Further characterization of *PGC-1α*, *PARIS* and *VPS13C*

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A thesis submitted to the

School of Graduate Studies

In partial fulfillment of the requirement for the degree of

Master of Science

Department of Biology

Memorial University of Newfoundland

2023

St. John's, Newfoundland and Labrador

Abstract

Parkinson Disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the *substantia nigra* of the brain and is often accompanied by the presence of Lewy bodies in surviving neurons. A number of genes have been identified to contribute, when expression is altered, to the pathogenesis of PD. This study focuses on *PGC-1α*, *PARIS*, and *VPS13C* which have all been implicated in pathways involved with mitochondrial biogenesis and lipid transport; two important processes that have been implicated in PD. Using *Drosophila melanogaster* as a model organism, I have investigated the consequences of altered gene expression using the homologues *spargel* (*srl*), *Paris*, and *Vps13* to further characterize the role that alterations to the expression of these genes play in disease and ageing. Most notably, this study found that enhanced expression of *srl* decreased longevity and locomotor ability when expressed in the motor neurons. Reduced expression of *srl* was found, in some instances, to increase longevity when expression was directed to the motor neurons, and glial cells. The overexpression of *Paris* in the motor neurons increased longevity, while overexpression in both the motor neurons and glial cells improved locomotor ability. Investigating altered *Vps13* expression yielded varying results. However, this study demonstrated that when expression of *Vps13* is enhanced in the motor neurons, dopaminergic neurons, and glial cells, longevity is decreased. Additionally, inhibition of *Vps13* in the motor neurons, dopaminergic neurons, and glial cells can increase longevity in *Drosophila*. These results help to further characterize these genes and their respective roles in models of human disease and ageing. I evaluated *UAS-LUC-RNAi* as a negative control for *RNAi*, as well as the potential for enhanced longevity using three *Gal4* lines which are new to the Staveley research group. Most notably, this study determined that *UAS-LUC-RNAi* is a suitable control for *RNAi* with the *Gal4* in longevity experiments and

should be used in future studies. As well, *C380-Gal4,* is not a suitable activating transgene for expression in the motor neurons during longevity experiments as this reduced lifespan when driving the expression of a *lacZ* control.

General Summary

Parkinson Disease (PD) is a progressive age-related disease which affects 1% of those over 60, and up to 4% in those over 80 years of age. PD targets the neurons of the brain which produce dopamine, a neurotransmitter and hormone. Dopamine is important in many functions including reward and motivation, memory, and movement. Therefore, people with PD exhibit physical symptoms such as muscle slowness, weakness, stiffness, and tremor. Currently, treatments for PD only target symptoms, as there is not yet a cure for the disease. Given the advances in global life expectancy, diseases such as PD are likely to increase in prevalence. Gaining a better understanding of PD and healthy ageing may lead to better treatments and quality of life for ageing people. Approximately 5 to 10% of PD cases are believed to have a genetic link, and several PD genes have been identified. In this study, fruit flies were used to study the fly versions of three genes, *PGC-1α, PARIS* and *Vps13C*, which have been shown in people to contribute to PD onset. These three genes play important roles in mitochondrial biogenesis, a process that generates new mitochondria, as well as lipid transport. Mitochondrial health is especially of interest, as the mitochondria are incredibly important cellular components which have several functions including the production of energy. Model organisms are species which are studied to understand a biological process, which may then give insight into other species including humans. *Drosophila melanogaster*, the common fruit fly, is one such organism which is important in genetic studies. The fruit fly contains about 100,000 neurons and can show complex behaviours such as courtship, navigation, and learning. In addition, many disease-causing genes have similar counterparts in the fruit fly. Modeling aspects of PD in fruit flies may give us great insight into how disease onset and ageing works in humans.

Acknowledgements

I would like to thank my supervisor Dr. Brian E. Staveley for his guidance and support, as well as my supervisory committee – Dr. Dawn Bignell and Dr. Helene Volkoff for their constructive feedback. I would also like to thank Dr. David Schneider for his assistance with statistics, as well as Allison Porter for making sense of it. Lastly, I would like to thank my family and friends for their belief in me (supporting the support).

This research was funded by the Department of Biology of Memorial University of Newfoundland Teaching Assistantship and a Memorial University of Newfoundland School of Graduate Studies Fellowship to MFK and by the Memorial University Seed Fund and by a Natural Science and Engineering Council of Canada (NSERC) Discovery Grant to BES.

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List of Abbreviations

AMPKα – AMP-activated protein kinase alpha

ATG2 – Autophagy-related protein 2

ATP – Adenosine triphosphate

BLAST – Basic Logical Alignment Search Tool

blastp – Protein Basic Logical Alignment Search Tool

CREB – cAMP response element-binding protein

D. melanogaster – Drosophila melanogaster

DA – Dopaminergic

DNA – Deoxyribonucleic acid

dsRNA – double-stranded RNA

dTOR – *Drosophila* target of rapamycin

ER – Endoplasmic reticulum

ERRα – Estrogen-related receptor alpha

ETC – Electron transport chain

FoxO – Forkhead box transcription factor (class O)

G6PD – glucose-6-phosphate dehydrogenase

GFP – Green fluorescent protein

GM1 – monosialotetrahexosylganglioside

H. sapiens – Homo sapiens

hSOD1 – Human superoxide dismutase 1

IMM – Inner mitochondrial membrane

KRAB – Krueppel-associated box

LRRK2 – Leucine-rich repeat kinase 2

LTP – Lipid transport protein

mRNA – messenger RNA

mtDNA – mitochondrial DNA

NCBI – National Centre for Biotechnology Information

NLS – Nuclear localization sequence

NRF – Nuclear respiratory factor

OMM – Outer mitochondrial membrane

PARIS – Parkin interacting substrate

PD – Parkinson Disease

PGC-1α – Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

PINK1 – PTEN-induced putative kinase 1

PSA – Pairwise sequence alignment

PTEN – Phosphatase and Tensin Homolog

RNA – Ribonucleic Acid

RNAi – RNA interference

ROS – Reactive oxygen species

SE – Standard error

siRNA – Small interfering RNA

SIRT1 – Sirtuin 1

SNpc – substantia nigra pars compacta

srl – spargel

TACO1 – Translational Activator of Cytochrome C Oxidase 1

TFAM – Mitochondrial transcription factor a

TH – Tyrosine hydroxylase

TIM23 – Mitochondrial import inner membrane translocase subunit 23

UAS – Upstream Activation Sequence

UPR – Unfolded protein response

VAB – Vps13 adaptor binding

VAP – VAMP-associated protein

VPS13 – Vacuolar protein sorting 13

VPS13C – Vacuolar protein sorting 13 homologue C

ZAD – Zinc finger associated domain

ZNF746 – Zinc finger protein 746

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Chapter 1: Introduction and Overview

1.1 Purpose

With advances in global life expectancy, the ageing population has demonstrated increased susceptibilities to age-related ailments, such as neurodegenerative disease. A prime example is Parkinson Disease which is prevalent and severely impacts quality of life. The goal of this study is to better investigate the consequences of altered expression of *PGC-1α*, *Paris*, and *Vps13C*, all of which have been identified as Parkinson Disease-related genes. How such alterations in *Drosophila melanogaster* as a model organism impact survivorship and locomotor ability is of interest. The relationship between mitochondrial function, lipid transport, and Parkinson Disease is of special importance in this study.

1.2 Parkinson Disease

Parkinson Disease (PD) is a progressive neurodegenerative disease which affects 1% of individuals above the age of 60, and up to 4% in those aged 80 and older (Antony *et* al., 2013; Gazewood *et al*., 2013). PD is characterized by the loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta* (*SNpc*) of the midbrain. Accompanying this is the accumulation of harmful protein aggregates, known as Lewy bodies, which consist largely of the protein α-Synuclein. The aggregation of α-Synuclein has been demonstrated to interfere with the necessary subcellular transport for synaptic function and neuronal homeostasis (Antony *et* al., 2013; Mhyre *et al*., 2012). Hallmark motor symptoms of PD include tremor, rigidity, bradykinesia, and postural instability. Non-motor symptoms may include depression, anxiety, apathy, constipation,

and memory loss among others (Armstrong & Okun., 2020; Zesiewicz., 2019). PD presents both sporadic and familial forms, with 5 to 10% of cases having a genetic link. Several PD genes have been identified, which includes *α-Synuclein* (*SNCA*), *parkin* and *PTEN-induced putative kinase 1* (*PINK1*), and *Leucine-rich repeat kinase 2* (*LRRK2*). These genes have shown importance in membrane trafficking dynamics and mitophagy. Mutations in PD genes have contributed to the development of the disease, especially in early-onset forms (Antony *et* al., 2013; Mhyre *et al*., 2012). At this time existing treatments do not cure or stop disease progression, but rather manage the symptoms of PD. Understanding the complex underlying cellular and molecular pathways involved in PD onset is imperative for the development of new and better treatments.

1.3 Mitochondria and Parkinson Disease

Mitochondria are conserved intracellular organelles that act as the site for aerobic respiration to provide energy for the cell through the synthesis of ATP via oxidative phosphorylation (Popov, 2020). These are dynamic organelles that can change in number, morphology, and function in response to physiological stressors (Golpich *et al.,* 2016). This is especially important for neurons, as these are complex cells with high energy demands, and about 20% of resting ATP production occurs in the brain (Kodavati *et al*., 2020; Wang *et al*., 2020). Mitochondria are essential for the regulation of calcium concentration for signal transduction, as neurons are cells that undergo excitation. In addition, mitochondria are vital in the regulation of cell survival and death under various conditions. Since neurons are long-lived cells, mitochondria are important for the protection of neuronal function throughout life (Wang *et al*., 2020; Golpich *et al.,* 2016). Other mitochondrial processes include amino acid and nucleotide metabolism, protein synthesis, fatty acid metabolism, ion homeostasis, and apoptosis

(Kodavati *et al*., 2020). Evidence shows that mitochondria play an important role in ageing and have been prominent in features of neurodegeneration.

Alterations in fission and fusion dynamics have been shown in patients with PD (Kodavati *et al*., 2020). Defective fusion in neurons causes swelling of mitochondria, which prevents entrance into smaller distal neuronal branches. This results in degradation in the axons and dendrites. Defective fission results in the failure to isolate damaged parts of the mitochondria which promotes their autophagic removal, promoting neuronal apoptosis (Kodavati *et al*., 2020). Deficiencies in Complex I of the electron transport chain (ETC) have been reported in the *SNpc* and frontal cortex of PD patients. This demonstrates that increased oxidative damage and reduced electron transfer rates occur through Complex I subunits (Henchcliffe and Beal., 2008; Kodavati *et al*., 2020). An increase in oxidative stress due to mitochondrial dysfunction can cause damage to lipids, proteins and DNA. This oxidative damage may induce α-Synuclein aggregation and impair the proteolytic system involving protein ubiquitination and degradation (Henchcliffe and Beal, 2008). Mitochondrial health is imperative for overall health of the cell, particularly in neurons.

