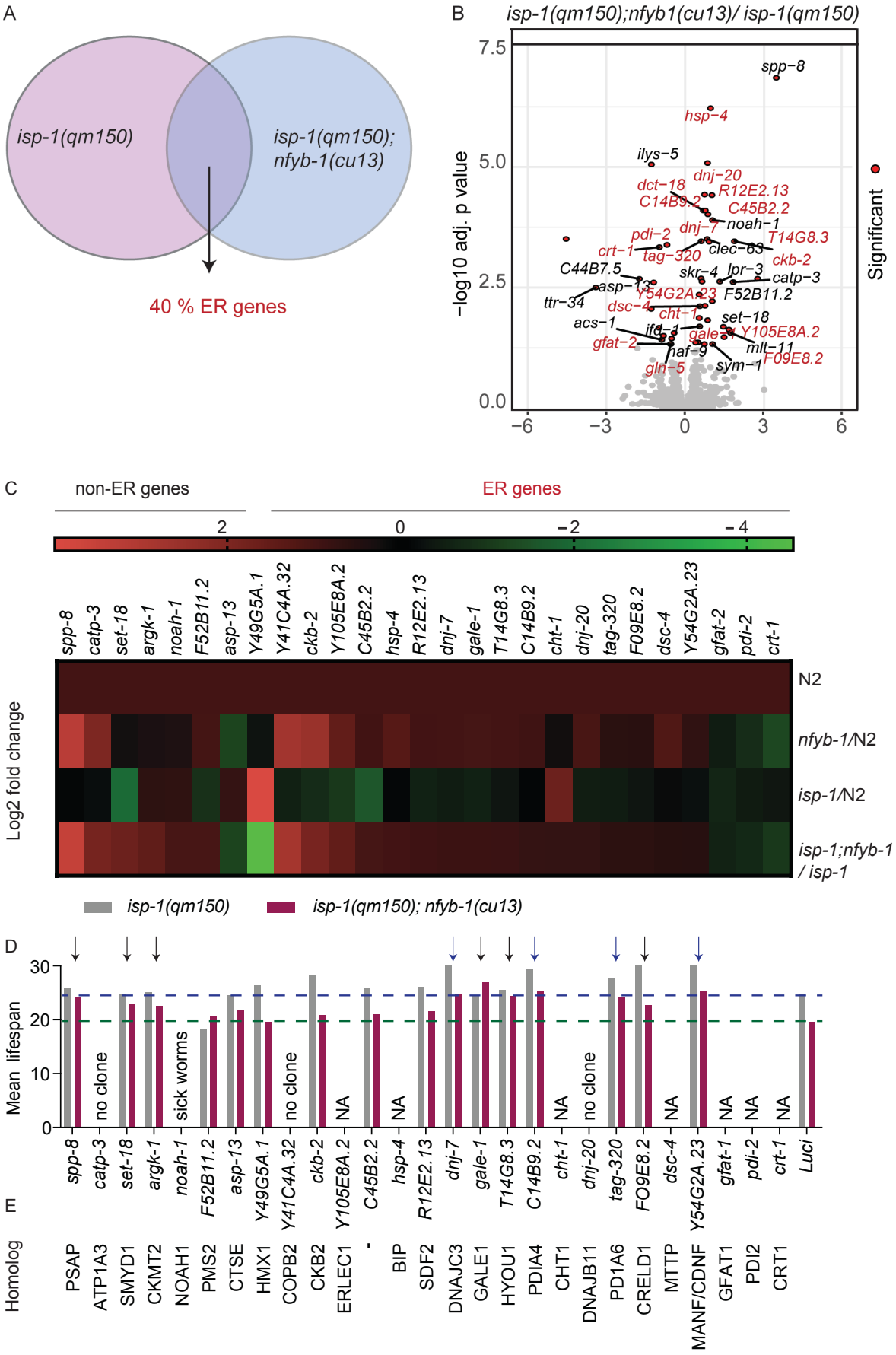


Figure 23: Regulation of mitochondrial longevity by modulating ER genes. Proteomic analysis of significantly regulated factors in *isp-1(qm150);*

nfyb-1(cu13) in comparison to *isp-1(qm150)* A) Venn diagram representation shows 40% ER associated genes, B) Volcano plot showing specific factors in particular ER genes (in red), C) Heat map representation of fold change in expression of respective comparisons clustered as non-ER and ER genes. D) RNAi lifespan screen of candidates from proteomics analysis for rescue of *isp-1(qm150); nfyb-1(cu13)* in comparison to *isp-1(qm150),* bar graphs represents mean lifespan n=1. RNAi candidates that rescue the lifespan of double mutant with (black arrow) & without (blue arrow) affecting *isp-1(qm150)* longevity (n=1, Mantel-Cox Log Rank test: refer appendix table 5 for statistics).

Proteomics and transcriptomics analysis for long lived mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* double mutant that abolish longevity induced by mitochondrial impairment very prominently indicated similar enrichment. Together these omics analysis primes to speculate that loss of NFYB-1 leads to deregulation of ER associated genes at the transcriptomic and proteomics levels thereby abolishing longevity induced by mitochondrial impairment. A comparison of transcriptome and proteomic changes also revealed that the majority of differentially expressed genes products were concordantly regulated such as in *hsp-4, dct-18, dnj-7, Y49G5A.1, T14G8.3, skr-4, clec-63, catp-3, spp-8, KO6G5.1, H34124.2, mlt-11, lrp-3, noah-1, C45B2.2* (Figure 22C).

Notably, 40% of the differentially regulated proteins between *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* were ER associated (Figure 23A-C) (refer appendix table 4), the majority of which were upregulated. Additionally, a few interesting non-ER proteins emerged, including *spp-8*/prosaposin, epigenetic SET domain factor *set-18*/SMYD1, *argk-1*/arginine kinase, F52B11.2/ phosphomannomutase 2, etc.

I hypothesized that factors differentially regulated in an *nfyb-1* dependent manner could contribute to *isp-1* longevity, and help elucidate mechanism. On the one hand, I reasoned that knocking down life extending genes that

showed a significant upregulation at the protein levels in long lived *isp-1(qm150)* mutants compared to *isp-1(qm150); nfyb-1(cu13)* double mutants should reduce *isp-1(qm150*) longevity. Some of the genes in this category include *Y49G5A.1, catp-3* and *asp-13.* On the other hand, knocking down life span inhibiting factors abnormally upregulated in *isp-1(qm150); nfyb-1(cu13)* might rescue longevity of the double mutant. This subset mainly includes ER genes *(Y441C4A.32, ckb-2, Y105E8A.2, C45B2.2, hsp-4, R12E2.12, dnj-7, gale-1, T14G8.3, C14B9.2, dnj-20, tag-320, F09E8.2 Y54G2A.23),* as well as a few non-ER genes (*spp-8, catp-3, set-18, argk-1*tand *F52B11.2)*.

To examine the effect on mitochondrial longevity of these candidates I performed lifespan screen by RNAi knockdown of the above-mentioned candidates on long lived mitochondrial mutant *isp-1(qm150)* and the normal lived *isp-1(qm150); nfyb-1(cu13)* double mutant. A few candidates (Y441C4A.32, *dnj-20* and *catp-3)* were excluded due to the unavailability of RNAi clone. Additionally, *Y105E8A.2* and *hsp-4* were not included in this screen due to their slow growing phenotype. Furthermore, a few of the ER genes such as *gfat-1, pdi-2, crt-1, dsc-4* and *cht-1* were excluded from the lifespan analysis because they were regulated in the same direction when comparing *isp-1(qm150)* to *isp-1(qm150); nfyb-1(cu13).*

RNAi knockdown of several factors significantly rescued longevity of otherwise short lived *isp-1(qm150); nfyb-1(cu13)* double mutant, including *spp-8*, *set-18*, *argk*-1, *gale-1* and *T14G8.3,* without affecting longevity of *isp-1(qm150).* F09E8.2 also partially rescued the longevity but slightly increased lifespan of *isp-1(qm150)*. *dnj-7, C14B9.2, tag-320* and *Y54G2A.23 also* rescued *isp-1(qm150); nfyb-1(cu13)* longevity but as well partially increased *isp-1(qm150)* (Figure 23D). Of note, this lifespan screen is preliminary and we are performing further lifespan analysis for the above-mentioned candidates. Interestingly most of the identified factors are

conserved and have a human homolog (Figure 23E). Hence further analysis will help us better understand the role of NFYB-1 in linking the regulation of mitochondrial and ER genes in higher organisms. In sum, these data imply that NFYB-1 could influence longevity through modulation of ER stress response, immunity and mitochondrial functions.

8 DISCUSSION

Mitochondria are the central organelle vital for generating energy and reducing power, but also globally coordinate metabolism, nutrient sensing and stress responses. Despite our current understanding of these fundamental processes, how their interplay impact cellular vitality, and organismal ageing remains elusive.

In this work, I sought to address three major questions:
1.How do quiescent states typified by the adult reproductive diapause impact mitochondrial biology, and how does mitochondrial biology affect ARD survival?
2. What are the ancestral factors governing *C. elegans* mitochondrial gene expression and biogenesis?
3. What specific role does transcription factor NFYB-1 play in mitochondrial physiology and longevity?

In short, I found that mitochondria become fused and hypometabolic under ARD, and that extended survival in ARD is impacted by mitochondrial fusion/fission factors. I identified several ancestral regulators of mitochondrial gene expression implicating NFYB-1, CREB signaling, NEF2 and other factors as major mediators. Focusing on NFYB-1, I discovered that this transcription factor promotes mitochondrial gene expression, physiology and longevity, and represses the ER stress response, thereby contributing to extended survival.

8.1 Mitochondrial dynamics are critical to ARD survival.

Upon starvation prior to sexual maturation *C. elegans* enter a quiescent state called the adult reproductive diapause (ARD*)*. Worms within ARD undergo a substantial remodelling of tissues and endure months in the

absence of food, yet upon refeeding can rejuvenate the tissues and reproduce. However, the molecular basis underlying ARD remodeling and extended survival remain unexplored.

The changes seen in ARD are not limited to cells and tissues but also organelles. We found that mitochondria are dramatically remodeled within ARD and rejuvenate upon exit. Notably, we observed that within ARD *C. elegans* maintain low oxygen consumption, low mtDNA copy number and form fused mitochondrial network. Several lines of evidence suggest that at least mitochondrial morphology is pivotal to ARD longevity. For instance, loss of the fission factor *drp-1* leads to a hyper fused mitochondrial network, higher mitochondrial copy number, and an extension of ARD longevity. Conversely, loss of the fusion factor *fzo-1* leads to a fragmented mitochondrial network, and a reduction of ARD longevity. Currently it is unclear how exactly mitochondrial dynamics impact survival under ARD. Mitochondrial fission and fusion have been implicated in regulating metabolism, mtDNA synthesis, respiration and mitophagy (Kujoth et al. 2005; Chan 2012; Bernhardt et al. 2015; Schiavi et al. 2015; Sebastián et al. 2017a). Mitochondria undergo fusion events to mix mtDNA, proteins, lipids and metabolites. *drp-1* has also been shown to have pro-apoptotic function (Breckenridge et al. 2008; Jagasia et al. 2005; Breckenridge et al. 2009; Sebastián et al. 2017b). Conceivably, mitochondria fusion may be required to prevent mitophagy and maintain mitochondrial copy number, which in turn could prevent apoptosis and maintain cellular integrity. Hence it will be interesting to further investigate how the process of mitophagy and apoptosis are regulated, or examine how mitochondrial dynamics impacts the metabolome under ARD.

We also observed that mitochondria undergo a remarkable rejuvenation upon ARD recovery. Mitochondrial copy number and oxygen consumption significantly increased upon refeeding, presumably to meet the high-energy demand for recovery. During recovery, ARD animals restore their germline,

where the bulk of mitochondrial biogenesis is thought to take place. Nevertheless, we also detect a notable increase in copy number with germline deficient *glp-4* worms, revealing biogenesis in somatic tissues. Interestingly upon recovery worms also form tubular mitochondrial structures similar to early adults fed under ad libitum, showing a restoration of mitochondrial morphology.

Taken together these observations reveal that upon ARD entry and exit *C. elegans* undergo an extraordinary change in mitochondrial structure, oxygen consumption and mtDNA levels leading to remarkable remodelling of mitochondrial function and metabolic process, and for the first time establish that factors regulating mitochondrial fission and fusion are critical to ARD survival.

8.2 Ancestral regulators of mitochondrial gene expression and biogenesis.

Although mitochondrial biogenesis and its regulation are reasonably well characterized in mammals, comparatively little is understood in *C. elegans*. Notably there are no clear homologs of major mammalian biogenesis factors such as PGC1 alpha, ERR-alpha, YY1, and others. This raises the question what are the ancestral factors that govern metazoan mitochondrial gene regulation, biogenesis, and function, and how might these factors impact cellular physiology and ageing? Identifying and characterizing such factors in an unbiased way is important since it would allow us to clarify the relationship between mitochondrial physiology and ageing in a simple model genetic system.

We leveraged the large changes seen in mitochondrial physiology upon ARD recovery to establish a genetic screen for factors involved in mitochondrial gene regulation and biogenesis. First we established that

several independent mitochondrial reporters were upregulated somatically during recovery, namely *cco-1;;gfp* and *par2.1::gfp*,. Second, we took advantage of this change as a tool to screen for alterations in mitochondrial gene expression upon knockdown of RNAi sub-libraries. Third, we followed up on factors to establish if the changes in gene expression correspond to changes in mitochondrial physiology.