1.4 Role of Ageing in Neurodegeneration

Ageing may be characterized by an accumulation of biological changes over time to result in the functional decline of an organism. This is generally coupled with cellular senescence (Abdul Halim *et al*., 2019; Kritsilis *et al*., 2018). Cellular senescence involves irreversible damage to DNA and is a normal part of ageing. An accumulation of senescent cells with age may be a contributor of disease pathogenesis. Cellular senescence is a preventative measure to stop damaged cells from multiplying and is a useful anti-tumor response. Evidence suggests that

cellular senescence contributes to the functional decline associated with ageing and diseases of the aged (Kritsilis *et al*., 2018). Initiators of senescence include oxidative stress, hyperoxia, impaired autophagy, and mitochondrial dysfunction. These can lead to telomeric or non-telomeric damage to DNA, or altered chromatin structure, and the activation of the DNA damage response. When cellular repair mechanisms are overwhelmed, cellular senescence is induced via the DNA damage response. Although senescent cells can be removed by apoptosis, these are viable and metabolically active, and increase in numbers with age (Popov, 2020; Abdul Halim *et al*., 2019; Kritsilis *et al*., 2018). Models of accelerated cellular senescence in mammals show premature ageing and increased age-related disease (Popov, 2020). Cellular senescence affects various processes in the mitochondria and is associated with impairment in mitochondrial biogenesis and bioenergetic potential, a decrease in mitochondrial dynamics, faulty quality control, failed mtDNA repair, an accumulation of mtDNA mutations, and a decrease in mitophagy. Mainly, it is the reduced activity of *AMPKα*, as well as the decreased expression of *PGC-1α*, *SIRT1* (which activates PGC-1α mediated transcription of nuclear and mitochondrial genes required for mitochondrial proliferation), *TFAM*, *NRF-1*, and *NRF-2*. The regulatory loop involving PGC-1α and NRF-2 interaction is also altered (Popov, 2020). Clearly ageing is a prominent risk factor for a plethora of diseases and is the most important risk factor for neurodegenerative disease.

1.5 *Drosophila melanogaster* as a Model Organism

Drosophila melanogaster has been used widely as a model organism for the past hundred years for studying concepts such as fundamental genetics, tissue development, and disease. A short lifespan and easy, inexpensive maintenance requirements are some advantages to using the fruit fly model. In addition, large numbers of offspring may be produced in a short amount of time, allowing for larger sample sizes (Jeibmann and Paulus, 2009). The *D. melanogaster* genome has been fully sequenced, and approximately 75% of disease-causing genes have functional homologues in this organism (Ugur *et* al., 2016; Aryal & Lee, 2019; Mirzoyan *et al*., 2019). With the use of various genetic techniques, information may be gained on ageing and disease pathogenesis from altering the expression of disease-causing genes in fruit flies.

1.6 *UAS-Gal4* System

Several genetic techniques have been developed to allow for the study of gene expression in model organisms such as *D. melanogaster*. One such method is the *UAS-Gal4* system which allows for the control of expression of a particular gene in a time and tissue-dependent manner. This consists of two transgenic components: the transactivator gene (*Gal4*) and the effector gene under the control of the upstream activating sequence (*UAS*). The *Gal4* gene encodes a protein (*Gal4*), first identified in *Saccharomyces cerevisiae*, that regulates expression of specific genes. When produced, *Gal4* binds to the *UAS* enhancer to activate the transcription of a target genes. This system operates under the understanding that *Gal4* is inert in most circumstances, and that gene expression is not induced without the presence of the *UAS* (Brand and Perrimon, 1993; Duffy, 2002; Barwell *et al*, 2017). In *D. melanogaster*, the *UAS* and *Gal4* components are

present in two different transgenic lines which allows for different expression combinations. When individuals from these parental lines are mated, critical class progeny are produced which carry both the *Gal4* and *UAS* elements in their genome. The *Gal4* protein can then bind to the *UAS* site to activate gene transcription in a time and tissue-specific manner (Duffy, 2002). This powerful genetic tool allows for the modelling of human disease in *Drosophila* by assessing various phenotypes that arise from altered gene expression.

1.7 RNA interference and function

RNA interference (*RNAi*) is a cellular mechanism which involves the degradation of the homologous mRNAs directed by double-stranded RNA (dsRNA) (Heigwer *et al*., 2018). A ribonuclease III enzyme named Dicer cleaves dsRNA into 21 to 23 nucleotide fragments known as small interfering RNAs (siRNAs). siRNA become inserted into a multiprotein complex called the RNA induced silencing complex (RISC) where it is unwound. This complex degrades mRNAs that are complementary to the siRNA sequence via action of the enzyme Slicer (Kavi *et al*., 2008; Yamaguchi and Yoshida, 2018). *RNAi* in combination with the *UAS-Gal4* system allows for the post-transcriptional knockdown of specific genes in a time and tissue-specific manner.

1.8 Research Goals

PGC-1α, *PARIS*, and *Vps13C* have been identified as candidate genes in the development of PD. This study aims to further characterize their role in disease. Manipulation of gene expression in *D. melanogaster* may help determine how the overexpression or inhibition of these genes in different tissues impact longevity and locomotor ability.

Chapter 2 – Materials and Methods

2.1 Bioinformatic Analyses

All bioinformatic analyses were conducted using sequences derived from *Drosophila melanogaster* and *Homo sapiens*. Human protein sequences were identified using the National Centre for Biotechnology Information (NCBI) gene search tool (*https://www.ncbi.nlm.nih.gov/*). The Basic Local Alignment Search Tool (BLAST) offered by NCBI, specifically 'blastp', was used to identify homologous protein sequences in *D. melanogaster*.

A pairwise alignment is used to identify any regions of similarity between two biological sequences to infer functional, structural or evolutionary relationships (Needleman & Wunsch, 1970). Particularly, apparent conserved domains and regions of conserved function are of interest. Pairwise sequence alignments (PSA) were done using the protein sequences obtained from NCBI, to identify the degree of similarity between them. This was conducted using the EMBOSS Needle pairwise alignment tool on default settings, with output format set to pairs (*https://www.ebi.ac.uk/Tools/psa/emboss_needle/*). Domains were identified using InterPro 92.0 using the default search parameters (*https://www.ebi.ac.uk/interpro/*).

2.2 *Drosophila melanogaster* Media and Stocks

Drosophila stocks and crosses were maintained on a standard media comprised of 65 g/L cornmeal, 15 g/L nutritional yeast, 5.5 g/L agar, and 50 ml/L Crosby's fancy grade molasses diluted in water with 5 ml of 0.1 g/ml methylparaben in ethanol and 2.5 ml of propanoic acid

(Staveley Lab *Drosophila* medium formula). Approximately 7 ml is poured into each plastic vial before being stored at 4 to 6°C. Media is prepared by Dr. Brian E Staveley.

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* stock centre at Indiana University (Indiana, USA) and the Vienna *Drosophila* Resource Centre as part of the Vienna Biocentre Core Facilities (Vienna, Austria).

2.3 *Drosophila melanogaster* Crosses

Stocks of all transgenic lines are maintained at room temperature (20°C±2), with media being changed every 21 days or so. Males from inhibition, overexpression, or control *UAS*–bearing lines were mated with virgin females from lines containing *Gal4* transgenes. Virgin females are collected by isolating newly eclosed females every 8 to 12 hours from stock vials. They are left for 6 to 7 days to ensure virginity. The males are collected and isolated for 24 hours. When mated, 2 to 3 males are placed on fresh media with 3 to 5 virgin females and left to breed. To increase breeding productivity, the parental *Drosophila* were placed onto new media every 2 to 3 days, for a total of three times before being discarded. Male offspring were collected as eclosion occurred. These critical class progenies were stored in incubators at 25°C. To eliminate the collection of F2 progeny with an undesired genotype, the collection vials were discarded after 18 days. Specific details on crosses and genotypes of flies used are to follow in subsequent chapters.

2.4 Longevity Assay

An analysis of *D. melanogaster* survivorship was conducted to determine the effect of altered gene expression on longevity when compared to control groups. Male progenies were collected from each critical class daily, placed in vials containing fresh media, and stored at 25°C. To avoid the negative effects of overcrowding, no more than 20 flies were placed in a single vial. Every 2 days the number of deaths in each vial was scored, and the media was changed after every death, or every 2-6 days. Flies were considered dead if no movement was observed, including when agitating the vial. Data was analyzed using the software Graphpad Prism 9.5.0 (Graphpad Software Inc.) using the log-rank (Mantel-Cox) test to compare survival curves. Significance was determined at 95%, with a P-value ≤ 0.05. Only statistically significant data was considered when reporting the results.

2.5 Locomotor Assay

An analysis of *D. melanogaster* locomotor ability was conducted to determine the effect of altered gene expression on motor function throughout life, when compared to control groups. A minimum of 50 male critical class progeny were collected and maintained at 25°C in cohorts of 10 in vials containing fresh media. These flies were transferred onto fresh media 1 to 2 times per week. Analysis began 7 days after collection with 5 cohorts per genotype being assessed for climbing ability. Each week, a maximum of 10 trials were conducted per cohort of 10 flies, for a total of 500 trials per genotype. This may be less as time progresses as these critical class flies die. The flies were scored based on their ability to climb up inside a 30 cm glass tube with a 1.5 cm diameter, which is marked with five 2 cm sections along a buffer zone. Each section

corresponds to a level, 1 being closest to the buffer zone, and 5 being the height from the last mark on the tube and higher.

A climbing index was calculated as Climbing index = Σ nm/N, where n represents the number of flies at a given level, m is the score of the level (between 1 and 5) and N is the total number of flies climbed in the trial (Todd and Staveley, 2008). The data was analyzed using RStudio 2021.09.0 Build 351 (Posit Software). A generalized linear model was used to generate curves with a 95% confidence interval. The slope of the curves represents the rate of decline in climbing ability. The slopes were compared using a Tukey test, a single step multiple comparison test. Statistical significance was determined based on an adjusted P-value. Significance codes are as follows: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' Results were not highlighted if not found to be statistically significant.

Chapter 3 – Further Characterization of *PGC*-1α

3.1 Introduction

3.1.1 Mitochondrial Biogenesis

Mitochondrial biogenesis is the generation of new mitochondria from already established mitochondria. This most often occurs in healthy cells (Popov, 2020). In addition to genes encoded by the nuclear genome, mitochondria contain their own circular genome which contains 13 genes important for mitochondrial function. Thus, coordination between mitochondrial and nuclear genomes is required for mitochondrial biogenesis (Simmons *et al*., 2020). Many conditions trigger mitochondrial biogenesis, which includes cell division and repair, alterations in physiological state, cold stress, energy limitations, mitochondrial damage, and oxidative stress (Golpich *et al*., 2016). Mitochondrial biogenesis is an important process that is vital for overall cellular health.

Mitochondrial biogenesis is a complex process that begins with the mtDNA transcription and translation. This is activated by several proteins from the PGC-1 family. Of these, *PGC-1α* is a nuclear-encoded gene that is regarded as the 'master regulator' of mitochondrial biogenesis. PGC-1α is activated by phosphorylation or deacetylation and stimulates the activation of nuclear respiratory factors, namely NRF-1, NRF-2, and ERR-α (Popov, 2020; Simmons *et al*., 2020; Rera *et al*., 2011). These transcription factors promote the transcription of mitochondrial genes that encode several subunits of the electron transport chain, such as ATP synthase, cytochrome oxidase IV, and mitochondrial transcription factor A (TFAM). Increased expression of TFAM allows for mtDNA transcription and translation. TFAM translocates to the mitochondrial matrix

where it stimulates mtDNA replication and gene expression (Simmons *et al*., 2020). The translation of mtDNA-encoded genes into proteins is aided by nuclear-encoded translation factors, elongation factors, translational factors, and recycling factors. Levels of mitochondrial proteins are regulated by the translational activation of cytochrome oxidase 1 (by TACO1) which binds mRNA (Popov, 2020). Thus, levels of the necessary proteins involved in mitochondrial function are maintained.

The next step in mitochondrial biogenesis involves the synthesis, import, and assembly of nuclear-encoded mitochondrial proteins. Preproteins are synthesized in the cytosol and contain an amino-acid cleavable mitochondrial targeting signal. The translocase TIM23 directs preproteins, through the signal, to the mitochondrial matrix for assembly. These proteins are sorted into specific locations in the matrix, or inner mitochondrial membrane (IMM) (Popov *et al*., 2020). The production of the outer mitochondrial membrane proteins (OMM) has been studied mainly in unicellular organisms. The OMM is important for dynamic changes in mitochondria, such as fission and fusion, as well as interactions with other organelles (Popov, 2020; Golpich *et al*., 2016). At this stage, it is likely that lipid transport proteins (LTPs) play an important role in transporting lipids to the mitochondria.