From RNAi, sub-libraries containing transcription factors, nuclear hormone receptors, chromatin regulators, phosphatases and kinases totalling >2200 clones, we identified 38 possible regulators of mitochondrial function. Most of these candidates reduced *cco-1::gfp* expression, while a handful increased it. From the screen, we identified several genes previously implicated in mitochondrial function, longevity, or both. Among them, the CREB signalling pathway stood out. We identified *crh-1* (human CREB homolog), *let-607* (human CREB3L3 homolog) and *K03H1.10* (CREB-binding protein) as positive regulators. CREB has been shown to be involved in mammalian mitochondrial gene expression and biogenesis via transcriptional regulation of PGC-1a (Wu et al. 2006) and has also been implicated in *C. elegans* mitochondrial function (Mair, Morantte, Rodrigues, Manning, Montminy, Reuben J Shaw, et al. 2011). We also identified several factors that might participate in an extended CREB signalling network. For example, FGFR-like/R151.4 tyrosine kinase in mammals regulates CREB activity and MAP kinase signaling also is known to act in a mitokine signaling pathway (Kimata et al. 2003; Dogan et al. 2014). Moreover, *C. elegans* R151.4 is transcriptionally regulated in mitochondrial ETC mutant *isp-1*(Yee et al. 2014). These results strongly indicate that CREB signalling comprises an ancestral mitochondrial regulatory network. We also identified *atfs-1,* a b-ZIP transcription factor required for mitochondrial unfolded protein response UPR^{mt} signalling (Nargund et al. 2012; Haynes et al. 2010). We also identified genes that are linked to oxidative stress response like *skn-1/NFE2* and its repressor *wdr-23.* Taken

together our candidate genes uncover many interrelated factors and phenotypes previously linked to mitochondrial function, proving that our screen can point to valid candidates.

We also identified several novel mitochondrial regulators. Among them are two-histone demethylase *jmjd-2* and *jhdm-1*; *ttx-1*, OTX1 homolog; two T-box transcription factors *tbx-40* and *tbx-34;* the VHP-1 phosphatase; nuclear hormone receptor *nhr-61*; *grh-1* GRainyHead Drosophila transcription factor homolog; nuclear transcription factor *nfyb-1,* as well as the transcription factor *drap-1 (*orthologue of human DRAP-1).

A subset of the identified genes is already known to be involved in determination of lifespan, including *jmjd-2, crh-1, ttx-1, skn-1, hsf-1; ncl-1* and *gei-3.* Histone demethylase *jmjd-2* and knockdown of *jmjd-2* results in lifespan extension (Ni et al. 2012). *crh-1* is essential to calcineurin and AMPK induced longevity and its knockdown extends lifespan (Mair, Morantte, Rodrigues, Manning, Montminy, Reuben J Shaw, et al. 2011). HSF-1 and SKN-1 are implicated in determination of lifespan (Robida-Stubbs et al. 2012; Munkácsy et al. 2016; Onken & Driscoll 2010; Tullet et al. 2008; Ackerman & Gems 2012; Steinkraus et al. 2008; Greer & Brunet 2009).

One of the more interesting candidates is *ttx-1*. *ttx-1/OTX1* is highly expressed in AFD thermosensory neurons also plays a role in thermotaxis and promotes long life at higher temperature (Lee & Kenyon 2009). *ttx-1* which has also shown to have 25% shorter lifespan at higher temperature (Lee & Kenyon 2009). Studies have also shown mitochondrial copy number increase with a shift to higher temperature (Dillin et al. 2002). we surmise that with *ttx-1* knockout mutants we might see an effect at higher temperature. We hypothesize that *ttx-1* is necessary for transcription

regulation of mitochondrial genes and is necessary for increase in copy number at higher temperature, absence of which leads to shorter lifespan.

Another interesting candidate is *jmjd-2*. In our hands, knockdown of *jmjd-2* affected transcript levels of multiple mitochondrial genes, and previous work has already shown that *jmjd-2* knockdown enhanced H3-K9Me3 and H3-K36Me3 methylation and increased lifespan (Ni et al. 2012; Black et al. 2010). We also identified a second histone demethylase, namely *jhdm-1*. Interestingly other jumonji proteins, namely *jmjd-1.2*/PHF8 and *jmjd-3.1*/JMJD3 have been recently shown to regulate mitochondrial function and longevity (Merkwirth et al. 2016; Tatar & Sedivy 2016). These findings provide a hint that a number of jumonji proteins coordinate mitochondrial biology.

Interestingly we see that *grh-1* GRainyHead Drosophila transcription factor homolog abolishes the lifespan extension of mitochondrial mutant *isp-1(qm150*) and also results in lower transcript levels of multiple mitochondrial genes. Also, T-box transcription factors *tbx-40 and tbx-34* partially reduces the *isp-1* lifespan extension but rather increases the mean WT lifespan and show lower mtDNA/nuDNA ratio. Where *tbx-40* show lower transcript level for multiple mitochondrial genes, *tbx-34* knockdown leads to activation of stress response genes of UPR^{mt}, antioxidant defence and oxidative stress response. This might indicate an interesting role of T-box transcription factors in regulation of lifespan of *isp-1,* mitochondrial stress response and regulation. Additionally, we also observe that another novel transcription factor identified from the RNAi screen *nfyb-1* has been shown to regulate T-box factors such as *tbx-2.*

A major factor regulating mitochondrial biogenesis is nutrient availability. We identified *ncl-1* which promotes dietary restriction mediated longevity by regulating nucleolar size and function (Tiku et al. 2016), providing a

potential starting point to further investigate how low food signals lead to changes in mitochondrial biology upon ARD induction. Another candidate, *gei-3,* is involved in the gonadal longevity pathway (S. Nakamura, O. Karalay, A. Antebi, unpublished). We found that knockdown of *gei-3* affects UPR^{mt} and antioxidant defence gene expression, and by inference, might disrupt the mitochondrial protein folding environment.

Taken together we see that many of our novel candidates have an effect on the transcript levels of multiple mitochondrial genes, activate mitochondrial stress response pathways and affect mitochondrial DNA content. A few of these novel candidates also partially affect the lifespan extension of mitochondrial mutant *isp-1* and are involved in lifespan determination in WT background.

8.3 NFYB-1 modulates mitochondrial function and longevity

We used a number of criteria to zoom in on the strongest candidates. We focused on those factors that affected transcription of multiple mitochondrial genes by qPCR, mitochondrial gfp marker expression, mitochondrial DNA content, respiration rate and longevity of WT and *isp-1.* Furthermore, we restricted our focus to evolutionarily conserved but relatively novel factors. Finally, we concentrated on loci with relatively few pleiotropies. One of the most prominent factors to emerge from this was nuclear transcription factor Y subunit beta (*nfyb-1)*, a transcription factors having high affinity for genes carrying CCAAT binding motif in the promoter regions.

Multiple lines of evidence strongly support the idea that NFBY-1 is critical to mitochondrial regulation and biology. First, we observed that loss of *nfyb-1* not only leads to a reduction in mitochondrial marker for nuclear encoded complex IV subunit *cco-1* but also to reduction of mitochondrial single stranded binding protein *mtss-1* under higher energy demand conditions such as ARD recovery, elevated temperature, as well as under ad libitum

conditions. These observations reveal a crucial role under various ambient conditions. Second, absence of *nfyb-1* leads to reduced oxygen consumption even in somatic tissues upon ARD recovery and under ad libitum. Third, *nfyb-1* plays a role in the regulation of mitochondria dynamics, since *nfyb-1* loss leads to fragmented mitochondria upon ARD recovery and under ad libitum conditions. Fourth, mtDNA levels are upregulated in *nfyb-1(cu13),* presumably revealing a perturbation of mitochondria biogenesis to compensate for mitochondrial dysfunction. Fifth, *nfyb-1* mutation abolished life extension by mitochondrial impairment via RNAi knockdown of *cco-1* or mitochondrial mutant *isp-1(qm150)*, showing that *nfyb-1* regulates mitochondrial longevity induced by at least two independent strategies. Sixth, *nfyb-1* diminished the expression of two major UPR^{mt} stress mediators, *atfs-1* and *dev-1*, responsible for promoting mitochondrial longevity. Finally, transcriptomic analysis revealed that *nfyb-1* mutation impacts the expression of many metabolic genes including those involved in mitochondria metabolism.

NFYB-1 is known to function in a complex with NFYA and NFYC. NFY subunit B forms dimer with subunit C a pre-requirement for binding of subunit A (Nardini et al. 2013; Sinha et al. 1995; Ceribelli et al. 2008; Fleming et al. 2013). Interestingly we only identified NFYB-1 and not NFYA or NFYC from our RNAi screen. This could indicate that NFYB-1 could form complexes with other factors to regulate mitochondrial function. Indeed, NFYB-1 has been shown to interact by yeast two-hybrid with DRAP-1, which also came out of our screen. Consistent with a common role, RNAi knockdown of *nfyb-1* and *drap-1* reduced mitochondrial longevity of *isp-1(qm150).* It will be interesting to further explore this interaction in the future.

8.4 NFYB-1 regulates life span across several pathways

Interestingly, we found that *nfby-1* was required for life span extension in all the major pathways examined. RNAi knockdown of *nfyb-1* not only abolished mitochondrial longevity of *isp-1(qm150)*, but also caused a partial reduction of *daf-2*/insulin-IGF signaling, a reduction of *glp-1/*germline signaling induced longevity and abolished *eat-2* /DR longevity. One interpretation of these results is that *nfyb-1* loss is deleterious, and generally makes animals sick. Arguing against this, *nfby-1* mutant animals did not display obvious pleiotropies and showed only modestly reduced life span. On the other hand, *nfyb-1; eat-2* double mutant animals were sick, and therefore loss of longevity in this case is likely a synthetic phenotype. Another interpretation is that *nfyb-1* is a convergent factor required to regulate downstream processes important for longevity in multiple pathways. This could reflect crucial outputs on mitochondrial physiology, metabolism or other functions important for extended survival. In particular, how mitochondrial function and metabolism are affected upon loss of NFYB-1 in these pathways would be worth pursuing. For instance, long-lived mutants *daf-2, eat-2, glp-1* show a delay in mitochondrial fragmentation (Regmi et al. 2014), while *nfyb-1* mutants show increased mitochondrial fragmentation. Hence it will be interesting to examine mitochondrial morphology in double mutants with *nfyb-1.* Alternately, perturbation in the ER stress response pathways could influence longevity in these pathways.

8.5 NFYB-1 regulates mitochondrial stress mediators but not UPR^{mt}

Mitochondrial impairment leads to activation of mitochondrial stress response pathways, such as the mitochondrial unfolded protein response UPR^{mt} and ROS signalling, and extension of life (Schulz et al. 2007; Durieux et al. 2011; Jensen & Jasper 2014; Lee et al. 2010)**.** Upon

mitochondrial stress, *C. elegans* UBL-5 and DVE-1 translocate into the nucleus along with ATFS-1 and induce the expression downstream target genes (Haynes et al. 2007; Haynes & Ron 2010; Nargund et al. 2012; Nargund et al. 2015; Pellegrino et al. 2013; Baker & Haynes 2011). We observed that NFYB-1 is essential for triggering nuclear localization of mitochondrial UPR factors DVE-1 and ATFS-1, but saw no effect on UBL-5. Moreover, mitochondrial heat shock *hsp-6* and *hsp-60* genes normally induced upon mitochondrial stress by ATFS-1 and DVE-1, were surprisingly unaffected by loss of *nfyb-1*. This unexpected result indicates that NFYB-1 can regulate UPR^{mt} factors but not UPR^{mt} heat shock response itself. Conceivably residual activity of DVE-1 or ATFS-1 in the *nfyb-1* background is sufficient for the UPR^{mt} response, or *nfyb-1* loss bypasses the requirement for these factors. Measuring expression of UPR^{mt} heat shock factors in *nfyb-1(cu13*) null mutants upon RNAi knockdown of DVE-1 and ATFS-1, as well as determining if NFYB-1 acts epistatically in the same pathway as these factors to regulate life span, may help address this question. An alternate explanation is that NFYB-1 may affect transcriptional outputs of DVE-1 and ATFS-1 other than the UPR^{mt} factors.

One possible common output of DVE-1 and NFYB-1 could be the mitochondria to cytosolic stress response. Both factors, as well genes involved in fatty acid metabolism and HSF-1, play a vital role in MCSR and longevity (Kim et al. 2016). Furthermore, NFYB-1 also affects the expression of genes involved in fatty acid synthesis. Because the MCSR stimulates HSF-1 and improves cytosolic protein homeostasis, it will be worth to test the ability of NFYB-1 to influence HSF-1 localization/activity and manage proteotoxic stress. Ultimately informatics analysis to examine the common downstream targets of NFYB-1with respect to DVE-1 and ATFS-1 may shed further light on this issue.

8.6 NFYB-1 regulates metabolism and ER stress

Transcriptomic and proteomic analyses of *nfyb-1(cu13)* reveal a number of interesting differential changes in gene expression. Among transcripts downregulated in *nyfb-1(cu13)* compared to wild type are GO terms enriched in metabolism of carbohydrates, RNA and siRNA, NAD metabolism, and proteolysis. Proteins downregulated in *nfyb-1(cu13)* are enriched in complex 1 of the mitochondria. Notably the effect on mitochondrial gene expression was surprisingly limited, given the global effects on mitochondrial physiology**.** Conceivably downregulation of *C. elegans* complex 1 or other mitochondrial functions are sufficient to trigger changes in mitochondrial physiology. Why complex 1 is the specific target of regulation is unclear. Disparate regulation of mitochondrial gene expression is seen in other species. On the one hand, yeast HAP3, the homolog of NFYB-1, is essential for the expression and import of nuclear encoded respiratory subunits as well as mitochondrial division that define the metabolic state of the cell (Buschlen et al. 2003). On the other hand, mammalian NFYB has only a minor effect on mitochondrial gene expression, and functions more to regulate lipid metabolism, glycolysis, and UPR genes (Donati et al. 2008; Donati et al. 2006; Benatti et al. 2016). Other specific gene products downregulated in *nfyb-1* that caught our attention were the dramatic reduction of three map kinase homologs, *pmk-1,2,3,* involved in innate immunity and stress response, *jmjd-1.2* implicated in mediating mitochondrial longevity, and *taco-1,* a factor regulating the translation of cytochrome c oxidase 1. How these factors contribute to *nfyb-1* physiology will be interesting to address in the future.

Transcripts upregulated in *nfyb-1* compared to wild type include those involved in ER stress, innate immunity, fatty acid synthesis, amino acid synthesis, calcium signaling, collagen, basement membrane, sugar transport. Several of these GO terms were corroborated in the proteomic analysis, particularly the upregulation of ER stress and innate immunity.

The most striking visible change was that of the ER stress response and secretory pathway components, including upregulation of various ER chaperones (*hsp-4*, *gale-1*, etc.). Notably, a number a key transcription factors regulating the ER stress response, including XBP-1 and ATF-5, were also upregulated. Thus, it would be important to test whether de-repression of the ER stress response seen in *nfyb-1(cu13)* mutants is dependent on these or other UPR factors, such as PERK or ATF-6. In particular, mammalian ATF-6 has been shown to regulate UPR target gene expression dependent on the physical proximity with CCAATT binding nuclear factor y (NF-Y) (Haze et al. 1999; Yoshida et al. 2001; Yoshida et al. 2000; Chen et al. 2002). Interestingly, another transcription factor upregulated in *nfyb-1* was the bZIP CCAAT/enhancer binding protein homolog CEBP-1. This factor binds similar elements as *nfyb-1* and its upregulation presumably reflects a compensatory mechanism. NFY has been proposed to be a pioneer factor (Nardini et al. 2013), which conceivably could initiate chromatin changes facilitating CEBP-1 entry. Exploring the genetic interactions between these two factors would be very much worthwhile.

Interestingly we found that proteomics and transcriptomics analysis comparing mitochondrial mutant *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* reveals a striking upregulation of ER associated genes. This raises the question how might NFYB-1 contribute to *isp-1* longevity? RNAi knockdown lifespan screen helped us to clarify if the differentially expressed ER genes modulate mitochondrial longevity. For instance, we found *isp-1(qm150); nfyb-1(cu13)* lifespan was partially rescued by RNAi knocking down of *spp-8/saposin*, *set-18/*SMYD1, *argk*-1/arginine kinase, while *gale-1*/GALE1 and *T14G8.3/HSP70* almost completely rescued the lifespan. Interestingly these candidates did not affect the longevity of *isp-1(qm150)* indicating that these factors might play a critical role in regulating mitochondrial longevity pathway downstream of NFYB-1. Whereas *dnj-7,*

C14B9.2/PDIA4, tag-320/PDIA6 and *Y54G2A.23/MANF* rescued *isp-1(qm150); nfyb-1(cu13)* longevity but also partially increased *isp-1(qm150).* *(*Note that these data are preliminary and are being repeated). Nevertheless, our results suggest that limiting ER stress response genes actually has benefits, and that ER stress induction impedes mitochondrial longevity. In the future, it will be important to see how these ER factors affect other aspects of mitochondrial physiology such as mitochondrial morphology, and oxygen consumption.

The other idea is that NFYB-1 is responsible for promoting mitochondrial gene expression, e.g. complex 1, and that further reduction of this expression places animals below a threshold for benefit. Rea and colleagues showed that mitochondrial longevity depends on an optimal reduction of mitochondrial activity too much or too little mitochondrial function abolished life extension (Durieux & Dillin 2007; Rea et al. 2007) . Perhaps knockdown of complex 1 subunits in the *isp-1* background could address this notion.

Although NFYB-1 regulation of ER and mitochondria could be entirely independent processes, we suggest rather a model that combines these two ideas whereby NFYB-1 promotes mitochondria gene expression, while suppressing ER stress response (Figure 24). In this view, NFYB-1 would coordinate ER-mitochondrial communication. Conceivably this may be a way to maintain a “firewall”, which blunts the spreading of mitochondrial stress to the ER. Alternately it may reveal how ER stress can thwart mitochondrial physiology under certain conditions. With this in mind, it would be appealing to see whether NFYB-1 activity or localization itself is regulated under different stress conditions. Communication between these two organelles has been observed previously through calcium signaling and ER-mitochondrial contact sites (Filadi et al. 2017; Murley & Nunnari 2016). Whether NFYB-1 visibly impacts these processes is unknown. Interestingly,

genetic screens for synthetic growth phenotypes with ER stress factors picked up a number of mitochondrial genes, as well as NFY-C (Sakaki et al. 2012; Shen et al. 2005). Yet clear transcriptional coordinators that modulate organellar communication in both directions have remained elusive.

Optimal protein homeostasis and efficient protein quality control depend on mechanisms ascribed to subcellular compartments (D'Amico et al. 2017; Broadley & Hartl 2008; Ruan et al. 2017). Though each organelle has a unique response, loss of homeostasis in one organelle can trigger responses in other cellular compartments, since crosstalk is essential to maintain cellular homeostasis. Our studies clearly show that an imbalance of ER factors has a major impact on mitochondrial biology, and NFYB-1 functions to maintain this balance. It also plays an important role in communicating mitochondrial stress to the cytosol through the MCSR. It will be interesting in the future to decipher the different levels of communication between these organelles and compartments, and how they influence organismal physiology and life span.

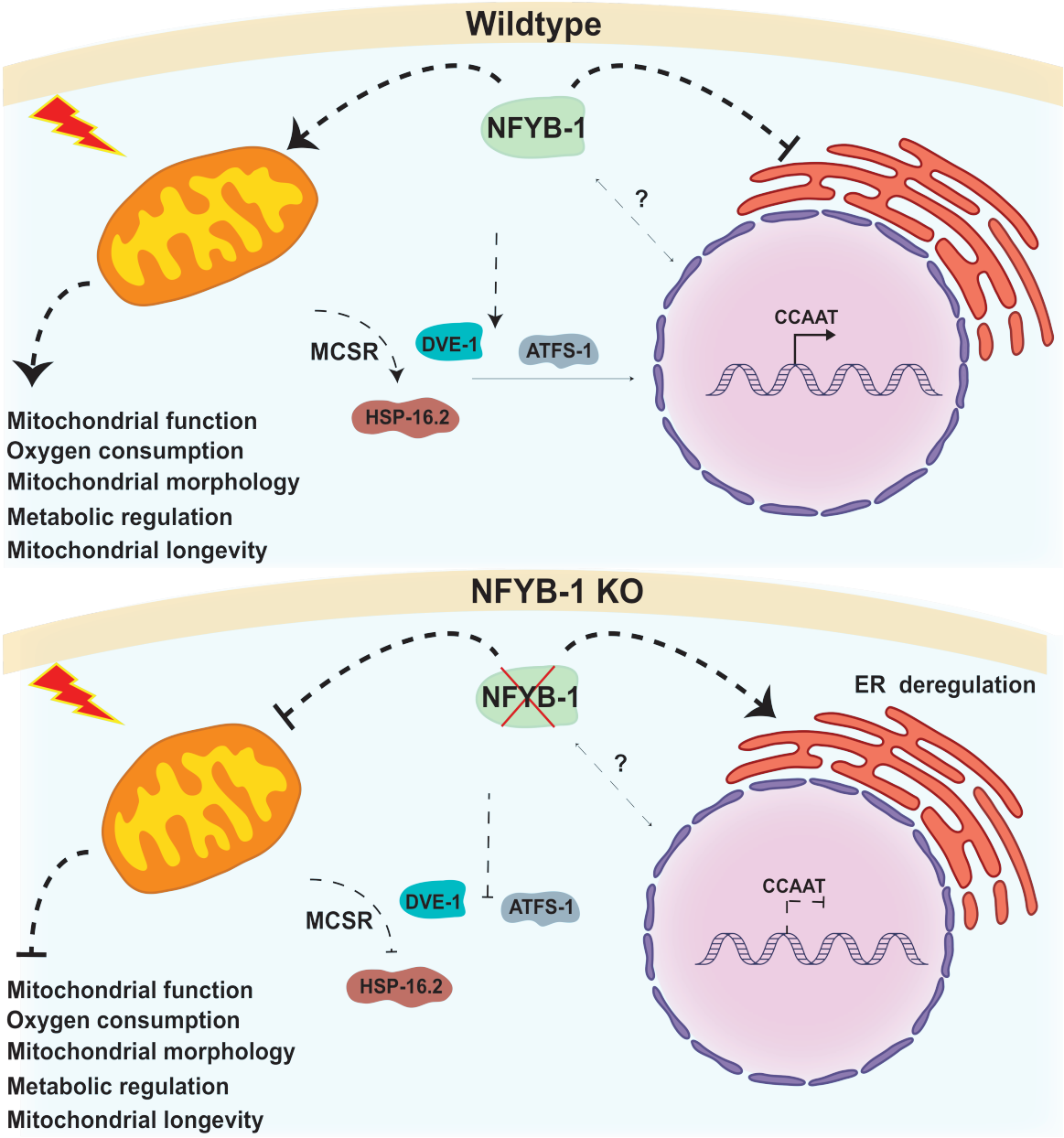


Figure 24: NFYB-1 regulates mitochondrial function and promotes longevity induced by mitochondrial impairment by modulating ER genes. We have identified an ancestral transcription factor NFYB-1 that can function as both activator and repressor of gene expression by binding to CCAATT binding region (Donati et al. 2008). Our data suggest that NFYB-1 function as activator of mitochondrial function and a repressor of ER associated genes under the wildtype condition. Hence upon loss of NFYB-1 mitochondrial function is disrupted leading to reduction of oxygen consumption and fragmented mitochondria. NFYB-1 is essential for longevity induced by mitochondrial impairment as in the case of *isp-1(qm150)* and RNAi knockdown of *cco-1*. Moreover, the nuclear localization

of UPR^{mt} factors DVE-1 and ATFS-1 upon mitochondrial stress is regulated by NFYB-1. Additionally, the induction of cytosolic stress response upon mitochondrial stress requires NFYB-1. Furthermore, our data clearly indicate that reduction in mitochondrial longevity upon loss of NFYB-1 is mediated by regulation of ER associated genes.

9 FUTURE PERSPECTIVE

9.1 Investigating regulation of *hlh-30/TFEB* and *drp-1* for ARD longevity

HLH-30 the TFEB homolog in *C. elegans* has shown to be essential to modulate longevity induced by Insulin/IGF signalling model *daf-2*, dietary restriction model *eat-2*, germline signalling model *glp-1* and mitochondrial mutant *clk-1* (Lapierre et al. 2013). Interestingly one of the factors that are known to play a key role in ARD longevity is *hlh-30/TFEB* (Birgit Gerisch, Adam Antebi et al unpublished). Additionally, we also observe fragmented mitochondria upon loss of HLH-30 under ARD in comparison to fused mitochondrial network in wildtype worms. Also, transcriptomic analysis shows a significant change in apoptotic genes upon HLH-30 loss in comparison to wild type under ARD (Birgit et al unpublished). As we observe that pro apoptotic *drp-1* loss leads to hyper fused mitochondrial network and better maintenance of mtDNA levels, we wondered if *drp-1* could rescue the short lifespan of *hlh-30* under ARD. If we observe a lifespan rescue we would further investigate how mitochondrial copy number and mitochondrial morphology is maintained under these conditions.

9.2 Investigating the mechanistic role of NFYB-1 and its function in a convergent network

The mechanism underlying the functional role of NFYB-1 is unclear in *C. elegans*. Interestingly, yeast two hybrid data from *C. elegans* indicate a conserved NFYB-1/DRAP-1 interaction. Strikingly we also identified *drap-1* as a potential candidate from our ARD recovery screen and RNAi knockdown of *drap-1* partially abolished mitochondrial longevity. To further investigate interacting partners of NFYB-1, we plan to construct an

endogenous NFYB-1 tagged CRISPR knock in strain and perform immunoprecipitation and subsequently detect associated interacting proteins by mass spectrometry. Additionally, by taking advantage of NFYB-1 reporter markers we plan to perform RNAi screen to identify upstream targets of NFYB-1. Once we have a potential list of interacting partners we plan to analyse these candidates by available mutants or RNAi knockdown. We will prioritise overlapping candidates from IP and RNAi screen, this approach would also help us to narrow down and built a network of factors. We hypothesize these factors might function in a convergent network with NFYB-1 to modulate ER genes expression, mitochondrial function, inter-organellar communication and longevity.

9.3 Deciphering the tissue specific role of NFYB-1 in modulating mitochondrial function and longevity.

Our findings clearly show an essential role of NFYB-1 in regulating longevity and mitochondrial function. We plan to further examine how longevity is regulated by NFYB-1 overexpression. We speculate that this loss of mitochondrial function and longevity can be rescued by an overexpression construct of NFYB-1. We plan to examine the expression pattern, tissue and subcellular location, and regulation in response to stress. It has been previously reported that benefits of mitochondrial longevity are also achieved in cell-nonautonoums manner. As it has been reported by (Durieux & Dillin 2010) that knockdown of *cco-1* just in intestine and neurons increases lifespan but not in muscles. Hence, we plan to further investigate tissue specific effects of NFYB-1 in regulating longevity, we intent to perform tissue specific knockdown of NFYB-1 using well established *sid-1* and *rde-1* tissue specific constructs. Once we can narrow down the tissue specific effect we plan to construct tissue specific overexpression strain to examine the tissue specific rescue of longevity. Interestingly knockdown of *cco-1* just in intestine results in an cell-

nonautonomous upregulation UPR^{mt} heat shock factor *hsp-6* in intestine (Durieux & Dillin 2010). Tissue specific constructs will additionally help us to better understand cross talk between organelles in different tissues.

9.4 Investigating the role of NFYB-1 in regulating cross talk between the subcellular compartments for protein quality control.