3.1.2 PGC-1α

Peroxisome Proliferator-activated receptor gamma co-activator 1-alpha (*PGC-1α*) is one member of a gene family, along with *PGC-1β* and *PRC/PPRC1* (Villena, 2015; Ng *et al.,* 2017) that are considered widely as the master regulators of mitochondrial biogenesis and energy metabolism. Each member is differentially expressed in mammalian tissues (George and Jacobs, 2019b). Particularly, *PGC-1α* is a key regulator and is expressed in highly energetic cells,

including the kidney, liver, heart and brain (Kang and Ji., 2012; Mukherjee *et al.,* 2014). Many studies reveal downregulated expression and activity of *PGC-1α* in neurodegenerative disease (Ross and Thompson, 2006; Chaturvedi *et* al., 2009; Wang *et al*., 2019). Therefore *PGC-1α* is thought to be particularly important in the understanding of PD pathogenesis and ageing.

PGC-1α shares a functional pathway with *Parkin* and *PINK1*, two recessive PD genes involved in mitophagy. *Parkin* encodes an E3 ubiquitin ligase which mediates mono- and polyubiquitylation of cellular components, to lead to destruction by the proteasome. Over 100 mutations exist in *parkin* which disrupts its activity, to result in the death of dopaminergic (DA) neurons. Likely, this is due to an accumulation of damaged mitochondria which often eventually causes cell death. *PINK1* encodes a serine/threonine kinase which recruits Parkin to damaged mitochondria (Scarffe *et al*., 2014; Merzetti and Staveley, 2015). Together, *PGC-1α*, *Parkin*, and *PINK1* regulate mitochondrial biogenesis and mitophagy.

The *PGC-1* genes share some functional homology in humans which can pose a challenge for the study of loss of function in human cells. In *D. melanogaster, spargel* (*srl*) is the single *PGC-1* family homologue which makes study of the interactions less complicated (Merzetti and Staveley, 2015). Like *PGC-1α* in humans, *srl* has been shown to regulate mitochondrial genes through *delg* (the *NRF-1* homologue) (Tiefenböck *et al*., 2009). Earlier studies found that *srl* overexpression coincided with increased mitochondrial oxygen consumption, ATP production, enhanced mitochondrial DNA content, increased enzyme activity, and protein production in the mitochondrial matrix (Mukherjee *et al.,* 2014; Rera *et al*., 2011). Reduction of *srl* function in *D. melanogaster* has been found to promote an age-dependent reduction in locomotor function (Merzetti and Staveley, 2015; Ng *et al*., 2017).

Exploring the effects of altered *srl* expression in a tissue-specific manner gives further insight into the mechanisms that influences the pathogenesis of neurodegenerative disease.

3.2 Materials and Methods

3.2.1 Bioinformatic Analysis

A bioinformatic analysis was conducted to determine the similarity between protein sequences of PGC-1α homologues in *D. melanogaster* (NP_730836.3), and *H. sapiens* (EAW92812.1). See Chapter 2, section 2.1 for details on Bioinformatic analyses.

3.2.2 *Drosophila melanogaster* Stocks and Crosses

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* stock centre at Indiana University (Bloomington, Indiana, USA) and the Vienna *Drosophila* Resource Centre as part of the Vienna Biocentre Core Facilities (Vienna, Austria). See Table 3.1 for list of genotypes used. See Chapter 2, section 2.3 for detail on *D. melanogaster* crosses.

3.2.3 Longevity Assay

An analysis on the survival of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in median lifespan. See Chapter 2, section 2.4 for full longevity assay methods.

3.2.4 Locomotor Assay

An analysis on the locomotor ability of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in locomotor ability over time. See Chapter 2, section 2.5 for full locomotor assay methods.

Table 3.1: Genotypes and location of expression patterns used in the analysis of altered expression of *srl*

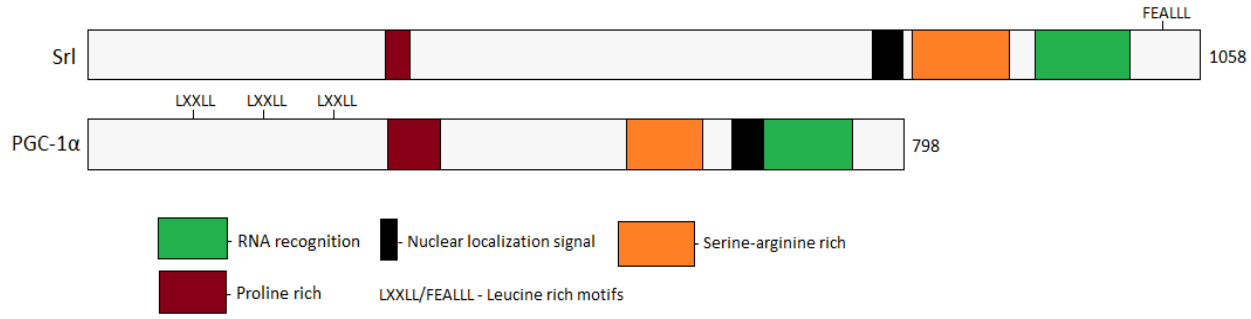
Abbreviated Genotype	**Location of Expression**	**Insertion Chromosome**	**Reference**
Control Lines			
UAS-lacZ	---	2	Brand *et al*., 1993
UAS-LUC-RNAi	---	3	Perkins *et al*., 2015
Driver Lines			
C380-Gal4	Motor neuron	X	Sanyal, 2009
D42-Gal4	Motor neuron	3	Parkes *et al*., 1998
OK6-Gal4	Motor neuron	2	RRID:BDSC_64199
TH-Gal4	Dopaminergic neuron	3	Inamdar *et al*., 2014
Repo-Gal4	Glial cell	3	RRID:BDSC_7415
Responder Lines			
UAS-srl-EY	---	3	Bellen *et al*., 2004
UAS-srl-RNAi^{33914}	---	3	Perkins *et al*., 2015
UAS-srl-RNAi^{33915}	---	3	Perkins *et al*., 2015

3.3 Results

3.3.1 Bioinformatic Analysis of PGC-1α

A pairwise alignment between *D. melanogaster* srl (NP_730836.3) and *H. sapiens* PGC-1α (EAW92812.1) shows conserved protein structure (Figure 3.1). While srl carries approximately 260 more amino acids, a pairwise sequence alignment revealed these proteins share 30.1% similarity. Each contains an N-terminal proline rich domain, a nuclear localization signal (NLS), and a C-terminal serine-arginine rich region (Mukherjee *et al*., 2014; Merzetti and Staveley, 2015). PGC-1α contains multiple leucine-rich motifs (LXXLL) which interact with nuclear receptors. *D. melanogaster* srl does not contain this motif, however it does possess another leucine-rich motif (FEALLL) (Matsuda *et al*., 2004; Wang *et al*., 2007). The use of Interpro to identify the presence of domains revealed a highly conserved RNA-recognition domain shared between srl and PGC-1α. This is composed of approximately 90 amino acids and is known to bind single-stranded RNA. RNA-binding proteins often regulate the expression of genes by controlling post-transcriptional processes such as splicing, cleavage and polyadenylation, localization, stability and translation of mRNAs (Van Nostrand *et al*., 2020). Given the similarities between the *D. melanogaster* and *H. sapiens* proteins, *srl* is ideal for studying *PGC-1α* function without the redundancy found within the *PGC-1* family in humans.

A)



B)

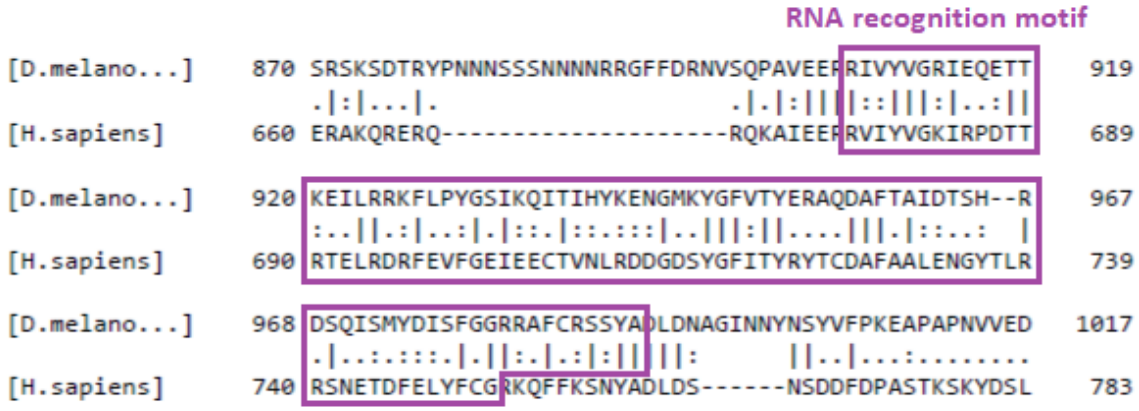


Figure 3.1: The PGC-1α protein in *H. sapiens* and srl protein in *D. melanogaster* share conserved domains. A) Aligned sequences show the position of each domain in *D. melanogaster* srl and *H. sapiens* PGC-1α. Green represents the RNA recognition motif, orange represents a serine-arginine rich region, black represents a nuclear localization signal, red represents a proline-rich region, and LXXLL/FEALLL represents leucine rich motifs. Elements of this figure were adapted from Merzetti and Staveley (2015). **B)** A pairwise alignment between srl and PGC-1α shows a high degree of sequence conservation within the RNA recognition motif found at the carboxyl terminal of each protein. "|" indicates identical amino acids in all sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions.

3.3.2 Overexpression of *srl* affects median lifespan and locomotor ability in a *Gal4*-dependent manner.

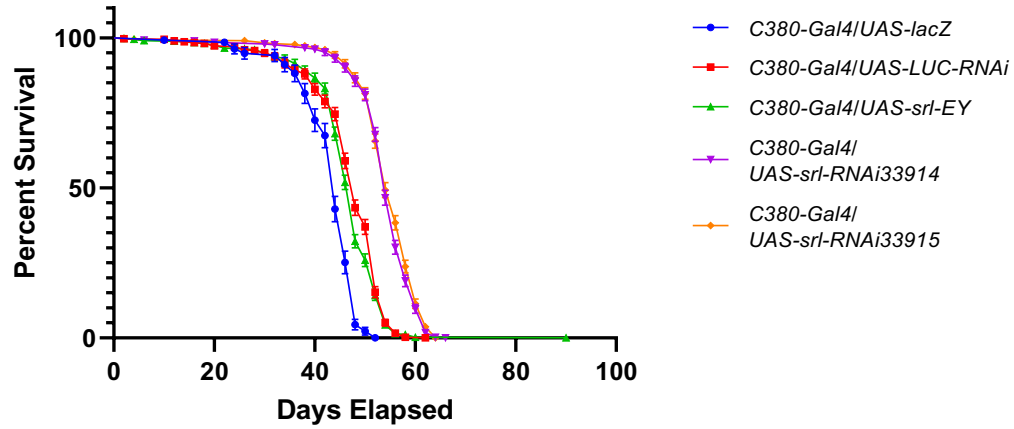
An analysis of the effect that *srl* overexpression has on lifespan and locomotor ability of *D. melanogaster* shows that overexpression affects median lifespan and locomotor ability depending on the expression pattern of the *Gal4* transgene. Overexpression of *srl* slightly increases median lifespan when expression is driven by the *C380-Gal4* transgene (Figure 3.2A) and markedly decreases lifespan when expression is driven by the *OK6-Gal4* transgene (Figure 3.4A), when compared to the *UAS-lacZ* control. When overexpression of *srl* is driven by *D42-Gal4*, locomotor ability is markedly lowered (Figure 3.3B). Overexpression through the *Repo-Gal4* (Figure 3.5) and *TH-Gal4* (Figure 3.6) transgenes did not have a statistically significant affect on lifespan or locomotor ability when compared to the *UAS-lacZ* control.

3.3.3 Inhibition of *srl* affects lifespan in a *Gal4*-dependent manner and does not significantly alter locomotor ability.