Efficient response mechanisms have been adapted by subcellular compartments to efficiently contain and resolve the protein misfolding stress (Pellegrino et al. 2013; Cohen et al. 2006; Haynes et al. 2007; Malhotra & Kaufman 2007). This helps to maintain optimal homeostasis and cellular function, never the less loss of homeostasis in one organelle can lead to detrimental effects of other compartment (Couvillion et al. 2016; D'Amico et al. 2017; Kim et al. 2016; Arantes-Oliveira 2002), hence cross talk between subcellular organelles are inevitable. Our data clearly suggest that NFYB-1 plays an essential role in regulating UPR^{mt} factors and MCSR, additionally. Additionally, most of the differentially expressed proteins encode ER associated genes, this further hint to the hypothesis of NFYB-1 being essential for cross talk between the organelles. Hence to better understand the role of NFYB-1 in cross talk between the organelles we plan to examine the UPR heat shock response of ER/*hsp-4*, mitochondria/*hsp-6* and cytosol/*hsp-16.2* under the wildtype condition and upon loss of NFYB-1. To do so we plan to induce 1) ER stress by tunicamycin feeding or *hsp-4* knockdown and examine the induction of mitochondrial/*hsp-6* and cytosolic/*hsp-16.2* expression. 2) mitochondrial stress by knockdown of *cco-1* or *hsp-6* and assess induction of cytosolic/*hsp-16.2* expression and specifically ER/*hsp-4*. As we already know that cytosolic stress response requires NFYB-1 we plan to examine 3) cytosolic proteotoxicity by using the strains Q35 and Q40. These strains contain polyglutamine repeats and they are used as Huntington's model in

C. elegans. As a read out for stress induction we use mitochondrial/*hsp-6* and ER/*hsp-4* expression in these models. Additionally, as our data hint to the possibility that NFYB could play an essential role in protein quality control and proteostasis we would further investigate the expression pattern, and subcellular location in response to mitochondrial, cytosolic and ER stress. Additionally, we would investigate whether loss of NFYB-1 makes the organism more vulnerable to proteotoxic disease using Aβ42/ Alzheimer's and α-synuclein/ Parkinson's disease model in *C. elegans*. This will shed light on cross talk between organelles under stress.

9.5 Deciphering how the ancestral transcription factor NFYB-1 regulates mitochondrial gene expression.

Nuclear encoded mitochondrial genes are translated in the cytosol and coordination between mitochondrial and nuclear encoded genes are pivotal for optimal mitochondrial function (Couvillion et al. 2016). Additionally, mitochondrial stress can initiate cytosolic stress response (Kim et al. 2016). Under protein stress GCN-2 mediated phosphorylation of eIF2α reduce the protein translation in the cytosol which in turn diminishes the load on mitochondrial chaperons. Moreover, upon longevity induced by mitochondrial stress as in the case with *isp-1(qm150)* the GCN-2 mediated eIF2α phosphorylation is enhanced, and RNAi knocking down of *gcn-2* abrogates mitochondrial longevity (Baker et al 2012).

Interestingly from the proteomics analysis for *N2 and nfyb-1(cu13)* we observe that GO term analysis for downregulated genes shows enrichment for NADH dehydrogenase activity indicating a probable role of NFYB-1 in regulating the protein levels of mitochondrial genes. Moreover, yeast homolog of transcription factor NFYB/HAP-3 function in regulating mitochondrial translation (Buschlen et al. 2003) hence we plan to investigate whether NFYB-1 is essential in regulating mitochondrial translation by

examining the eIF2α phosphorylation under mitochondrial stress. This will help us to better understand how NFYB-1 regulates mitochondrial function and gene expression.

9.6 Deciphering the role of NFYB-1 in modulating ER genes and its implications for mitochondrial function and longevity.

UPR^{ER} are mediated by (inositol-requiring protein 1) IRE-1, activating transcription factor 6 (ATF6), and protein kinase RNA (PKR)-like ER kinase (PERK). These UPR^{ER} factors regulate the downstream target genes to improve ER capacity and resolve protein stress (Malhotra & Kaufman 2007; Kaufman et al. 2002; Frakes & Dillin 2017a). Strikingly the activity and specificity of ATF-6 cytosolic fragment to regulate the target gene is controlled by its physical interaction with factors such as CCAATT binding nuclear factor y (NF-Y) (Haze et al. 1999; Yoshida et al. 2001; Yoshida et al. 2000; Chen et al. 2002). Hence, we plan to perform bioinformatics analysis to examine the overlap in downstream target genes between ATF-6 and NFYB-1.

Additionally, as we observe an induction of ER heat shock factor HSP-4, we plan to examine if the regulation of ER stress response upon loss of NFYB-1 is mediated by already known UPR^{ER} factors or by any of the deferentially expressed proteins from the proteomics analysis. Additionally, UPR^{ER} is essential to prevent larval arrest in *C. elegans*, interestingly a subset of mitochondrial genes and NFYC subunit were identified as essential factors for development of ER stress sensitive *ire-1* mutant worms (Sakaki et al. 2012; Shen et al. 2005). Similarly, as mitochondrial mutants show delayed development we plan to analyze synthetic growth phenotype in the mitochondrial background upon knockdown of UPR^{ER} factors and deferentially expressed proteins. Additionally, we plan to examine how the known UPR^{ER} factors regulate mitochondrial longevity upon *nfyb-1* loss.

Our data clearly suggest that NFYB-1 promotes mitochondrial gene expression, while suppressing ER stress response. We also observed that mitochondrial longevity is regulated by differentially expressed ER genes. Once we narrow down the candidates that significantly rescue the longevity without affecting *isp-1(qm150)* lifespan, we will further examine whether these factors can also rescue other mitochondrial phenotypes that we observe upon NFYB-1 loss such as mitochondrial morphology.

9.7 Investigating the role of NFYB-1 in metabolic and epigenetic regulation.

Additional it has been reported that long-lived mitochondrial mutants have similar metabolic footprints such as in the case of alpha-ketoacids. We are currently performing metabolomics analysis for *nfyb-1(cu13)* and long lived mitochondrial mutant *isp-1(qm150)* and shorter lived *isp-1(qm150); nfyb-1(cu13).* Metabolic dysfunction additionally leads to methylation changes to the chromatin structures (Jiménez-Chillarón et al. 2012). Remarkably mitochondrial stress leads to the remodelling of chromatin structures by H3K3 di-methylation (Tian et al. 2016b). Additionally, family of Jumanji proteins *jmjd1.2* and *jmjd 3.1* histone lysine demethylase have been described as epigenetic factors essential for mtUPR and mitochondrial longevity in *C. elegans* (Merkwirth et al. 2016). NF-Y transcription factor contains histone fold motif that specifically bind CCAATT, and are like to histone modifications (Donati et al. 2008; Nottke et al. 2009; Nardini et al. 2013). Moreover, similar to *nfyb-1* histone lysine demethylase Jumanji protein *jmjd-2* was also identified from our ARD recovery RNAi screen and interestingly mitochondrial mutant exometabolites inhibits *jmjd-2* (Falk et al. 2008; Mishur et al. 2016; Butler et al. 2013; Butler et al. 2010). As NFYB-1 plays an essential role in mitochondrial longevity, it will be particularly interesting to investigate whether these factors function

together in a network along with NFYB-1. These studies will give insight into metabolomic and epigenetic regulation of NFYB-1 to modulate mitochondrial function

9.8 Investigating the role of NFYB-1 in infection response.

Upon mitochondrial stress ATFS-1, UPR^{mt} factor is translocated into the nucleus where it activates downstream target genes to re-establish homeostasis, interestingly in addition to activation of mitochondrial associated genes. ATFS-1 also regulates innate immune response genes. Additionally, upon infection *C. elegans* mitochondria undergo fusion for protection. Moreover, loss of ATFS-1 makes the worms susceptible to infectious pathogens such as *P. aeruginosa* (Pellegrino et al. 2014). Additionally, it has been reported by (Liu et al. 2014) that mitochondrial impairment can be perceived by *C. elegans* as a pathogenic attack and thereby mitochondrial dysfunction leads to activation of innate immune response gene.

Interestingly proteomic and transcriptomic analysis has shown enrichment for activation of innate response genes upon loss of NFYB-1. Additionally, we also observe that NFYB-1 also regulates mitochondrial morphology and the nuclear localization of ATFS-1. Additionally, preliminary analysis indicates that loss of NFYB-1 makes the worms more sensitive to infection by *S. aureus* (Figure 25A). Another well-established pathway that regulates innate immune response is the MAP Kinase pathway. We additionally observe that transcript levels of *pmk-1, pmk-2* and *pmk-3* are downregulated upon NFYB-1 loss (Figure 24B). We hypothesize that NFYB-1 plays a pivotal role in infectious response by regulation of mitochondrial function and acts upstream of both ATFS-1 and PMK. We also plan to further examine how NFYB-1 differentially expressed ER genes regulate mitochondrial function in response to infection. This will also help us shed

light on how cross talk between compartments is regulated in response to external pathogenic infections.

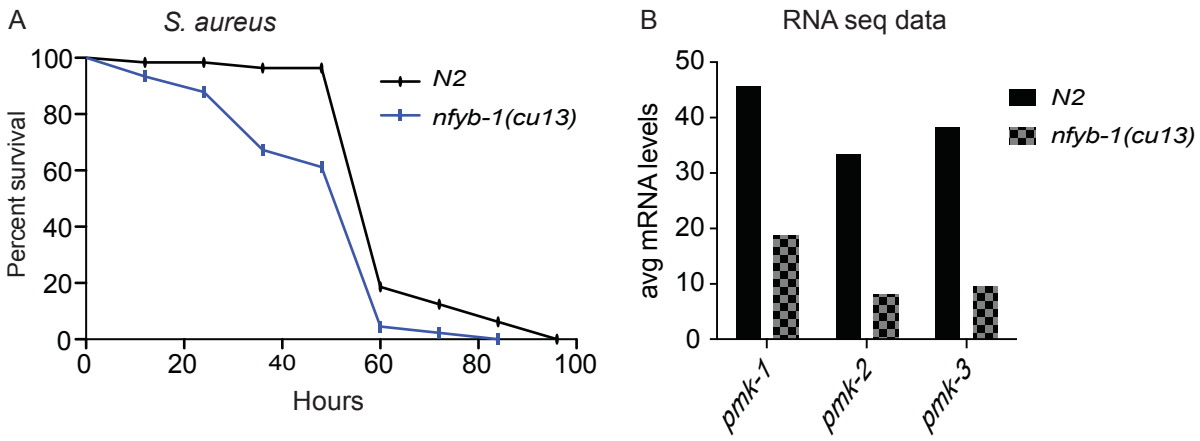


Figure 25: NFYB-1 regulates *C. elegans* immune response. A) *nfyb-1(cu13)* makes the worm sensitive to *S. aureus* infection in comparison to wildtype at 25 °C, n=1 performed by Varnesh Tiku. B) RNA seq analysis at ad libitum day 1 shows reduction in *pmk1, pmk-2* and *pmk-3* in *nfyb-1(cu13)* in comparison to N2.

11 MATERIAL AND METHODS

11.1 Methods

11.1.1 *C. elegans* strains

All strains were maintained at 20 °C on NGM plates seeded with a lawn of *E. coli* strain OP50. We used the following strains (refer Table.1) provided by *C. elegans* Genomic Center (CGC), other than ATR0011 (*p(par2.1)::gfp;rol6)* a kind gift from Dr. Aleksandra Trifunovic laboratory, University of Cologne. AGD643 (N2 *nthIs208[cco-1p::pestGFP, unc-54 3' utr, rol-6])* was provided by The Howard Hughes Medical Institute / The Salk Institute, Cal, USA. EX *zc376.7pr::zc376.7::gfp* was a kind gift from Dr. Haynes laboratory at UMASS Medical School, MA, USA. *(nfyb-1cu13)* was outcrossed at least three times to Antebi laboratory N2 wildtype strain and crossed into the respective genotypes. To decontaminate the strain and to obtain a synchronized population worms were bleached (sodium hypochlorite, potassium hydroxide and water). To do so plates were washed off with M9 buffer and collected in a falcon tube. Once the worms settled down supernatant was discarded and bleach was added and incubated at room temperature for 5-10 minutes. Samples were washed twice with M9 buffer at least three times and eggs that survived were transferred to plates containing *E. coli* OP50 bacteria.

Table. 1: Strains used for the experiments described in this thesis.

STRAIN NAME	GENOTYPE
SS104	*glp-4(bn2) I*
AA4385	*fzo-1(tm1133) II.*
AA4386	*drp-1(tm1108) IV.*
AA4387	*drp-1(tm1108) IV; zcIs14(myo-3::gfp(mt))*
SJ4103	*zcIs14(myo-3::gfp(mt))*
	pmyo-2::tomm-20::rfp
AA4715	*fzo-1(tm1133) II.; pmyo-2::tomm-20::rfp*
AGD643	*N2 nthIs208[cco-1p::pestGFP, unc-54 3' utr, rol-6]*
ATR0011	*p(par2.1)::gfp;rol6*
AA4714	*isp-1(qm150) IV*
CB1370	*daf-2(e1370) III*
AA3868	*eat-2(ad465) II*
AA3561	*glp-1(e2141ts)*
SJ4005	*zcIs4 [hsp-4::GFP] V*
SJ4058	*zcIs9[hsp-60::GFP]*
SJ4100	*ZCIS13(phsp-6::gfp)*
CL2070	*dvIs70 CL25 (phsp-16-2::GFP,pRF4(rol-6(su1006))*
SJ4197	*zcIs39([Pdve-1::dve-1::GFP]) II.*
SJ4151	*zcIs42([Publ-5::GFP]) X*
CMH1	*EX zc376.7pr:zc376.7::gfp*
AA4376	*nfyb-1(cu13)*
AA4377	*nfyb-1(cu13); N2 nthIs208[cco-1p::pestGFP, unc-54 3' utr, rol-6]*
AA4381	*nfyb-1(cu13); p(par2.1)::gfp;rol6*
AA4712	*nfyb-1(cu13); glp-4(bn2) I*
AA4378	*nfyb-1(cu13); zcIs14(myo-3::gfp(mt))*

AA4382	*nfyb-1(cu13); isp-1(qm150) IV*
AA4383	*nfyb-1(cu13); eat-2(ad465) II*
AA4384	*nfyb-1(cu13); daf-2(e1370) III*
AA4713	*nfyb-1(cu13); glp-1(e2141ts)*
AA4379	*nfyb-1(cu13); zcIs4 [hsp-4::GFP] V*
AA4380	*nfyb-1(cu13); zcIs9[hsp-60::GFP] V*
AA4564	*nfyb-1(cu13); ZCIS13(phsp-6::gfp)*
AA4565	*nfyb-1(cu13); dvIs70 CL25 (phsp-16-2::GFP,pRF4(rol-6(su1006))*
AA4566	*nfyb-1(cu13); zcIs39([Pdve-1::dve-1::GFP]) II.*
AA4710	*nfyb-1(cu13); zcIs42([Publ-5::GFP]) X*
AA4711	*nfyb-1(cu13); EX zc376.7pr:zc376.7::gfp*

11.1.2 ARD Induction and recovery

ARD induction: Adult worms were bleached to obtain a synchronized population. Animals in mid L3 grown on Nematode Growth Medium (NGM) plates seeded with a lawn of *E. coli* strain OP50 were analysed under DIC microscopy to confirm the stage. The worms are washed of the plates using M9 buffer and transferred to 1,5 ml eppendorf tube. The worms are allowed to settle down and they are washed four times, 20 minutes in M9 each to remove bacteria. Animals were then transferred to NGM agarose plates with 50 mg/ml ampicillin wrapped in parafilm and stored at 20 °C.

ARD recovery**:** ARD recovery was carried out by transferring ARD worms (day 9-10) on OP50 seeded NGM plates. For RNAi screening ARD worms were transferred to RNAi seeded (*E. coli* HT115) NGM plates containing 1 M Isopropyl β-D-1-thiogalactopyranoside (IPTG) to induce dsRNA expression.

11.1.3 Transcription factor RNAi screening

HT115 bacteria transformed with vectors expressing dsRNA of the genes of interest were obtained from the Ahringer library (Boutros & Ahringer 2008). RNAi colonies were grown overnight at 37 °C in Luri Broth with 50 µg/ml ampicillin and 10 µg/ml tetracycline, the cultures were spun down at 4000 rpm 4 °C for 10 min. 500 µL of one-fold concentrated culture was seeded onto agar plates containing 1M IPTG to induce dsRNA expression. HT115 (DE3) containing the empty vector L4440 and RNAi against gfp were used as controls for gfp expression analysis. *unc-22* RNAi which produces a visible "twitching" or uncoordinated (unc) phenotype was used as a control for RNAi induction.

11.1.4 Screening with copas biosorter

Screening was performed using the strain AGD643 (*pcco-1::gfp*) expressed in the pharynx. For screening day 10 ARD worms (150-200) were recovered by transferring to RNAi seeded plates. The recovery was carried out at 20 °C and the gfp expression was measured after 48 hours using the Copas Biosorter (worm sorter). The gfp expression is captured using Union Biometric Copas Biosorter and measured using the program Flowjo. Worms grown on RNAi plates were washed off after 48 hours with M9 buffer, and individual samples were run through the worm sorter. For *pcco-1::gfp* 600 PMT used as the laser voltage. The real-time analysis of these measured parameters is stored. Using the program Flowjo the mean gfp expression (Median Fluorescence Intensity- MFI) of the worms was measured. The MFI is measured by gating the region of desired size for ARD recovery worms (>450 on a scale for TOF 1024) and for reproductive adults (>500 on a scale for TOF 1024).

11.1.5 Confocal microscopy

For examining mitochondrial morphology under ARD and upon recovery *SJ4103 (zcIs14(myo-3::gfp(mt));*N2 was induced for ARD and around day

10 of ARD, worms were recovered on OP50 plates. Recovered worms were collected after 24 hours, along with day 10 ARD, day 1 and day 10 reproductive adult of SJ4103 *(zcIs14(myo-3::gfp(mt));* N2. To examine mitochondrial morphology under ARD for fission and fusion mutants N2*; (zcIs14(myo-3::gfp(mt)), drp-1(tm1108); (zcIs14 (myo-3::gfp(mt*)), N2*; pmyo-2::tomm-20:;rfp* and *fzo-1(tm1133)II; pmyo-2::tomm-20:;rfp* were induced for ARD and morphology was examined around day 10 of ARD. For analysing mitochondrial morphology of *nfyb-1(cu13)* upon ARD recovery N2*; (zcIs14(myo-3::gfp(mt)) and nfyb-1(cu13; (zcIs14 (myo-3::gfp(mt))* were induced for ARD and recovered worms were collected after 24 hours. And for reproductive adult bleached day 1 adult worms were used for an 4-5 hours egg lay to obtain a synchronized population, next generation day 1 worms were transferred daily till day 5 to new plates. Worms were mounted on 5% agarose pads using 20 mm levamisol as anaesthetic. A minimum of 15-25 worms were examined per strain and per condition per repeat. Mitochondrial morphology was observed using Leica TCS SP5-X confocal microscope with 100 times magnification.

11.1.6 Microscopy imaging

For examining nuclear localization *nfyb-1(cu13); EX zc376.7pr:zc376.7::gfp* and N2*; EX zc376.7pr:zc376.7::gfp* were grown on *hsp-6* and control *luci* RNAi from egg on, as described in (Nargund et al. 2012). From each genotype L3-L4 worms were mounted on 5% agarose pads using 20 mm levamisol as anaesthetic. Nuclear localisation was examined using 60 times magnification with Axio Imager Z1. A minimum of 15-20 worms were examined per strain, per condition and per repeat. The number of nuclear localization per number of nuclei observed was quantitated.

11.1.7 mtDNA copy number measurement

For measurement of mtDNA copy number under reproductive condition samples were collected at different larval stages L3, L4, young adult and

Day 1, *polg-1(tm2685)* was used as control. For ARD wildtype worms were induced to enter ARD, at day 10 of ARD worms were recovered on OP50 plates. Recovered worms were collected after 24 hours for mtDNA measurement. To examine mtDNA copy number in somatic tissues reproductive condition egg lay was performed for *glp-4(bn-2)* worms maintained at 15 °C and transferred to 25 °C along with wildtype under same condition and samples were collected at different larval stages L3, L4, young adult and day 1. Whereas for ARD recovery *glp-4(bn-2)* worms maintained at 15 °C were induced to enter ARD and recovered around day 10 ARD at 25 °C for 18 hours, wildtype worms were treated similarly. For measuring mtDNA copy number in fission and fusion mutants *drp-1(tm1108), fzo-1(tm1133)II* and N2 under ARD and under reproductive conditions , worms were induced for ARD and around day 10 of ARD worms (10 worms) were collected per well for mtDNA measurement. For reproductive condition, bleached day 1 adult worms were used for an 4-5 hours egg lay to obtain a synchronized population of *drp-1(tm1108), fzo-1(tm1133)II* and N2. Late L4 stage worms were collected for mtDNA measurement. For quantitating the levels of mtDNA copy number of RNAi knockdown ARD recovery screen candidates and synchronized wildtype worms were grown on candidate RNAi from egg on to late L4. Single worms from respective RNAi knockdown was collected for measurement and compared empty vector L4440 RNAi control. For analysing mtDNA copy number in *nfyb-1(cu13)* somatic tissues the double mutant *glp-4(bn-2); nfyb-1(cu13)* maintained at 15 °Cwere induced for ARD and transferred to 25° C along with wildtype under same condition. Around day 10, ARD worms were recovered at 25 °C for 18 hours. Worms were singled out and lysed using standard protocol. Quantification was carried out using RT-PCR to obtain absolute mtDNA copy number and relative mtDNA/nuDNA ratio as described in (Bratic et al. 2010).

11.1.8 Oxygen consumption assay

For quantitating oxygen consumption under ARD and upon recovery-synchronized N2 worms were induced for ARD and around day 10 of ARD, worms were recovered on OP50 plates. ARD, ARD recovered and synchronized population of day 1 N2 worms and *isp-1(qm150)* were used for comparison. BCA protein assay (kit- Thermo scientific) analysis was performed to quantitate the protein levels of total number of worms used for measuring oxygen consumption. For measurement of respiration under ARD and reproductive for fission and fusion mutants *drp-1(tm1108), fzo-1(tm1133) II* and N2 were induced for ARD and around day 10 of ARD 1000 worms were collected for measurement. Whereas for reproductive conditions bleached day 1 adult worms were used for an 4-5 hours egg lay to obtain a synchronized population of *drp-1(tm1108), fzo-1(tm1133) II* and N2. Day 1 stage worms were collected for measurement of respiration rate. For measuring oxygen consumption of *nfyb-1(cu13)* upon ARD recovery N2 and *nfyb-1(cu13)* were induced for ARD and around day 10 ARD worms were recovered on OP50 plates and worms were collected after 24 hours of recovery. For reproductive conditions, bleached day 1 adult worms were used for an 4-5 hours egg lay to obtain a synchronized population and day 1 worms were collected for measurement of respiration rate. Under all conditions 300 worms (mentioned otherwise) were transferred to unseeded plates and collected in M9. Once the worms settled they were transferred to the chambers and O2 flux per volume was measured for 10-15 min at 20 °C using the Oxygraph-2k from Oroboros Instruments.

11.1.9 Lifespan assay

Lifespan analyses were performed at 20 °C as previously reported (Gerisch et al., 2001). RNAi screen candidate lifespan analyses were performed using egg lay synchronized N2 and *isp-1(qm150)* upon knockdown of candidate RNAi from egg on. Similarly, for proteomics RNAi lifespan screen *isp-1(qm150) and isp-1(qm150); nfyb-1(cu13)* were fed with RNAi from egg

on. Lifespan analysis was performed at 20 °C with l*uci* as control. For examining lifespan upon RNAi knockdown of *cco-1* synchronized N2 and *nfyb-1(cu13)* upon RNAi knockdown from egg on and *luci* as control. For lifespan assays of longevity mutant models *isp-1(qm150) IV/nfyb-1(cu13); isp-1(qm150)IV, daf-2(e1370) III/ nfyb-1(cu13); daf-2(e1370)III and eat-2(ad465)II/nfyb-1(cu13); eat-2(ad465) II* bleached day 1 adult worms were used for an 4-5 hours egg lay to obtain synchronized population along with N2 and *nfyb-1(cu13).* Next generation worms were used and lifespan analysis was performed at 20 °C. Whereas for lifespan assay of germline deficient worms N2, *nfyb-1(cu13), glp-1(e2141ts)* and *nfyb-1(cu13); glp-1(e2141ts)* maintained at 15 °C were used for an 4-5 hours egg lay to obtain a synchronized population and transferred to 25 °C for 52 hours and transferred to 20 °C for lifespan analysis. A minimum 120 to 150 age-synchronized (4-5 hours) worms were used per condition and strain/RNAi. Experiments were carried out double blinded. Worms were scored every second day and transferred to new plates till end of reproductive period. Worms that had undergone internal hatching, vulva bursting, or that had crawled off the plates were censored. Data was plotted to calculate mean, median, and maximum lifespans. To determine significance between the lifespan curves log-rank (Mantel-Cox) analysis was used (Refer Table 3).

11.1.10 Measurement of GFP expression by copas biosorter

For measurement of mitochondrial marker expression upon ARD recovery *nfyb-1(cu13); p(par2.1)::gfp;rol6,* N2*; p(par2.1)::gfp;rol6, nfyb-1(cu13);nthIs208[cco-1p::pestGFP, unc-54 3' utr rol-6]* and N2*;nthIs208[cco-1p::pestGFP, unc-54 3' utr rol-6])* were induced for ARD and around day 10 of ARD worms were recovered on OP50 seeded NGM plates, recovered worms were collected after 24 hours. For measurement of gfp expression of at higher temperature synchronized population of above mentioned strains were shift to 25 °C after 4-5 hours egg lay at 20 °C and samples were collected for measurement after around 52 hours. Whereas for ad libitum

conditions synchronized population of day 1 adult worms at 20 °C were collected for measuring expression levels. For quantitating expression of UPR^{mt} reporter lines the following genotypes *nfyb-1(cu13); zcIs9[hsp-60::GFP]V,* N2*; zcIs9[hsp-60::GFP] V, nfyb-1(cu13); ZCIS13(phsp-6::gfp),* N2*; ZCIS13(phsp-6::gfp), nfyb-1(cu13); zcIs42([Publ-5::GFP])X,* N2*; zcIs42([Publ-5::GFP]) X,nfyb-1(cu13); zcIs39([Pdve-1::dve-1::GFP])II* and N2*; zcIs39([Pdve-1::dve-1::GFP])II* were bleached to obtain synchronized population of day 1 adults which were used to perform 4-5 hours egg lay on *cco-1* and *luci* control RNAi. Day 1 samples were collected for measurement of gfp expression of respective samples. For measurement of MCSR *nfyb-1(cu13); dvIs70 CL25(phsp-16-2::GFP,pRF4(rol-6(su1006))* and N2*;dvIs70 CL25 (phsp-16-2::GFP,pRF4(rol-6(su1006))* were bleached to obtain synchronized population of day 1 adults which were used to perform 4-5 hour egg lay on OP50 plates, on day 1 of adulthood worms were transferred to *hsp-6* and *luci* control RNAi. Until day 3 worms were transferred to fresh plates and on day 3 samples were collected for measurement of gfp expression (Kim et al. 2016). BodiPY staining (2μm working stock, thermos fisher scientific ref: D3823) was similarly done using N2 and *nfyb-1(cu13)* on *hsp-6* and *luci* control RNAi. To measure UPR^{ER}, heat shock protein expression synchronized population of *nfyb-1(cu13); zcIs4 [hsp-4::GFP]V* and N2;*zcIs4 [hsp-4::GFP]V* were grown on OP50 seeded NGM plates till day 1 and worms were transferred to 10 ug tunicamycin plates to induce UPR^{ER}. After 6 hours of tunicamycin treatment gfp expression was measured along with control strains on OP50 seeded NGM plates without tunicamycin. The gfp intensity was kept constant for every genotype throughout the repeats of every independent experiment. All samples were washed off the plate and collected in M9 buffer, and individual samples were run to capture the *gfp* expression using Union Biometric Copas Biosorter and measured using the program Flowjo.

11.1.11 Tunicamycin survival assay

For examining survival upon tunicamycin stress 100 eggs were transferred on 3 cm plates containing NGM with tunicamycin (10ug/ml). *gfat-1(dh785)* gain-of-function mutant was used as a control for the assay (Denzel & Antebi 2015). 72 hours after transferring the number of unhatched eggs and worms in different larval stages L1, L2, L3, L4 and day 1 were counted.