The analyses of the effects of *srl* inhibition on *D. melanogaster* lifespan and locomotor ability overtime were carried out using two *srl* inhibition responder transgenes: *UAS-srl-RNAi*^{33914} and *UAS-srl-RNAi*^{33915}. When *srl-RNAi* are expressed, the affect on *D. melanogaster* lifespan is dependent on the expression pattern of the *Gal4* transgene as well as the responder line. Expression of both *srl* inhibition transgenes increases median lifespan when driven by the *C380-Gal4* transgene (Figure 3.2A) and the *OK6-Gal4* transgene (Figure 3.4A) when compared to the *UAS-LUC-RNAi* control. Median lifespan decreases when *srl* is inhibited using the transgene *D42-Gal4* (Figure 3.3A). When expressed using the glial cell-specific transgene *Repo-*

Gal4, median lifespan decreased with *UAS-Srl-RNAi*^{33914} expression and significantly increased median lifespan with *UAS-srl-RNAi*^{33915} expression (Figure 3.5A). There is no statistically significant effect of *srl* inhibition on lifespan when expressed using *TH-Gal4* (Figure 3.6A), though it is worthwhile to mention that the reported median lifespan of *UAS-srl-RNAi*^{33914} is much lower than the *UAS-LUC-RNAi* control despite the statistical insignificance. Inhibition of *srl* does not significantly affect locomotor ability when compared to a *UAS-LUC-RNAi* control.

A)



B)

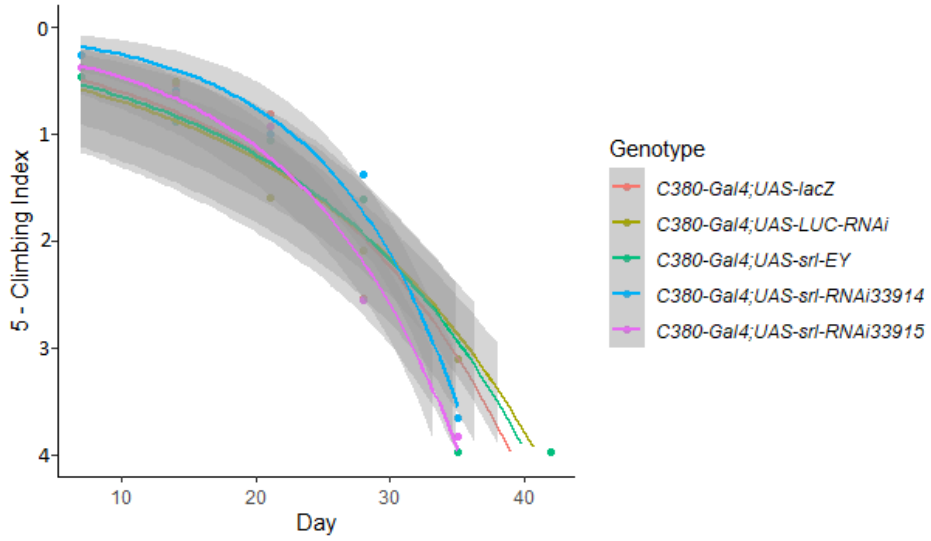
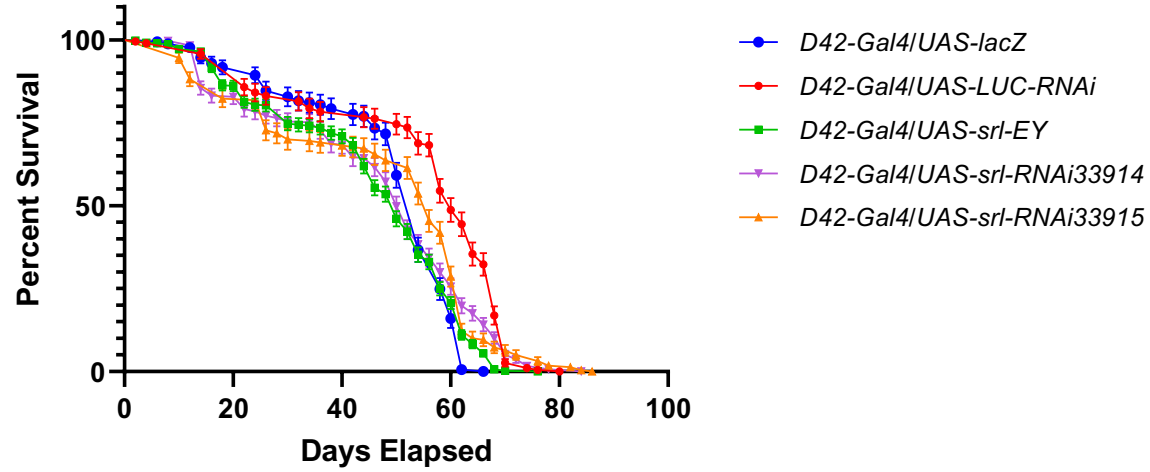


Figure 3.2: Altered expression of *srl* directed through the *C380-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *C380-Gal4/UAS-lacZ* (n=135)*, C380-Gal4/UAS-LUC-RNAi* (n=373), *C380-Gal4/UAS-srl-EY* (n=432), *C380-Gal4/UAS-srl-RNAi*^{33914} (n=394), *C380-Gal4/UAS-srl-RNAi*^{33915} (n=404). **B:** Locomotor assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

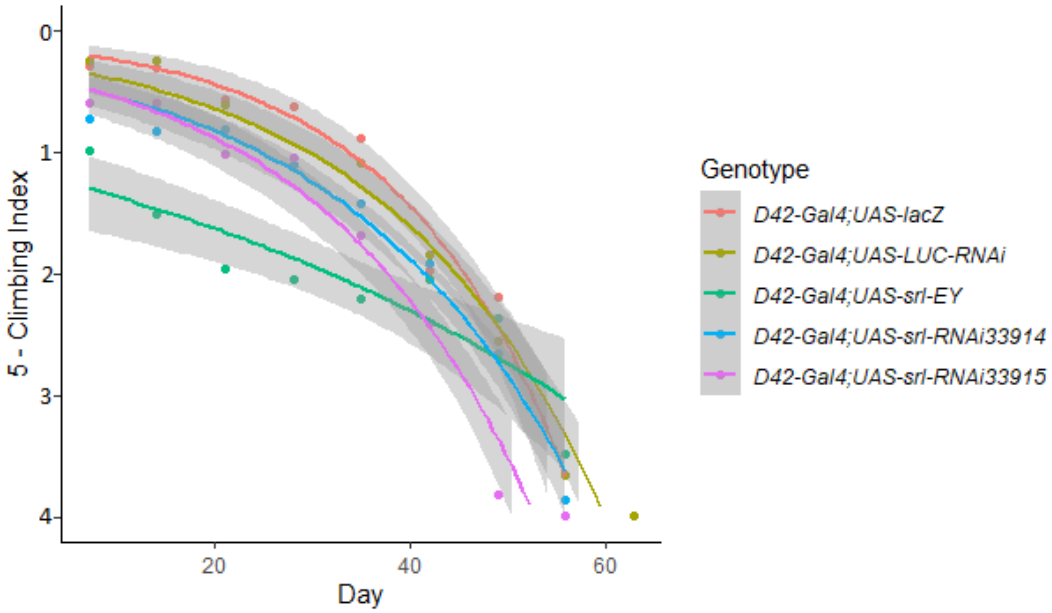
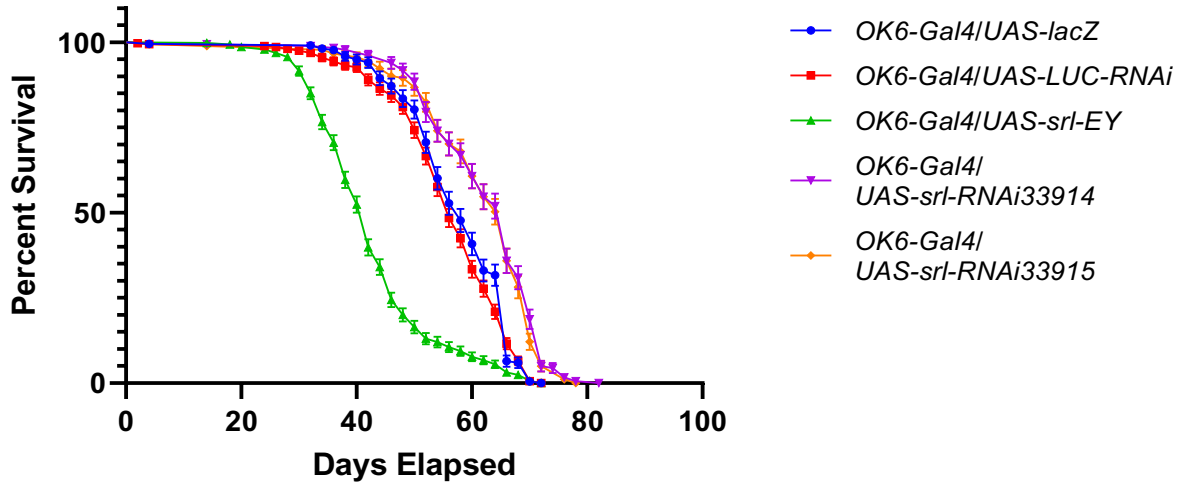


Figure 3.3: Altered expression of *srl* directed through the *D42-Gal4* transgene affects lifespan and locomotor ability. **A:** Longevity assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *D42-Gal4/UAS-lacZ* (n=169), *D42-Gal4/UAS-LUC-RNAi* (n=189), *D42-Gal4/UAS-srl-EY* (n=460), *D42-Gal4/UAS-srl-RNAi*^{33914} (n=297), *D42-Gal4/UAS-srl-RNAi*^{33915} (n=220). **B:** Locomotor assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

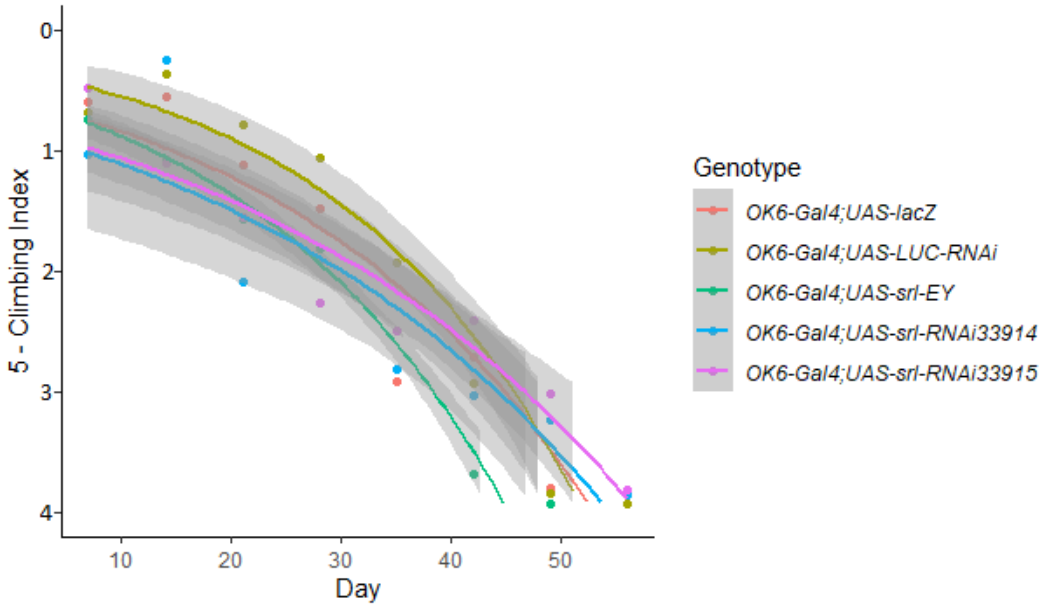
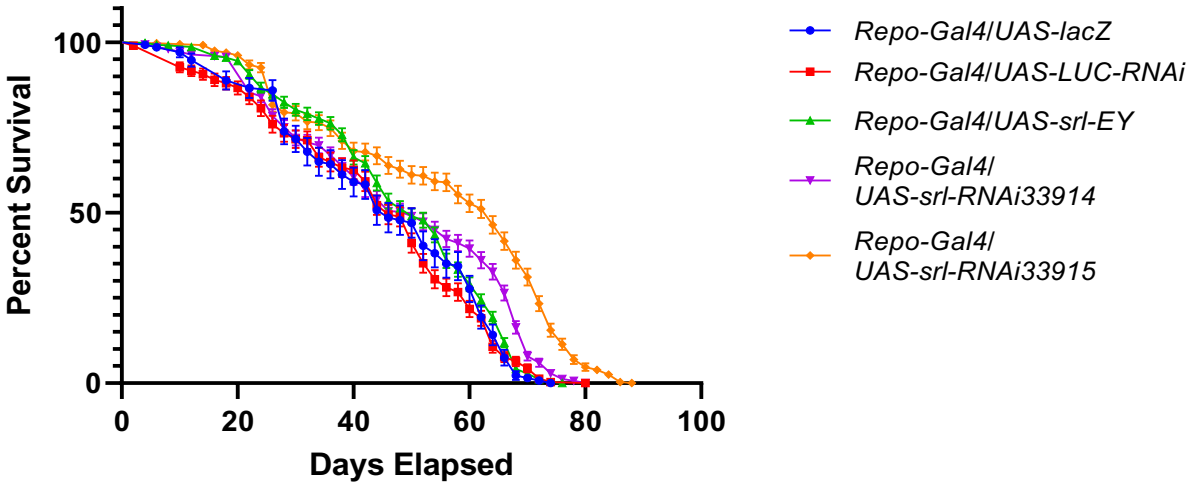


Figure 3.4: Altered expression of *srl* directed through the *OK6-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *OK6-Gal4/UAS-lacZ* (n=218), *OK6-Gal4/UAS-LUC-RNAi* (n=353), *OK6-Gal4/UAS-srl-EY* (n=414), *OK6-Gal4/UAS-srl-RNAi*^{33914} (n=181), *OK6-Gal4/UAS-srl-RNAi*^{33915} (n=181). **B:** Locomotor assay of *D. melanogaster* males displaying altered *srl* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

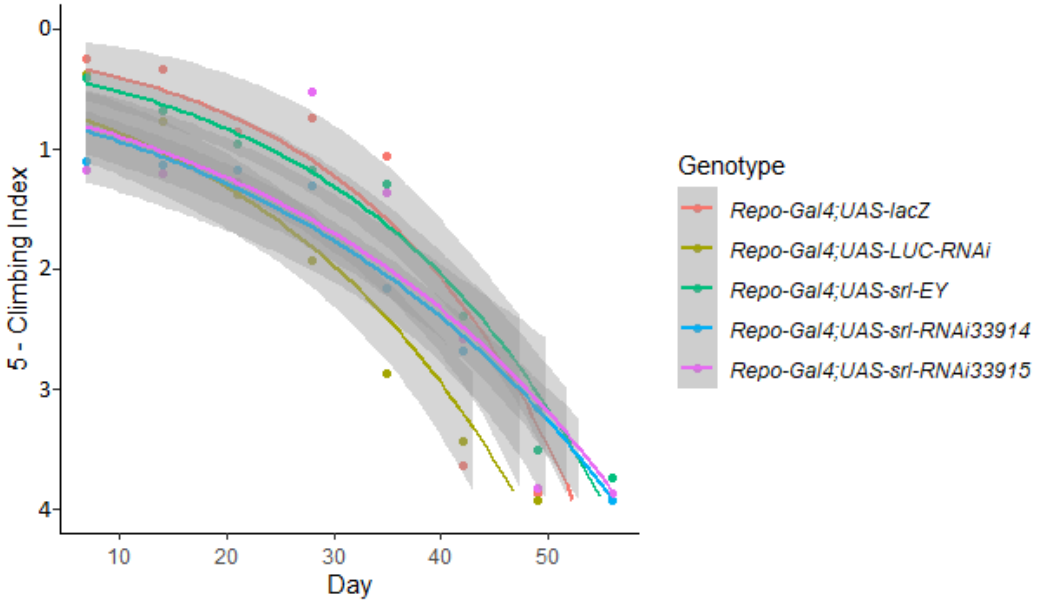
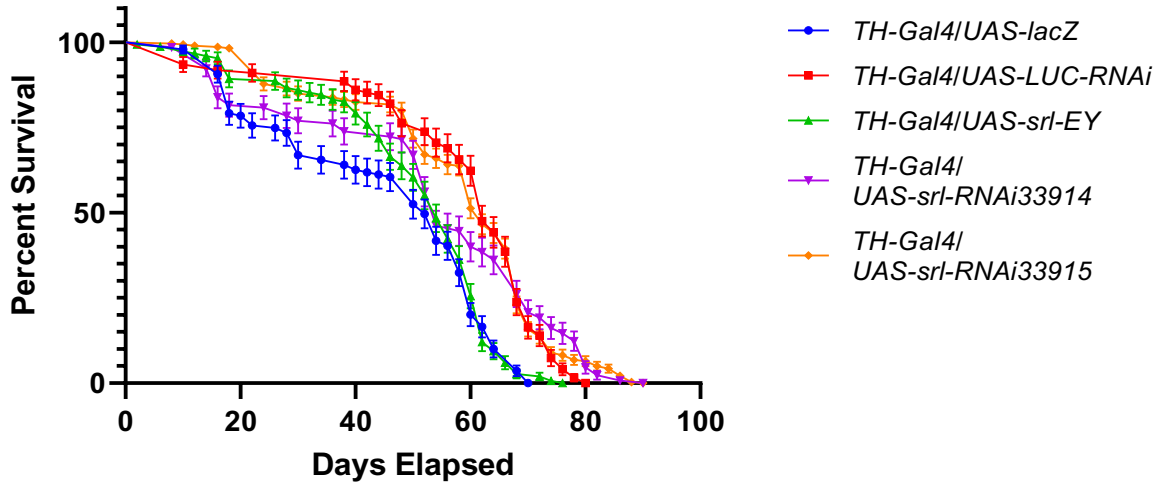


Figure 3.5: Altered expression of *srl* directed through the *Repo-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *srl* expression in glial cells. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *Repo-Gal4/UAS-lacZ* (n=134), *Repo-Gal4/UAS-LUC-RNAi* (n=299), *Repo-Gal4/UAS-srl-EY* (n=504), *Repo-Gal4/UAS-srl-RNAi*^{33914} (n=416), *Repo-Gal4/UAS-srl-RNAi*^{33915} (n=360). **B:** Locomotor assay of *D. melanogaster* males displaying altered *srl* expression in glial cells. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

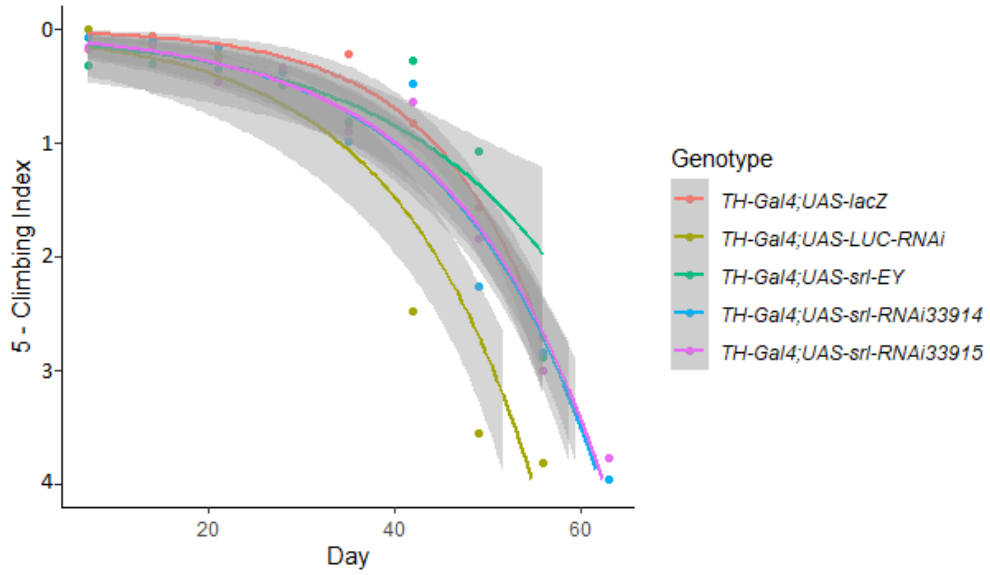


Figure 3.6: Altered expression of *srl* directed through the *TH-Gal4* transgene does not significantly affect lifespan or locomotor ability. **A:** Longevity assay of *D. melanogaster* males displaying altered *srl* expression in the dopaminergic neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *TH-Gal4/UAS-lacZ* (n=139), *TH-Gal4/UAS-LUC-RNAi* (n=122), *TH-Gal4/UAS-srl-EY* (n=149), *TH-Gal4/UAS-srl-RNAi*^{33914} (n=130), *TH-Gal4/UAS-srl-RNAi*^{33915} (n=279). **B:** Locomotor assay of *D. melanogaster* males displaying altered *srl* expression in the dopaminergic neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

3.4 Discussion

Parkinson disease is a progressive neurodegenerative disease. There is no cure for this debilitating disease, so understanding the underlying complexities in PD pathogenesis is important in the development of more superior treatments and therapies. Mitochondria have been heavily implicated in ageing and neurodegeneration. This makes *PGC-1α*, particularly interesting given the role it plays in regulating mitochondrial biogenesis. Bioinformatic analysis shows that PGC-1α shares conserved protein structure with the *D. melanogaster* homologue srl. Each protein contains an N-terminal proline rich domain, an NLS, and a C-terminal serine-arginine rich region (Mukherjee *et al*., 2014; Merzetti and Staveley, 2015). Additionally, a highly conserved RNA-recognition domain is found in both protein sequences. These domains are around 90 amino acids long and are known to bind single stranded RNA. This RNA-recognition domain is important in regulating gene expression by controlling post-transcriptional processes (Van Nostrand *et al*., 2020). PGC-1α contains multiple leucine-rich motifs (LXXLL) which interact with nuclear receptors. While srl does not contain this motif, it does possess another leucine-rich motif (FEALLL) which has demonstrated interaction with nuclear receptors thus providing a similar function (Matsuda *et al*., 2004; Wang *et al*., 2007). The similarities between these two proteins, particularly the conservation of several domains/motifs allow for the use of *D. melanogaster* to create models of altered *PGC-1α* expression.

The overexpression of *srl* influenced longevity and locomotor ability in *D. melanogaster* when expressed through specific *Gal4* transgenes. When overexpressed through the motor neuron-specific transgene *C380-Gal4*, a slight increase in longevity is seen in *D. melanogaster*. However, when *srl* overexpression is driven by *OK6-Gal4*, another motor neuron-specific directing transgene, there is a significant decrease in longevity. Overexpressing *srl* in the motor

neurons produces a significant decrease in locomotor ability, specifically when driven using the *D24-Gal4* transgene. Earlier studies found that *srl* overexpression coincided with increased mitochondrial oxygen consumption, ATP production, enhanced mitochondrial DNA content, increased enzyme activity, and protein production in the mitochondrial matrix (Mukherjee *et al.,* 2014; Rera *et al*., 2011). In some cases, *srl* overexpression is enough to rescue disease phenotypes in *D. melanogaster* (Ng *et al*., 2017). In other cases, *srl* overexpression does little to rescue disease phenotypes in *D. melanogaster* (George and Jacobs, 2019a). Overexpression of *srl* on its own does little to alter climbing ability, dopaminergic neuronal integrity, neuronal mitochondrial size, or dopamine levels (Ng *et al*., 2017). Overall, a decrease in longevity and locomotor ability is found in this study when *srl* is overexpressed in certain tissues. This is not unlike previous research done by Merzetti and Staveley (2015), which found that the induced expression of *srl* cause a severe decrease in median lifespan and locomotor ability. Tiefenbock *et al* (2009) found that *srl* overexpression leads to a small-size phenotype in cells. It is possible that changes to the expression of *srl* are poorly tolerated during normal development and cell viability.