11.1.12 Western blot analysis

For western blot analysis synchronized population of *zcIs39([Pdve-1::dve-1::GFP])II, nfyb-1(cu13);zcIs39([Pdve-1::dve-1::GFP])II,* N2 and *nfyb-1(cu13)* was used for performing 4-5 hours egg lay on *cco-1* RNAi and control luci RNAi. Additionally, *hsp-6, dve-1* and *gfp* RNAi were used for respective experiment as controls. Day 1 adult worms were collected in M9 (1.5 and 2-fold more worms were collected for N2 and *nfyb-1(cu13)* for samples upon *cco-1* RNAi knockdown). Laemmli lysis buffer was added to the sample, snap-frozen in liquid nitrogen and stored at -80 °C. The samples were thawed at room temperature water-bath and frozen in liquid nitrogen for five to seven cycles. Then boiled at 95 °C for 5 minutes, ultra-sonicated for 10 cycles and loaded on 4-15 % Mini-PROTEAN® TGX™ Precast Protein Gels. After separation, proteins were blotted on a nitrocellulose membrane using Trans-Blot® Turbo™ Transfer System (Bio-Rad). The membranes were then blocked for an hour at room temperature in 5 % milk in Tris-buffered Saline and Tween20 (TBST) and probed with the following antibodies against: HSP-60 (BD, 611562, 1:5000), GFP (takara, 632381,1:2000) and anti-histone (Abcam, ab9108, 1:5000). The antibodies were diluted in TBST with 1 % Bovine Serum Albumin (BSA). The membranes were incubated in the primary antibody overnight at 4 °C. Specific secondary antibodies (mouse or rabbit) were used at a concentration of 1:4000 in TBST with 1 % BSA at room temperature for one hour. The membranes were developed with Western Lightening® Plus-Enhanced Chemiluminescence Substrate (PerkinElmer). The membranes were imaged then with ChemiDoc Imager (BioRad).

11.1.13 Real Time- quantitative PCR and RNA sequencing analysis

For quantification of transcript levels of RNAi screen candidates synchronized WT worms were grown on candidate RNAi from egg on to late L4. Empty vector L4440 was used as the sample control. Samples were collected by late L4 stage in TRIzol (Invitrogen) and frozen in liquid nitrogen and stored at –80 °C. For RNA isolation samples were thawed in 37 °C water bath and frozen in liquid nitrogen for five to seven cycles. Samples were shaken (50 hz) with around 100 µl of glass-beads using TissueLyser LT (QIAGEN) at 4 °C. Afterwards chloroform was added to the samples and tubes were spun. Supernatant was used for mRNA preparation in accordance to RNeasy Mini Kit (QIAGEN) manual. For mRNA isolation 70 % EtOH was used. DNase treatment was performed each time with RNase-Free DNase (QUIAGEN) for 15 min at RT. Isolated RNA was measured with Nano Drop 2000c (peqLab) to determine quality and purity of each.

With respect to the quantity of each RNA sample cDNA was prepared with reverse transcriptase using iSCRIPT protocol and subsequently diluted with ddH2O to 1 ng/µl. For preparation of 384 well mRNA plates JANUS automated workstation (PerkinElmer) was used. Power SYBR Green (Applied Biosystems) was used for RNA quantitation and samples were analyzed in technical quadruplicates with 7900HT Fast Real-Time PCR System. A standard program for comparative CT values including Melting curve was used on both qPCR machines. For primer validation, standard curve program was used. *act-1* was used as internal control for mRNA (Refer Table 2 for primer sequence).

For RNA seq analysis a minimum of 5000 day 1 worms for each genotype were collected in TRIzol. Samples were prepared as described above. Libraries were quantified followed by sequencing-by-synthesis on a HiSeq2500 at the Max Planck Genome Center, Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). Reads were adapted and quality

trimmed using Flexbar version 2.5(1). After trimming reads were mapped to the reference genome (GRCm38, release 79) using hisat2 version 2.0.4 (2). Guided transcript assembly was performed with StringTie version 1.3.0 (2) and respective assemblies merged with cuffmerge version 2.2.1 (4). After feature quantification with cuffquant version 2.2.1 (4) differential gene expression was done with Cuffdiff version 2.2.1(4). Differential expressed genes (*q*-value<0.05) of each pairwise comparison was identified. GO annotation and enrichment analysis was performed using the DAVID (version 5) bioinformatics resources database.

11.1.14 Proteomics

For sample collection and preparation each genotype was bleached to obtain a synchronized population of worms. Day 1 worms were used for 5-6 hours of egg lay to obtain highly synchronized population of N2*, nfyb-1(cu13), isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* strains with a minimum of 5000 worms. Samples were collected on day 1 in cold M9 and washed thrice in M9 and once in distilled water and directly frozen in liquid nitrogen, samples were then stored at – 80°C. Lysis buffer (refer material section) was added to sample (20 µl to 10 µl of worm pellet). Sample was heated for Heat at 95^{o}C for 5-10 min and the sample was lysed with the Bioruptor (30s sonication, 30s breaks, 10 cycles). This step was repeated two to three times. Sample was then centrifuged at 20000g for 20 min. 2µl of the protein supernatant was then diluted at least 10 times with 20mM Tris to 20µl to reduce the concentration of GuCL to lower than 0.6M. Protein concentration was then measured by Nano Drop. The sample was then diluted 10 times and digested with trypsin (1:2000) (Promega, Mass Spec Grade) overnight at 37^{o} C. the digestion was stopped by adding 50% of FA to 1% (final concentration).

For LC-MS/MS analysis peptides were separated on a 25 cm, 75 µm internal diameter PicoFrit analytical column (New Objective) packed with 1.9

µm ReproSil-Pur 120 C18-AQ media (Dr. Maisch) using an EASY-nLC 1200 (Thermo Fisher Scientific). The column was maintained at 50°C. Buffer A and B were 0.1% formic acid in water and 0.1% formic acid in 80% acetonitrile. Peptides were separated on a segmented gradient from 6% to 31% buffer B for 120 min and from 31% to 50% buffer B for 10 min at 200 nl/min. Eluting peptides were analyzed on a QExactive HF mass spectrometer (Thermo Fisher Scientific). Peptide precursor m/z measurements were carried out at 60000 resolutions in the 300 to 1800 m/z range. The top ten most intense precursors with charge state from 2 to 7 only were selected for HCD fragmentation using 25 % normalized collision energy. The m/z values of the peptide fragments were measured at a resolution of 30000 using a minimum AGC target of 8e3 and 55 ms maximum injection time. Upon fragmentation, precursors were put on a dynamic exclusion list for 45 sec.

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.

11.2 Material

Solution	Composition
LB medium	10 g NaCl 10 g tryptone 5 g yeast Fill up to one liter Adjust pH to 7.0 using 1N NaOH
NG Agarose plates	25 g Agarose 25 ml KPO_4 1M 3 g NaCl 2.25 g Peptone fill up to one liter add 1 ml $CaCl_2$ 1M, 5mg/ml $MgSO_4$ 1M cholesterol 50mg/ml Amp (after autoclaving)
RNAi plates	17 g Agarose 25 ml KPO_4 1M 3 g NaCl 2.5 g Peptone fill up to one liter add 1 ml $CaCl_2$ 1M, 5mg/ml $MgSO_4$ 1M cholesterol 50mg/ml Amp and IPTG 1M (after autoclaving)
M9 buffer	3 g KH_2PO_4 1 ml $MgSO4$ 1M 5 g NaCl 6 g Na_2HPO_4

	fill up to one liter add $MgSO_4$ after autoclaving
Single Worm Lysis buffer	50 mM KCl 2.5 mM $MgCl_2$ 0.45 % NP-40 (or Triton-X100) 10 mM Tris pH 8.3 0.45 % Tween
Lysis buffer for proteomics	6M Guanidinium chloride 2.5mM TCEP (Tris(2-carboxyethyl) phosphine) 10mM CAA (chloroacetamide) 100mM Tris-HCl

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13 APPENDIX

Table 2: Primers used for qPCR, mtDNA measurement and PCR genotyping designed using online tool primer 3plus

GENE	SEQUENCE
qPCR primers	
fzo-1 F	TCTGCAGGTTGAAGGTTCAGAAGGC
fzo-1 R	AGTCGGCATTCCCCTGATTCCG
opa-1 F	TCGCGGCTAGAACGTGGTATGA
opa-1 R	CTTGGCCATCCATTCTGCCCA
drp-1 F	AGGCTGAAGCCCACCAATGAGC
drp-1 R	ACCGGTTGTAGCTGCGCTCT
dnj-10 F	GCGGGCTCATTCATCGATCTGTAC
dnj-10 R	CAGATTTTTTGTCGACACCCAAAG
tim-23 F	CAACTGAAATCTGCTGGAGTAGGAG
tim-23 R	GGCATAATGTATTGCGGCTGC
skn-1 F	TCCACCAGCATCTCCATTCG
skn-1 R	CTCCATAGCACATCAATCAAGTCG
hsp-6 F	AACCACCGTCAACAACGCCG
hsp-6 R	AGCGATGATCTTATCTCCAGCGTCC
sod-3 F	CTAAGGATGGTGAACCTTCA
sod-3 R	CGCGCTTAATAGTGTCCATCAG
sod-2 F	ACAGGAGTCGCTGCTGTTCGC
sod-2 R	TCCTTTGGAGACCGCCTCGTGA
gas-1 F	GTTGCGTCTCGTGTTGAAACTGG
gas-1 R	TCGAAGTATGGGAGAGCCTGAG
mev-1 F	GGAACATCGATCGTCACCAAGTCC
mev-1 R	CGCTGCTTCAACAGGTATTCCC
isp-1 F	TCTTGGATGTGTCCCAATTGCC

isp-1 R	CGTCGTAGTGAGATCCGTGACAAG
atp-2 F	TATGGCTTCGCGTTCGTTAGCC
atp-2 R	TTGTTTGAGCTGAGGCGGATGG
clpp-1 F	ATTAACAGTCCAGGCGGCAGTG
clpp-1 R	TTTCACCGGCACAAAGCAGCAG
hmg-5 F	AAGAAGTACACAGACGAAGCGAAG
hmg-5 R	TGTTCGGCTTCCCATTTCTGGAG
rpom-1 F	TCGTTGAATTGTGCACAGAGAGG
rpom-1 R	GAATTGGCGTGCGTCCAGAAAC
tomm-40 F	TATCAAACCGAGCTGCCAGAGG
tomm-40 R	TCGAATACACCACCAACAGTCCAG
mtss-1 F	CAACCGAGCAAGCAAGAGGTTG
mtss-1 R	ATGCATGCCGTCTTTGTTCTGG
acs-2 F	AGATCACCGCGGAGCACATTAAG
acs-2 R	TGGAACCTTGTAGTGAGCCATTCC
ant 1.4 F	CCAATCGGACTCTACAGAGGTTTC
ant 1.4 R	AGTTTCTTGCCATCAGCAGTGAAC
nd-1 F	GAACTTAACCGGGCGCCATTTG
nd-1 R	AAAGCTACTCTGGCAAACTCCAC
ctb-1 F	TTCCAATTTGAGGGCCAACT
ctb-1 R	AACTAGAATAGCTCACGGCAATAAAAA
nd-5 F	TTAGCAAGTTTGGTCGAAGAAGATT
nd-5 R	GGCCCAAAGTAACTATTGAAAAACC
act-1 F	CCCACTCAATCCAAAGGCTA
act-1 R	ATCTCCAGAGTCGAGGACGA
hsp-16.2 F	TAGATGTTGGTGCAGTTGCTTCG
hsp-16.2 R	TGGCGCTTCAATCGAAAGTTTAC
MT COPY NO	
act-3 F	F: TGCGACATTGATATCCGTAAGG
act-3 R	R: GGTGGTTCTCCGGAAAGAA

nd-1 F	F: AGCGTCATTTATTGGGAAGAAGAC
nd-1 R	R: AAGCTTGTGCTAATCCCATAAATGT
PCR genotyping primers	
nfyb-1(cu13) F	caatttttgagctggaagcc
nfyb-1(cu13) R	cccggttttacctgcagtaa
nfyb-1(cu13) I	TGAAAAATCATCATTCTCAGTACTTCA
drp-1 (tm1108) F	tgaagagcaaatccgtgaaa
drp-1 (tm1108) R	taactcgattcgcaactgctt
fzo-1 (tm1133) F	agagaatcctgtcccattgc
fzo-1 (tm1133) R	agtggttcattatcggcagc

Table 3: Relative mtDNA levels of WT worms on RNAi knockdown of candidates. RNAi treatment from egg on to late L4 stage, t. test, n=3.