The inhibition of *srl* via *RNAi* alters longevity in a *Gal4* dependent manner. However, in most cases *srl* loss-of-function leads to improved longevity. Specifically, when using the glial-specific driver *Repo-Gal4* to drive *UAS-srl-RNAi*^{33915}, a rather significant increase in median lifespan is observed. The exception of this is the inhibition of *srl* through the *D42-Gal4* transgene which resulted in a slight decrease in longevity. Many studies reveal downregulated expression and activity of *PGC-1α* in neurodegenerative disease (Ross and Thompson, 2006; Chaturvedi *et* al., 2009; Wang *et al*., 2019). Bioenergetic genes which respond to *PGC-1α* activity are underexpressed in cases of PD (Zheng *et al*., 2010). With regards to *Drosophila*, a

loss of TH-positive neurons in two dopaminergic clusters was observed which coincided with depleted dopamine levels in the brain. Additionally, mitochondria appeared much smaller in *srl* deficient flies (Ng *et al*., 2017). It is unusual that *srl* inhibition using *RNAi* resulted in an increased lifespan. However, previous research done by this lab has also implicated *srl* inhibition with increasing *D. melanogaster* lifespan in a tissue-specific manner (Merzetti and Staveley, 2015; Baker, 2018). Overall, this increase in longevity could be attributed to the activation of the unfolded protein response (UPR) or an ROS-dependent mitohormesis (Merzetti and Staveley, 2015). Mitohormesis is a biological response through which the induction of mild or moderate mitochondrial stress may promote the viability and wellness of a cell, tissue or organism. Various model organisms have shown an increase in lifespan when the mitohormetic response is triggered (Barcena *et al*., 2018; Vo *et al*., 2022). The formation of ROS at lower levels could elicit antioxidants, potentially causing a stronger response to future ROS exposure (Merzetti and Staveley, 2015). Although *srl* inhibition did not alter locomotor ability in this study, knockdown of *srl* function in *D. melanogaster* has been found to promote an age-dependent reduction in locomotor function (Merzetti and Staveley, 2015; Ng *et al*., 2017). This shows that altered expression of *srl* responds in a tissue-specific manner.

Chapter 4 – Further characterization of *PARIS*

4.1 Introduction

4.1.1 PARIS

In humans, *PARIS* is a gene that encodes a 644 amino acid protein known as parkin interacting substrate (PARIS) or zinc finger protein 746 (ZNF746). PARIS contains a Krueppel-associated box (KRAB) at the N-terminus and a C2HC/C2H2 type zinc finger at the C-terminus. It is widely distributed throughout human tissues, with differential expression in the brain. Namely, it is localized to dopaminergic (DA) neurons in the *SNpc.* It is a transcriptional repressor that regulates the expression of *PGC-1α* by binding to its promoter (Castillo-Quan, 2011; Shin *et al*., 2011). One of the main genes coregulated by *PGC-1α* and *PARIS* is *NRF-1* which is important for mitochondrial functioning and oxidant species metabolism (Stevens *et al*., 2015; Castillo-Quan, 2011). PARIS binds to and is regulated by Parkin and PINK1. PINK1 does so by mediating phosphorylation of PARIS which primes it for subsequent ubiquitylation by Parkin (Lee *et al.,* 2017). Parkinson Disease (PD)-associated Parkin mutations seem to have decreased ubiquitylation activity targeting PARIS (Castillo-Quan, 2011; Pirooznia *et al*., 2020; Stevens *et al*., 2015). This leads to an accumulation of PARIS protein which drives PD pathogenesis through the suppression of mitochondrial biogenesis (Brahmachari *et al*., 2019). This accumulation can lead to *PGC-1α*-dependent deficiency in mitochondrial respiration (Stevens *et al*., 2015). Ultimately, in healthy conditions Parkin ubiquitylates PARIS which causes its proteasomal degradation, allowing an increase in *PGC-1α* expression and mitochondrial biogenesis (Castillo-Quan, 2011; Pirooznia *et al*., 2020). A *PARIS* homologue

exists in *D. melanogaster*, referred to as *Paris* (or *dPARIS*), which is most similar in structure and function than other proposed homologues. *Paris* shows importance for the function of dopaminergic neurons in *D. melanogaster* (Merzetti and Staveley, 2016). Altered *Paris* expression in flies may give insight into interactions between *PARIS* and *PGC-1α*. This may hold key information for the development of neuroprotective therapies for patients with PD.

4.2 Materials and Methods

4.2.1 Bioinformatic Analysis

A bioinformatic analysis was conducted to determine the similarity between protein sequences of Paris homologues in *D. melanogaster* (NP_608840.1) and *H. sapiens* (NP_001381127.1). See Chapter 2, section 2.1 for detail on Bioinformatic analyses.

4.2.2 *Drosophila melanogaster* stocks and crosses

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* stock centre at Indiana University (Indiana, USA) and the Vienna *Drosophila* Resource Centre as part of the Vienna Biocentre Core Facilities (Vienna, Austria). See Table 4.1 for list of genotypes used. See Chapter 2, section 2.3 for detail on *D. melanogaster* crosses.

4.2.3 Longevity Assay

An analysis on the survival of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in median lifespan. See Chapter 2, section 2.4 for full longevity assay methods.

4.2.4 Locomotor Assay

An analysis on the locomotor ability of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in climbing ability over time. See Chapter 2, section 2.5 for full locomotor assay methods.

Table 4.1: Genotypes and location of expression patterns used in the analysis of altered expression of *Paris.*

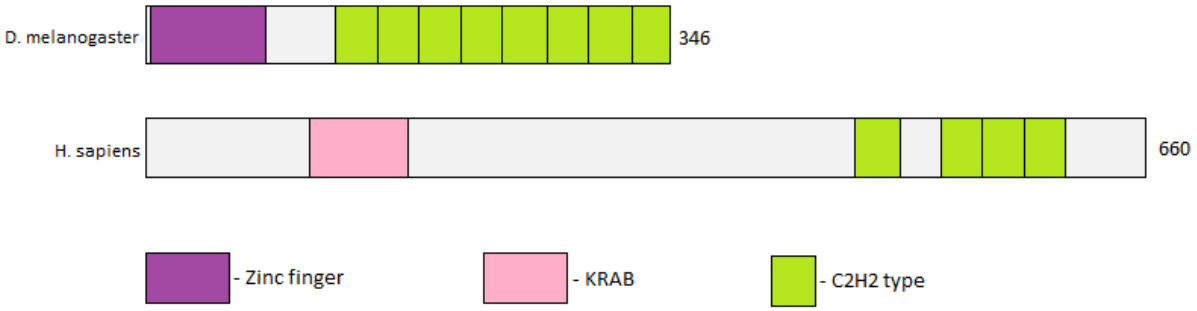
Abbreviated Genotype	**Location of Expression**	**Insertion Chromosome**	**Reference**
Control Lines			
UAS-lacZ	---	2	Brand *et al*., 1993
UAS-LUC-RNAi	---	3	Perkins *et al*., 2015
Driver Lines			
C380-Gal4	Motor neuron	X	Sanyal, 2009
D42-Gal4	Motor neuron	3	Parkes *et al*., 1998
OK6-Gal4	Motor neuron	2	RRID:BDSC_64199
TH-Gal4	Dopaminergic neuron	3	Inamdar *et al*., 2014
Repo-Gal4	Glial cell	3	RRID:BDSC_7415
Responder Lines			
UAS-paris-ORF	---	3	Bischof *et al*., 2014
UAS-paris-RNAi	---	2	Dietzl *et al*., 2007

4.3 Results

4.3.1 Bioinformatic Analysis of *Paris*

A pairwise sequence alignment between Paris homologues in *D. melanogaster* (NP_608840.1) and *H. sapiens* (NP_001381127.1) show some conserved protein structure with 31.3% similarity (Figure 4.1). The use of Interpro to identify the presence of domains revealed that each protein contains several conserved C2H2-type zinc finger domains. These C2H2-type domains are relatively small and contain finger-like projections. These are understood to possess the ability to bind DNA, RNA, protein and lipid substrates (Hall, 2005). *H. sapiens* PARIS contains a KRAB domain at the N-terminal which may function in the ability of PARIS to be a transcriptional repressor (Urrutia, 2003). Alternatively, *D. melanogaster* Paris contains an N-terminal zinc-finger associated domain (ZAD). It is likely that the ZAD works in a similar fashion to the KRAB domain found most often in vertebrates. Given the structural and functional similarities of the PARIS protein in both species, particularly within these conserved domains, *D. melanogaster* can be used to create models of human disease related to the function of *Paris* in regulating mitochondrial biogenesis.

A)



B)

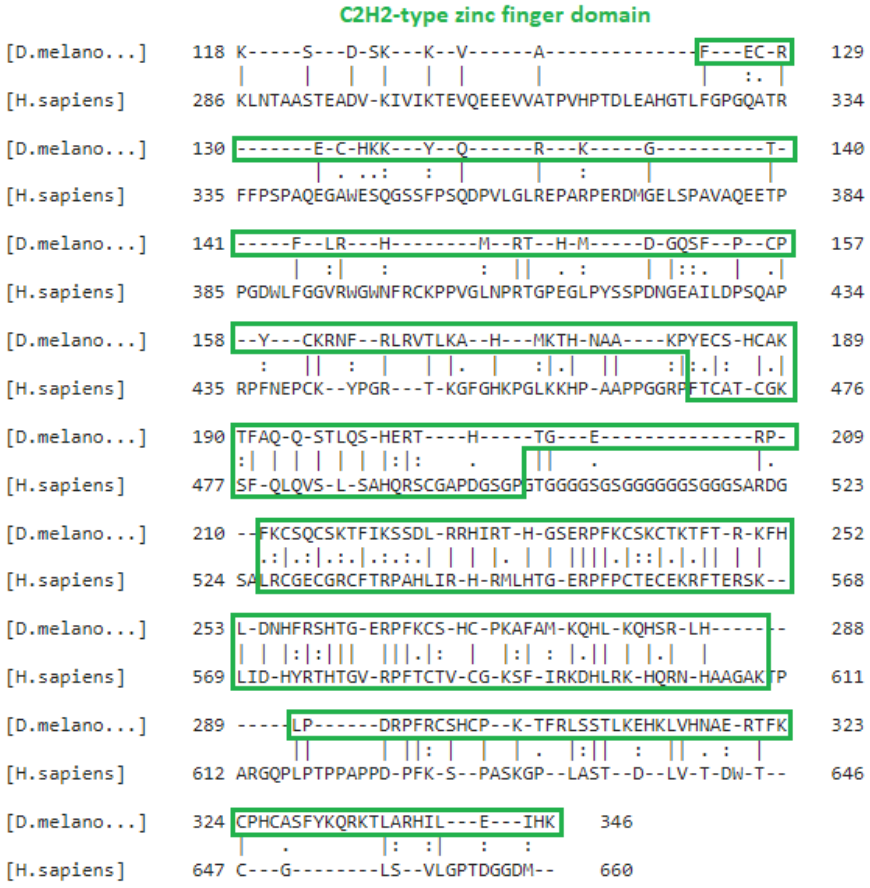


Figure 4.1: The Paris protein in *D. melanogaster* and *H. sapiens* share conserved domains. **A)** Aligned sequences show the position of each domain in *D. melanogaster* and *H. sapiens* Paris. Purple represents the zinc finger domain, pink represents the Krueppel-associated box (KRAB) domain, and green represents the C2H2-type zinc finger domain. **B)** A pairwise alignment between *D. melanogaster* and *H. sapiens* shows a high degree of sequence conservation within the C2H2-type zinc finger domain. "|" indicates identical amino acids in all sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions.

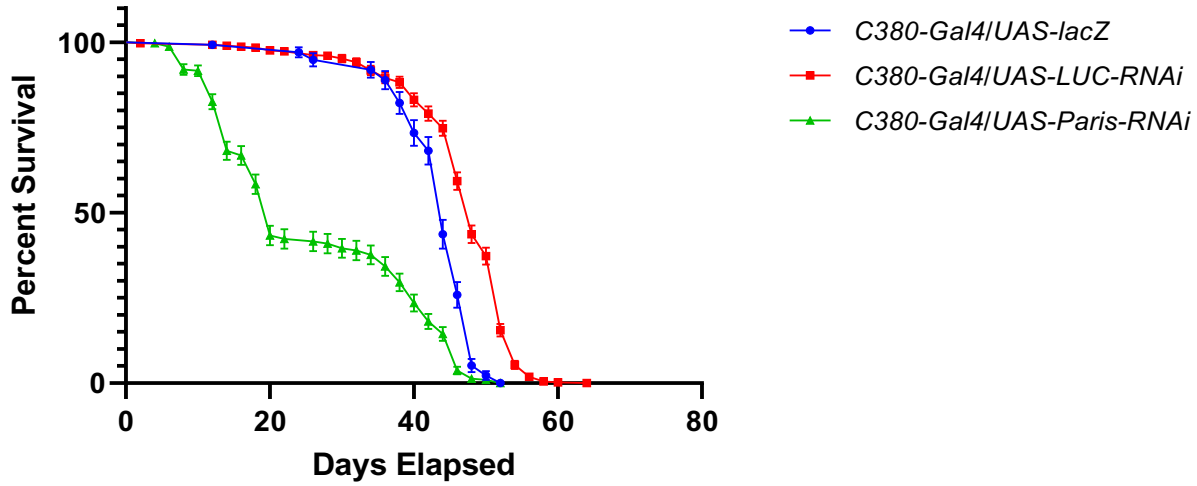
4.3.2 Overexpression of *Paris* increases median lifespan and locomotor ability over time.

An analysis of the effect that *Paris* overexpression has upon lifespan and locomotor ability of *D. melanogaster* shows that overexpression increases median lifespan and locomotor ability over time when compared to a control (*UAS-lacZ*). When expressed with the *D42-Gal4* (Figure 4.3A) and *OK6-Gal4* (Figure 4.4A) transgenes, *Paris* overexpression increased median lifespan. A slight increase in locomotor ability is exhibited when *Paris* is expressed with the *D42-Gal4* (Figure 4.3B) and *Repo-Gal4* (Figure 4.5B) transgenes. There was no statistically significant affect on lifespan and locomotor ability in *D. melanogaster* when *Paris* is expressed using the *TH-Gal4* transgene (Figure 4.6). In addition, the *C380-Gal4;UAS-Paris-ORF* line did not produce any progeny for the longevity assay.

4.3.3 Inhibition of *Paris* decreases median lifespan and locomotor ability over time.

An analysis of the effect that *Paris* inhibition has on lifespan and locomotor ability of *D. melanogaster* shows that inhibition decreases median lifespan and locomotor ability over time when compared to a control (*UAS-LUC-RNAi*). Expression of the *Paris* inhibition transgenes resulted in a significantly reduced median lifespan when expressed using the *C380-Gal4* transgene (Figure 4.2A). Inhibition of *Paris* using the *D42-Gal4* (Figure 4.3A) and *TH-Gal4* (Figure 4.6A) transgenes also reduced median lifespan. Locomotor ability was shown to decrease slightly when *Paris* is inhibited using the *D42-Gal4* (Figure 4.3B) and *OK6-Gal4* (Figure 4.4B) transgenes. No statistically significant alterations in median lifespan and locomotor ability were exhibited when using the *Repo-Gal4* transgene (Figure 4.5).

A)



B)

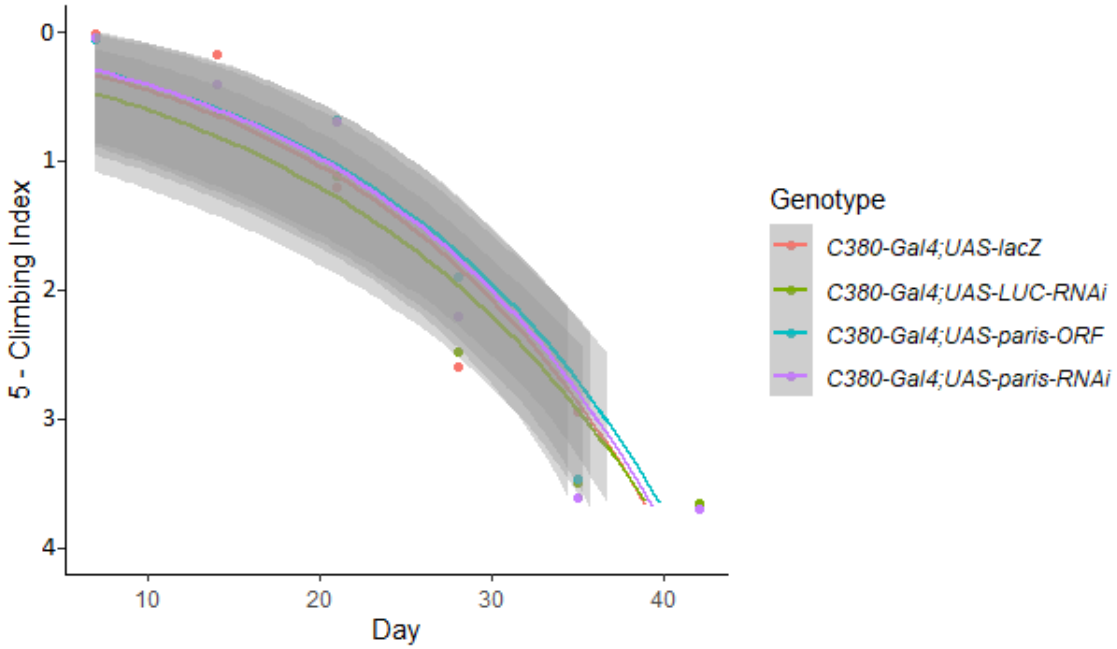
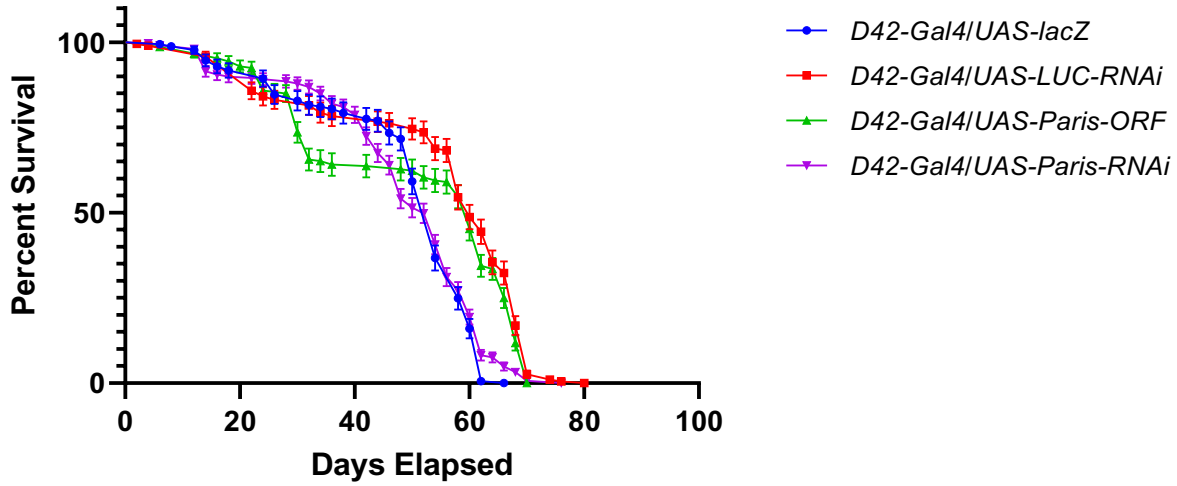


Figure 4.2: Altered expression of *Paris* directed through the *C380-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *C380-Gal4/UAS-lacZ* (n=135), *C380-Gal4/UAS-LUC-RNAi* (n=373), *C380-Gal4/UAS-Paris-RNAi* (n=298). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

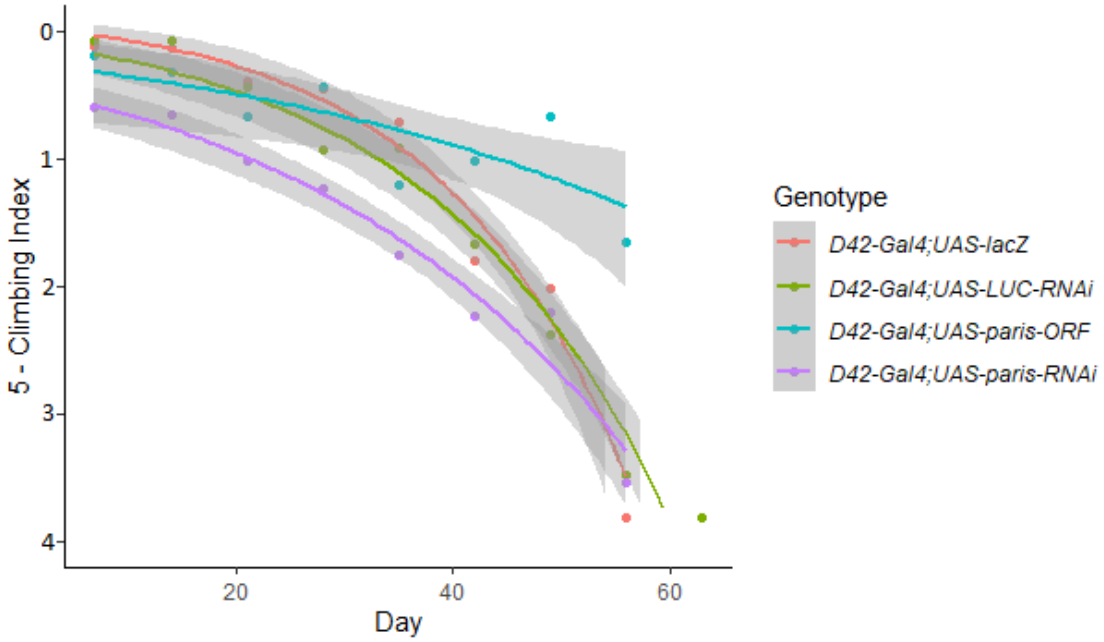
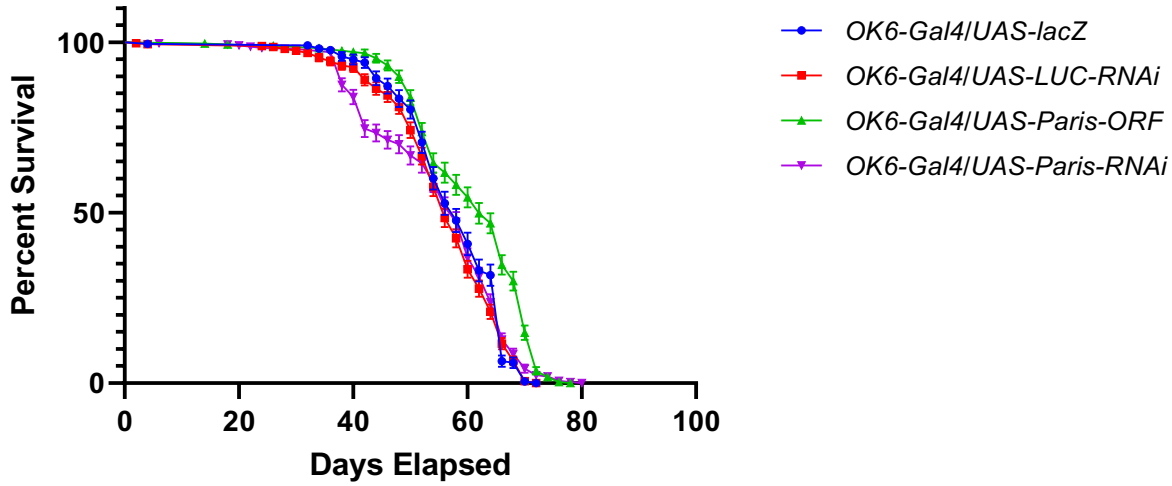