RNAi CONDITION	R1	R2	R3	P.VALUE	AVERAGE	% CHANGE
l4440	62.73364	65.24128	59.98201		62.65231	
pqn-80	57.01754	32.28701	35.6161	0.028162013	41.64021667	33.5376195
msp-49	33.41998	10.97843	12.0195	0.002102892	18.80597	69.98359677
ubl-1	20.64376	15.23361	14.57869	2.40154E-05	16.81868667	73.15552026
let-607	23.20327	17.77966	31.25088	0.000389441	24.07793667	61.56895625
skn-1	145.452	98.70753	41.06145	0.171932763	95.07366	-51.74805207
Y95B8A.7	61.02449	44.14292	57.77626	0.098395327	54.31455667	13.30797433
zip-12	74.24632	71.25617	74.50928	0.002198888	73.33725667	-17.05435389
gei-3	188.813	91.15546	36.37447	0.195849301	105.4476433	-68.30607416
nfyb-1	85.84049	56.66413	95.38998	0.114709301	79.2982	-26.5686772
his-34	14.33455	46.41225	41.08723	0.022982773	33.94467667	45.82055048
elt-7	32.16571	26.38155	28.53412	6.05213E-05	29.02712667	53.6695029
lim-6	56.37655	51.49641	74.44741	0.402770707	60.77345667	2.998857238
R151.4	51.34018	30.34446	37.64153	0.01128786	39.77539	36.51408863
hsf-1	57.01052	105.729	56.24565	0.281691642	72.99505667	-16.50816493
F18F11.4	37.02987	53.6972	48.95504	0.018055033	46.56070333	25.68397984
nhr-61	36.21638	28.89024	22.17324	0.000746803	29.09328667	53.56390424
mag-1	48.64593	26.0689	39.58423	0.010918826	38.09968667	39.18869605

ncl-1	71.65451	58.39635	58.27046	0.490295805	62.77377333	-0.193868883
drap-1	58.55489	36.72366	38.15145	0.032682303	44.47666667	29.01033231
jhdm-1	69.31264	65.3126	64.24475	0.084079444	66.28999667	-5.806149313
vhp-1	32.0071	29.4766		0.000343742	30.74185	50.93261525
K03H1.10	42.06158	35.0055	40.42126	0.000426663	39.16278	37.49188178
dmd-6	84.60249	40.15134	62.8423	0.49651001	62.53204333	0.191958871
jmjd-2	80.12637	45.08763	74.57829	0.368756666	66.59743	-6.296846836
atfs-1	67.04004	37.06734	26.60965	0.096596316	43.57234333	30.45373214
mnat-1	61.23954	44.58304	71.20773	0.334619787	59.01010333	5.813363732
ttx-1	70.76228	81.51459	40.42344	0.452331085	64.23343667	-2.523652626
crh-1	42.9749	53.48277	60.6403	0.063443607	52.36599	16.41810174
wdr-23	68.99814	58.24627	47.51547	0.264357611	58.25329333	7.021316
grh-1	36.4133	62.46926	85.8275	0.471755182	61.57002	1.727454263
tbx-40	57.79906	45.57921	66.05952	0.185787144	56.47926333	9.852863632
rpn-11	67.33588	36.03228	35.76115	0.099555066	46.37643667	25.97808977
Y39G10AR.8	56.729	25.08027	32.66659	0.032141941	38.15862	39.09463195
F15A8.7	72.63573	63.71865	32.11521	0.313853096	56.15653	10.36798164
cky-1	105.147	82.18154	43.49487	0.236475986	76.94113667	-22.80654403
tbx-34	53.37485	41.28523	72.93177	0.253889144	55.86395	10.83497161
gfp	61.92097	63.61317	58.83711	0.296815484	61.45708333	1.907713645

Table 4: Proteomics data indicated 40% of the differentially regulated proteins between *isp-1(qm150)* and *isp-1(qm150); nfyb-1(cu13)* were ER associated (marked in red).

	nfyb-1/ N2	***isp-1/ N2***	***isp-1;nfyb-1/ N2***	***isp-1;nfyb-1/ isp1***	***nfyb-1/ N2***	***isp-1/ N2***	***isp-1; nfyb-1/ N2***	***isp-1; nfyb-1/ isp1***
GENE	**log FC**	**log FC**	**log FC**	**log FC**	**SIG***	**SIG***	**SIG***	**SIG***
Y49G5A.1	-0.24	3.99	-0.54	-4.53		+		+
ttr-34	-3.74	0.04	-3.36	-3.40	+		+	+
C44B7.5	-0.34	0.67	-1.06	-1.73			+	+
C39H7.1	0.23	-1.55	-2.84	-1.28		+	+	+
ilys-5	-0.11	-0.95	-2.23	-1.28		+	+	+
asp-13	-1.18	0.78	-0.40	-1.19	+	+		+
C30G12.2	-1.21	-1.32	-2.30	-0.98	+	+	+	+
crt-1	-1.26	-0.29	-1.26	-0.97	+		+	+
acs-1	-0.39	-0.58	-1.46	-0.88		+	+	+
T13F3.6	-1.34	-0.30	-1.11	-0.81	+		+	+
pdi-2	-0.77	-0.38	-1.06	-0.68	+	+	+	+
gfat-2	-0.40	-0.60	-1.15	-0.55		+	+	+
gln-5	-0.13	0.13	-0.40	-0.53			+	+
C39D10.7	-0.14	0.50	0.00	-0.50		+		+
Y18D10A.11	-0.30	-0.73	-1.14	-0.40	+	+	+	+

K06G5.1	0.27	-0.21	0.21	0.42				+
haf-9	0.35	-0.03	0.48	0.51			+	+
Y54G2A.23	0.72	-0.21	0.34	0.55	+		+	+
H34I24.2	0.39	-0.01	0.55	0.55	+		+	+
ifd-1	0.43	0.06	0.63	0.57	+		+	+
dsc-4	1.19	0.17	0.75	0.58	+		+	+
F09E8.2	0.59	-0.27	0.35	0.62	+	+	+	+
tag-320	0.61	-0.40	0.23	0.63	+	+	+	+
skr-4	0.40	0.19	0.85	0.66	+		+	+
dct-18	0.81	-0.44	0.27	0.71	+	+	+	+
C39H7.4	0.65	0.05	0.80	0.75	+		+	+
dnj-20	1.09	-0.44	0.32	0.76	+	+	+	+
cht-1	0.23	1.69	2.46	0.77		+	+	+
C14B9.2	0.94	-0.05	0.73	0.78	+		+	+
clec-63	0.72	0.83	1.69	0.86	+	+	+	+
T14G8.3	1.01	-0.29	0.59	0.87	+	+	+	+
gale-1	1.04	-0.51	0.36	0.88	+	+		+
dnj-7	1.00	-0.40	0.49	0.89	+	+	+	+
R12E2.13	0.98	-0.52	0.41	0.93	+	+	+	+
hsp-4	1.29	-0.09	0.90	0.99	+		+	+
C45B2.2	0.96	-1.54	-0.50	1.04	+	+	+	+
F52B11.2	1.01	-0.82	0.23	1.05	+	+		+
noah-1	0.40	0.69	1.75	1.06		+	+	+
sym-1	-0.19	0.93	2.00	1.06		+	+	+
lpr-3	0.10	0.61	1.96	1.34		+	+	+
ARGK-1	0.37	0.65	2.13	1.48			+	+
Y105E8A.2	1.51	-0.97	0.54	1.51	+	+		+
set-18	0.27	-1.95	-0.26	1.69		+		+
mlt-11	0.25	0.51	2.26	1.76			+	+
catp-3	2.01	-0.16	1.70	1.85	+		+	+
ckb-2	2.53	-0.74	1.16	1.90	+	+	+	+
Y41C4A.32	2.73	-0.50	2.29	2.78	+		+	+
spp-8	3.22	0.10	3.60	3.50	+		+	+

Table 5: 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	MEAN	MEAN % CHANGE	MEDIAN	MEDIAN % CHANGE	CENSORED	DEATHS	SIG*	REF. CONTROL
Mito FISSION & FUSSION								
N2	21		21		51	60		
drp-1	22	5	22	5	48	69	0.0506	*vs.* N2
N2/ ARD	56		56		46	399		
drp-1/ ARD	67	20	68	21	28	276	0.0e+00	*vs.* N2 ARD
N2	19		20		45	71		
drp-1	21	11	20	0	35	86	0.0014	*vs.* N2
N2/ ARD	62		64		47	692		
drp-1/ ARD	69	11	64	0	25	261		*vs.* N2 ARD
N2	18		20		42	78		
drp-1	20	11	21	5	48	72	0.7686	*vs.* N2
N2/ ARD	51		48		30	618		
drp-1/ ARD	58	14	55	15	42	768	0.0e+00	*vs.* N2 ARD
N2	19		20		45	71		
fzo-1	19	0	20	0	56	65	0.9786	*vs.* N2
N2/ ARD	62		64		47	692		
fzo-1 / ARD	49	-21	50	-22	41	773	0.0e+00	*vs.* N2 ARD
N2	18		20		42	78		
fzo-1	20	11	21	5	48	72	0.7686	*vs.* N2
N2/ ARD	59		56		26	295		
fzo-1 / ARD	44	-25	44	-21	38	476	0.0e+00	*vs.* N2 ARD
N2	19		19		38	77		
fzo-1	18	-5	19	0	58	59	0.1244	*vs.* N2
N2/ ARD	59		56		76	923		
fzo-1 / ARD	43	-27	43	-23	4	289	0.0e+00	*vs.* N2 ARD
ARD RECOVERY CANDIDATE RNAI								
N2 luci	16.92	0.00	15.00	0.00	54.00	66.00		
N2 tbx-40i	22.35	32.06	22.00	46.67	55.00	65.00	< 0.0001	vs N2 luci
N2 grh-1i	19.02	12.36	19.00	26.67	58.00	62.00	0.00	vs N2 luci
N2 tbx-34i	20.28	19.82	19.00	26.67	77.00	43.00	< 0.0001	vs N2 luci
N2 let-607i	10.43	-38.38	12.00	-20.00	72.00	28.00	< 0.0001	vs N2 luci
N2 ttx-1i	18.50	9.31	19.00	26.67	88.00	32.00	0.03	vs N2 luci
N2 mnat-1i	17.81	5.24	19.00	26.67	83.00	37.00	0.02	vs N2 luci
N2 crh-1i	19.82	17.10	19.00	26.67	65.00	55.00	< 0.0001	vs N2 luci
N2 atfs-1i	18.72	10.61	19.00	26.67	52.00	68.00	0.00	vs N2 luci
N2 jmjd-2i	19.97	18.02	19.00	26.67	44.00	76.00	< 0.0001	vs N2 luci
N2 Luci	20.42	20.68	20.00	0.00	81.00	68.00		
N2 dmd-6i	21.31	25.94	22.00	10.00	109.00	41.00	0.59	vs N2 luci
N2 gei-3i	14.04	-17.06	14.00	-30.00	97.00	53.00	<0.0001	vs N2 luci
N2 zip-12i	20.67	22.12	20.00	0.00	91.00	59.00	0.99	vs N2 luci
N2 elt-7i	19.94	17.81	20.00	0.00	78.00	72.00	0.51	vs N2 luci
N2 his-34i	17.76	4.93	18.00	-10.00	88.00	62.00	0.00	vs N2 luci

N2 nfyb-1i	19.35	14.31	18.00	-10.00	80.00	70.00	0.22	vs N2 luci
N2 drap-1i	24.78	46.44	24.00	20.00	111.00	39.00	0.00	vs N2 luci
N2 nhr- 61i	20.83	23.10	24.00	20.00	40.00	110.00	0.86	vs N2 luci
N2 luci	21.44	0.00	23.00	0.00	72.00	53.00		
N2 Y95B8A.7i	24.95	16.34	26.00	13.04	94.00	31.00	0.00	vs N2 luci
N2 F15A8.7i	24.92	16.21	26.00	13.04	94.00	31.00	0.00	vs N2 luci
N2 F15811.4	33.25	55.08	33.00	43.48	77.00	48.00	<0.0001	vs N2 luci
N2 Y39610AR.8	22.22	3.62	18.00	-21.74	69.00	56.00	0.94	vs N2 luci
N2 jhdm-1	21.18	-1.20	23.00	0.00	94.00	31.00	0.54	vs N2 luci
N2 mag-1i	26.16	22.01	26.00	13.04	90.00	35.00	<0.0001	vs N2 luci
N2 rpn-1I	10.27	-52.08	8.00	-65.22	64.00	61.00	<0.0001	vs N2 luci
N2 R151.4I	24.76	15.47	23.00	0.00	86.00	39.00	0.00	vs N2 luci
N2 KO3H1.10i	15.53	-27.58	16.00	-30.43	102.00	23.00	<0.0001	vs N2 luci
N2 luci	21.31	0.00	18.00	0.00	85.00	35.00		
N2 cky-1i	20.90	-1.93	0.00	-100.00	29.00	91.00	0.75	vs N2 luci
N2 ubl-1	22.30	4.66	22.00	22.22	79.00	42.00	0.42	vs N2 luci
N2 wdr-23i	18.01	-15.48	18.00	0.00	55.00	65.00	0.00	vs N2 luci
N2 msp-49i	21.36	0.23	22.00	22.22	68.00	52.00	0.91	vs N2 luci
n2 vhp-1 i	17.16	-19.50	18.00	0.00	77.00	43.00	0.03	vs N2 luci
isp-1(qm150) luci	23.23	0.00	26.00	0.00	89.00	130.00		
isp-1(qm150) tbx- 40i	21.48	-7.55	19.00	-26.92	55.00	65.00	0.02	vs i*sp-1(qm150)* luci
isp-1(qm150) grh-1i	18.95	-18.41	19.00	-26.92	55.00	65.00	0.02	vs i*sp-1(qm150)* luci
isp-1(qm150) tbx-34i	21.22	-8.68	19.00	-26.92	56.00	64.00	< 0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) let-607i	11.47	-50.64	15.00	-42.31	55.00	65.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) ttx-1i	27.06	16.49	28.00	7.69	90.00	30.00	< 0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) mnat-1i	24.83	6.89	19.00	-26.92	87.00	33.00	0.19	vs i*sp-1(qm150)* luci
isp-1(qm150) crh-1i	23.86	2.70	19.00	-26.92	61.00	59.00	0.68	vs i*sp-1(qm150)* luci
isp-1(qm150) atfs-1i	18.69	-19.54	19.00	-26.92	50.00	70.00	0.62	vs i*sp-1(qm150)* luci
isp-1(qm150) jmjd-2i	22.94	-1.23	19.00	-26.92	65.00	55.00	< 0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) luci	30.22	0.00	30.00	0.00	44.00	76.00		
isp-1(qm150) dmd-6i	27.13	-10.21	30.00	0.00	82.00	58.00	< 0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) gei-3i	19.56	-35.28	20.00	-33.33	115.00	35.00	0.10	vs i*sp-1(qm150)* luci
isp-1(qm150) zip-12i	24.69	-18.28	24.00	-20.00	141.00	9.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150 elt-7i	22.55	-25.38	20.00	-33.33	108.00	42.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) his-34i	24.76	-18.06	24.00	-20.00	102.00	33.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) nfyb-1i	20.70	-31.49	20.00	-33.33	56.00	55.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) drap-1i	24.55	-18.74	24.00	-20.00	47.00	43.00	<0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) Y95B8A.7i	22.64	-25.07	20.00	-33.33	91.00	44.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) F15A8.7i	22.90	-24.21	22.00	-26.67	121.00	29.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) cky-1i	23.47	-22.33	20.00	-33.33	122.00	28.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) nhr-61i	24.28	-19.66	24.00	-20.00	113.00	37.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) luci	30.39	0.00	31.00	0.00	31.00	49.00		
isp-1(qm150) F18F11.4i	38.13	25.46	39.00	25.81	78.00	47.00	<0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) Y39610AR.8i	27.75	-8.70	26.00	-16.13	99.00	26.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) jhdm-1i	28.42	-6.50	29.00	-6.45	67.00	58.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) mag-1i	34.87	14.72	36.00	16.13	106.00	19.00	0.35	vs i*sp-1(qm150)* luci
isp-1(qm150) rpn-11i	9.07	-70.15	10.00	-67.74	86.00	39.00	0.03	vs i*sp-1(qm150)* luci
isp-1(qm150) R151.4i	28.91	-4.88	26.00	-16.13	103.00	22.00	0.60	vs i*sp-1(qm150)* luci
isp-1(qm150) K03H1.10i	14.92	-50.90	14.00	-54.84	79.00	46.00	<0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) ubl-1	26.15	-13.97	26.00	-16.13	91.00	34.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) wdr-23i	29.16	-4.07	29.00	-6.45	88.00	37.00	0.25	vs i*sp-1(qm150)* luci
isp-1(qm150) msp-49i	23.09	-24.02	29.00	-6.45	101.00	22.00	0.49	vs i*sp-1(qm150)* luci
isp-1(qm150) vhp-1i	27.80	-8.52	26.00	-16.13	93.00	32.00	0.09	vs i*sp-1(qm150)* luci
LONGEVITY PATHWAYS								
N2	19.97	0.00	18.00	0.00	32.00	88.00		
nfyb-1(cu13)	15.15	-24.13	15.00	-16.67	43.00	77.00	0.09	vs N2
isp-1(qm150) IV	26.49	32.63	29.00	61.11	62.00	58.00	<0.0001	vs N2
eat-2(ad465) II	23.27	16.50	22.00	22.22	88.00	32.00	<0.0001	vs N2
daf-2(e1370) III	33.50	67.75	38.00	111.11	84.00	36.00	<0.0001	vs N2

nfyb-1(cu13); isp-1(qm150) IV	18.18	-31.35	19.00	-34.48	65.00	55.00	<0.0001	vs i*sp-1(qm150)*
		-8.95		5.56			0.16	vs N2
nfyb-1(cu13); eat-2(ad465) II	15.33	-34.12	15.00	-31.82	47.00	73.00	<0.0001	vs *eat-2(ad465) II*
		-23.24		-16.67			0.01	vs N2
nfyb-1(cu13); daf-2(e1370) III	27.27	-18.60	26.00	-31.58	110.00	10.00	0.01	*vs daf-2(e1370) III*
		36.55		44.44			<0.0001	vs N2
N2	16.96	0.00	16.00	0.00	87.00	73.00		
nfyb-1(cu13)	15.80	-6.83	16.00	0.00	82.00	78.00	0.06	vs N2
isp-1(qm150) IV	22.26	31.26	21.00	31.25	112.00	48.00	<0.0001	vs N2
eat-2(ad465) II	21.02	23.96	21.00	31.25	50.00	90.00	<0.0001	vs N2
daf-2(e1370) III	33.36	96.72	37.00	131.25	169.00	71.00	<0.0001	vs N2
nfyb-1(cu13); isp-1(qm150) IV	14.94	-32.87	16.00	-23.81	126.00	32.00	0.00	vs i*sp-1(qm150)*
		-11.88		0.00			0.93	vs N2
nfyb-1(cu13); eat-2(ad465) II	13.80	-34.37	14.00	-33.33	96.00	61.00	<0.0001	vs *eat-2(ad465) II*
		-34.37		-12.50			0.00	vs N2
nfyb-1(cu13); daf-2(e1370) III	26.62	-20.21	28.00	-24.32	217.00	23.00	0.00	*vs daf-2(e1370) III*
				75.00			<0.0001	vs N2
N2	21.33	0.00	22.00	0.00	120.00	80.00		
nfyb-1(cu13)	18.22	-14.56	17.00	-22.73	127.00	73.00	0.00	vs N2
isp-1(qm150) IV	27.05	26.81	25.00	13.64	151.00	49.00	<0.0001	vs N2
eat-2(ad465) II	23.54	10.34	25.00	13.64	89.00	111.00	0.00	vs N2
daf-2(e1370) III	35.41	65.99	38.00	72.73	125.00	75.00	<0.0001	vs N2
nfyb-1(cu13); isp-1(qm150) IV	19.74	-27.02	17.00	-32.00	149.00	51.00	<0.0001	vs i*sp-1(qm150)*
		-7.45		-22.73			0.16	vs N2
nfyb-1(cu13); eat-2(ad465) II	18.94	-19.51	17.00	-32.00	145.00	51.00	<0.0001	vs *eat-2(ad465) II*
		-11.19		-22.73			0.01	vs N2
nfyb-1(cu13); daf-2(e1370) III	25.77	-27.22	25.00	13.64	170.00	30.00	<0.0001	*vs daf-2(e1370) III*
		20.81		-100.00			0.01	vs N2
N2	20.63	0.00	22.00	0.00	56.00	64.00		
nfyb-1(cu13)	20.52	-0.52	22.00	0.00	83.00	37.00	0.75	vs N2
glp-1(e2141ts)	29.30	42.04	26.00	18.18	68.00	52.00	<0.0001	vs N2
nfyb-1(cu13); glp-1(e2141ts)	20.80	-29.02	20.00	-23.08	57.00	63.00	<0.0001	vs *glp-1(e2141ts)*
		0.83		-9.09			0.28	vs N2
N2	20.63	0.00	22.00	0.00	56.00	64.00		
nfyb-1(cu13)	20.52	-0.52	22.00	0.00	83.00	37.00	0.75	vs N2
glp-1(e2141ts)	27.19	31.81	26.00	18.18	55.00	65.00	<0.0001	vs N2
nfyb-1(cu13); glp-1(e2141ts)	23.49	-13.60	22.00	-15.38	71.00	49.00	0.01	vs *glp-1(e2141ts)*
		13.88		0.00			0.01	vs N2
N2	20.77	0.00	21.00	0.00	78.00	82.00		
nfyb-1(cu13)	18.90	-9.01	18.00	-14.29	95.00	65.00	0.04	vs N2 luci
N2 cco-1i	23.35	12.39	23.00	9.52	102.00	58.00	0.01	vs N2 luci
nfyb-1(cu13) cco-1i	20.17	-13.63	18.00	-21.74	74.00	86.00	0.00	vs N2 cco-1
		-2.92		-14.29			0.62	vs N2 luci
N2	19.34	0.00	21.00	0.00	82.00	38.00		
nfyb-1(cu13)	18.72	-3.23	18.00	-14.29	76.00	44.00	0.63	vs N2 luci
N2 cco-1i	24.20	25.09	23.00	9.52	53.00	67.00	<0.0001	vs N2 luci
nfyb-1(cu13) cco-1i	18.54	-23.39	18.00	-21.74	65.00	65.00	0.00	vs N2 cco-1
		-4.17		-14.29			0.26	vs N2 luci
PROTEOMICS CANDIDATE RNAI								
isp-1(qm150) Luci	24.72	0.00	26.00	0.00	33.00	117.00		
isp-1(qm150) Y49G5A.1i	26.34	6.57	28.00	7.69	38.00	62.00	0.16	vs i*sp-1(qm150)* luci
isp-1(qm150) F52B11.2i	18.17	-26.48		-100.00	23.00	77.00	0.00	vs i*sp-1(qm150)* luci
isp-1(qm150) ckb-2i	28.28	14.42	28.00	7.69	11.00	89.00	0.37	vs i*sp-1(qm150)* luci
isp-1(qm150) C45B2.2i	25.84	4.54	28.00	7.69	18.00	82.00	0.84	vs i*sp-1(qm150)* luci
isp-1(qm150) R12E2.13i	26.11	5.64	28.00	7.69	24.00	76.00	0.68	vs i*sp-1(qm150)* luci
isp-1(qm150) asp-13i	24.48	-0.97	22.00	-15.38	47.00	53.00	0.16	vs i*sp-1(qm150)* luci
isp-1(qm150) argk-1i	25.10	1.54	28.00	7.69	37.00	63.00	0.09	vs i*sp-1(qm150)* luci
isp-1(qm150) FO9E8.2i	30.05	21.59	35.00	34.62	29.00	71.00	0.01	vs i*sp-1(qm150)* luci

isp-1(qm150) set-18i	24.83	0.46	22.00	-15.38	31.00	69.00	0.95	vs i*sp-1(qm150)* luci
isp-1(qm150) spp-8i	25.76	4.22	28.00	7.69	31.00	69.00	0.39	vs i*sp-1(qm150)* luci
isp-1(qm150) tag-320i	27.78	12.41	35.00	34.62	26.00	74.00	0.18	vs i*sp-1(qm150)* luci
isp-1(qm150) T14G8.3i	25.51	3.23	28.00	7.69	21.00	79.00	0.81	vs i*sp-1(qm150)* luci
isp-1(qm150) dnj-7i	31.16	26.06	35.00	34.62	23.00	77.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) C14B9.2i	29.35	18.76	28.00	7.69	18.00	82.00	0.01	vs i*sp-1(qm150)* luci
isp-1(qm150) Y54G2A.23i	31.71	28.30	38.00	46.15	40.00	60.00	<0.0001	vs i*sp-1(qm150)* luci
isp-1(qm150) gale-1i	24.51	-0.83	28.00	7.69	40.00	60.00	0.03	vs i*sp-1(qm150)* luci
isp-1(qm150); nfyb-1(cu13) Luci	19.58	0.00	22.00	0.00	31.00	119.00		
isp-1(qm150); nfyb-1(cu13) Y49G5A.1i	19.64	0.30	22.00	0.00	8.00	92.00	1.00	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) F52B11.2i	20.59	5.17	22.00	0.00	18.00	82.00	0.60	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) ckb-2i	20.88	6.64	22.00	0.00	11.00	89.00	0.66	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) C45B2.2i	21.07	7.61	22.00	0.00	8.00	92.00	0.42	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) R12E2.13i	21.62	10.40	22.00	0.00	13.00	87.00	0.34	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) asp-13i	21.78	11.21	22.00	0.00	18.00	82.00	0.35	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) argk-1i	22.53	15.03	28.00	27.27	11.00	89.00	0.07	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) FO9E8.2i	22.69	15.89	22.00	0.00	17.00	83.00	0.09	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) set-18i	22.81	16.50	22.00	0.00	9.00	91.00	0.09	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) spp-8i	24.03	22.72	22.00	0.00	16.00	82.00	0.02	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) tag-320i	24.30	24.10	22.00	0.00	26.00	74.00	0.03	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) T14G8.3i	24.41	24.66	28.00	27.27	19.00	81.00	0.01	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) dnj-7i	24.63	25.77	22.00	0.00	34.00	66.00	0.01	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) C14B9.2i	25.27	29.06	28.00	27.27	17.00	83.00	0.01	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) Y54G2A.23i	25.39	29.67	28.00	27.27	13.00	87.00	0.01	vs *isp-1; nfyb-1* luci
isp-1(qm150); nfyb-1(cu13) gale-1i	26.88	37.28	35.00	59.09	13.00	87.00	0.05	vs *isp-1; nfyb-1* luci

14 ACKNOWLEDGEMENTS

I would like to express my gratitude to Prof. Adam Antebi for giving me the great opportunity to perform my doctoral research in his wonderful laboratory. His guidance, trust and support always motivated me to be dedicated to my project. His curiosity for science sets a unique example of being a true scientist which will always inspire me to be committed to my work. I would also like to thank my PhD committee: Prof. Thomas Langer, Prof. Aleksandra Trifunovic for always giving suggestions for my project. I want to specially thank my thesis committee chair Prof. Mats Paulsson for finding time to evaluate my thesis work.

I would like to extend my thankfulness to Dr. Birgit Gerisch for introducing me to ARD, teaching me biosorter work and for all her suggestions for my project and thesis. Special thanks to Raymond Laboy for teaching me basic illustrator skills. I would further like to thank the entire A-team for all the scientific inputs and for creating a great working atmosphere, especially to the encouraging PhD crew. I would like to thank all former Antebi Lab members especially Dr. Varnesh Tiku for all his suggestions for my project. Special thanks to former Antebi Lab member Dr. Ben Becker for translating the abstract to German.

I have to specially thank Dr. Christoph Geisen for all his scientific advices for my project and thesis, specifically for prompting me to follow safe laboratory conduct and being a great friend. I personally want to convey my gratefulness to the wonderful friends I have gained from the lab especially Dr. Andrea Annibal, Gabriel Gurrero, Jennifer Mak and Christian Latza. Specifically, Dr. Oezlem Karalay for all her scientific advices and being a fun bench mate. I would like convey my gratitude to my mother, my cousin Daniel Varghese and the rest of the huge family for all the love, encouragement and well wishes. Last but not least I want to thank my dear

friends especially Dr. Trieu-My Van, Dr. Vipin Babu, Vicky Klein and Dr. Estella Cepeda for all the love, support and wonderful memories.

15 WORK CONTRIBUTION

All experiments described in this thesis were performed by myself. Except for ARD and reproductive longevity of mitochondrial fission and fusion mutants, which was performed by Dr. Birgit Gerisch, who also helped me in with scoring proteomics lifespan RNAi screen. Additionally, I had assistance from our master internship student Lea Schneider in performing Tunicamycin stress resistance assay. Infection assay was performed by Dr. Varnesh Tiku. RNA seq analysis was performed at Max-Plank Genomic Center, Koeln. Bioinformatics analysis on transcriptomics data was performed by Dr. Jorge Bouças and Dr. Rafeal Cuadrat at the bioinformatics core facility at Max-Plank for Biology of ageing. Proteomics assay and analysis was performed by Dr. Ilian Atanassov at the proteomic core facility at Max-Plank for Biology of ageing.

16 ERKLÄRUNG

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