Figure 4.3: Altered expression of *Paris* directed through the *D42-Gal4* transgene affects lifespan and locomotor ability. **A:** Longevity assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *D42-Gal4/UAS-lacZ* (n=169), *D42-Gal4/UAS-LUC-RNAi* (n=189), *D42-Gal4/UAS-Paris-ORF* (n=212), *D42-Gal4/UAS-Paris-RNAi* (n=305). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

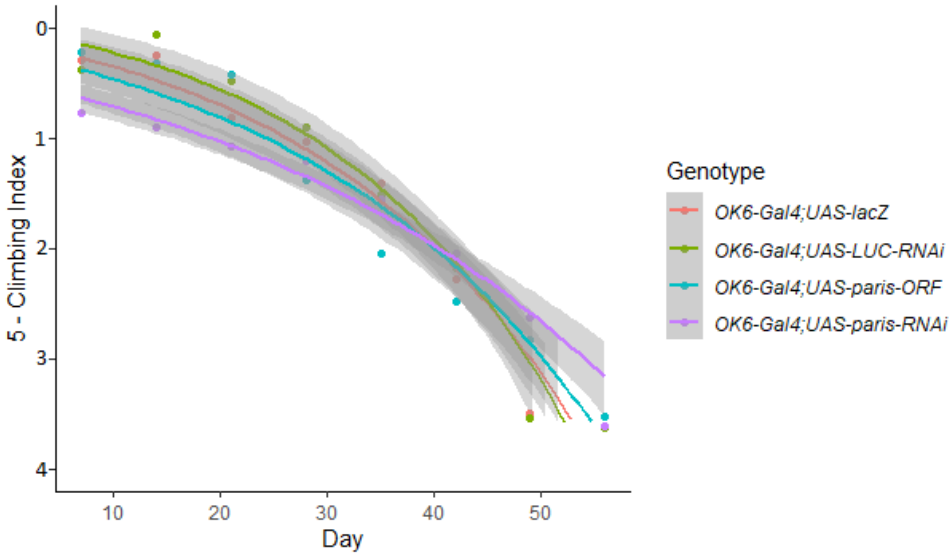
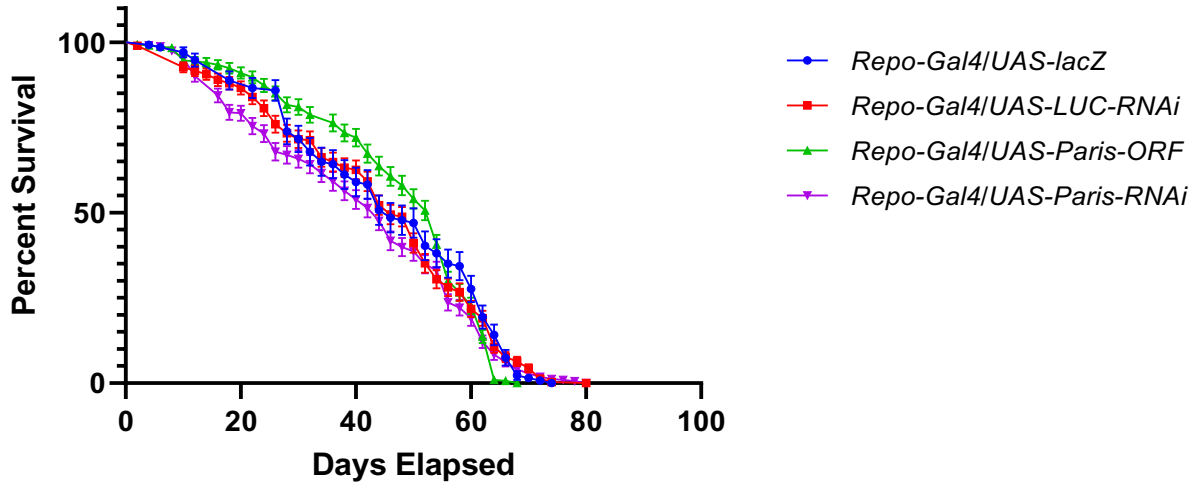


Figure 4.4: Altered expression of *Paris* directed through the *OK6-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *OK6-Gal4/UAS-lacZ* (n=218), *OK6-Gal4/UAS-LUC-RNAi* (n=353), *OK6-Gal4/UAS-Paris-ORF* (n=277), *OK6-Gal4/UAS-Paris-RNAi* (n=304). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Paris* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

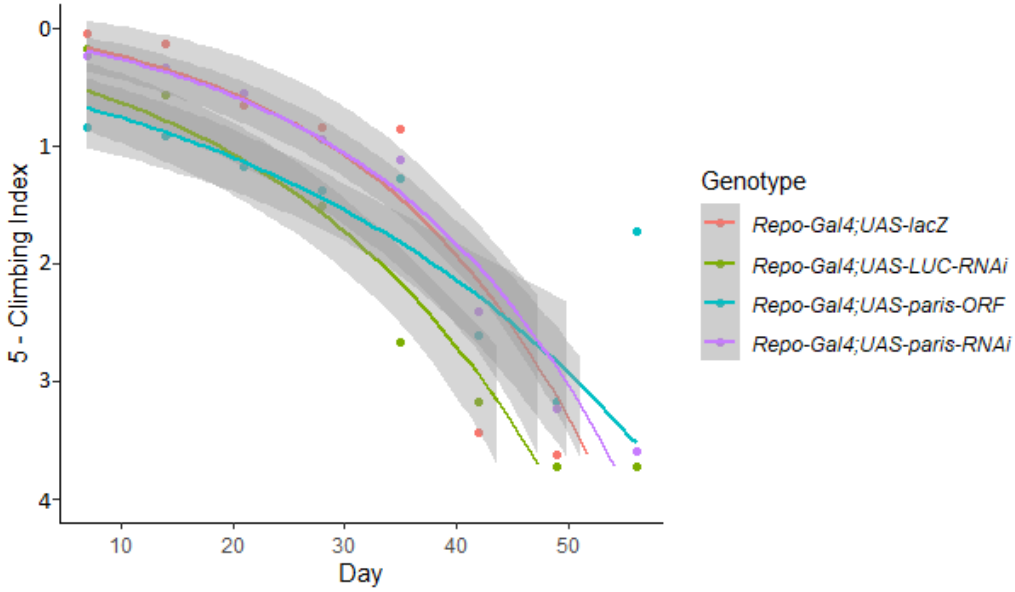
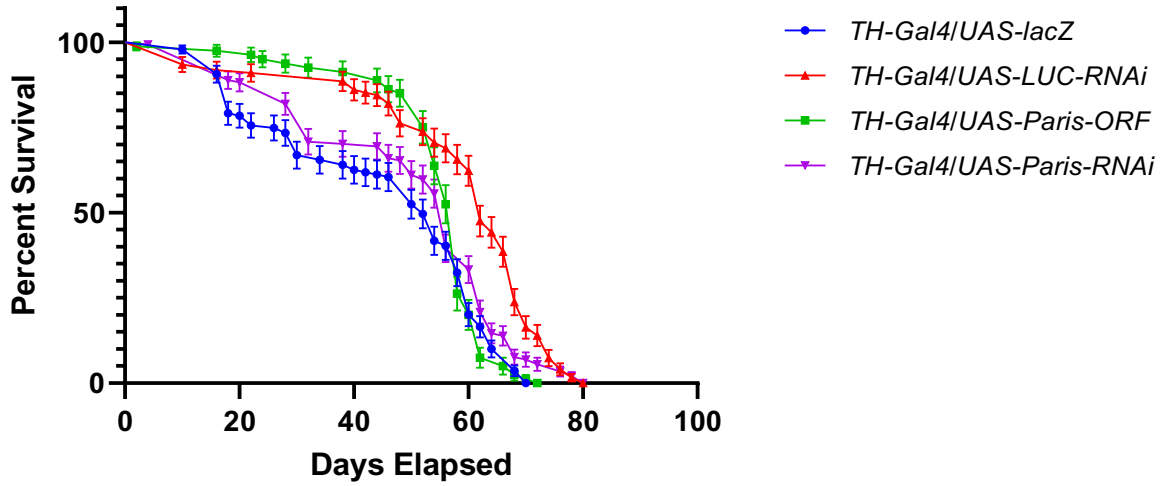


Figure 4.5: Altered expression of *Paris* directed through the *Repo-Gal4* transgene affects locomotor ability. **A:** Longevity assay of *D. melanogaster* males displaying altered *Paris* expression in glial cells. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *Repo-Gal4/UAS-lacZ* (n=134), *Repo-Gal4/UAS-LUC-RNAi* (n=299), *Repo-Gal4/UAS-Paris-ORF* (n=300), *Repo-Gal4/UAS-Paris-RNAi* (n=321). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Paris* expression in glial cells. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

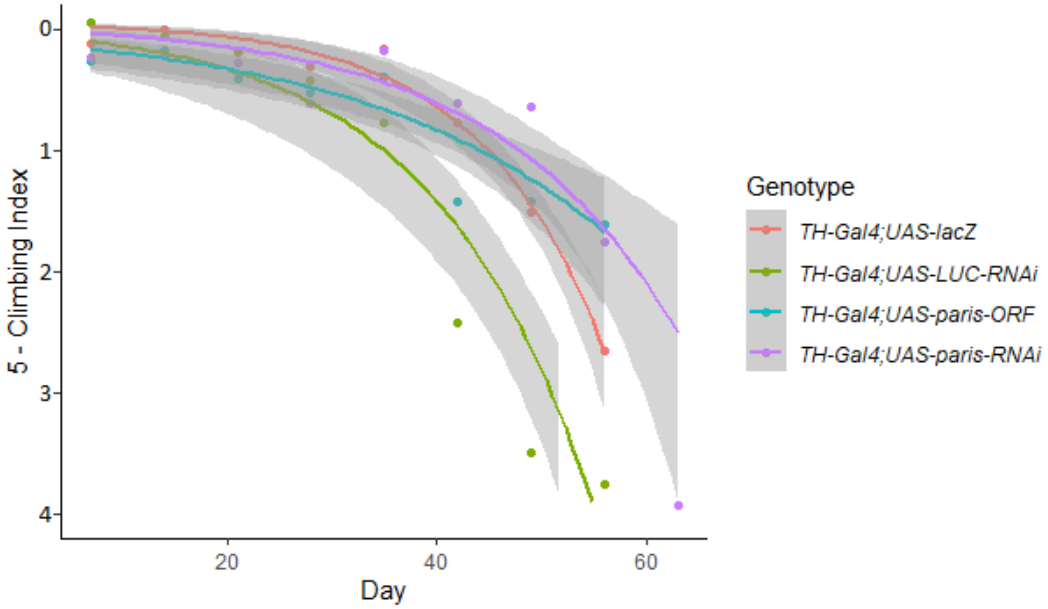


Figure 4.6: Altered expression of *Paris* directed through the *TH-Gal4* transgene affects lifespan and locomotor ability. **A:** Longevity assay of *D. melanogaster* males displaying altered *Paris* expression in dopaminergic neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *TH-Gal4/UAS-lacZ* (n=139), *TH-Gal4/UAS-LUC-RNAi* (n=122), *TH-Gal4/UAS-Paris-ORF* (n=80), *TH-Gal4/UAS-Paris-RNAi* (n=144). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Paris* expression in the dopaminergic neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

4.4 Discussion

Neurons are highly energetic cells which are highly dependent on mitochondria for both their energy requirements and the regulation of calcium concentration for signal transduction (Wang *et al*., 2020; Golpich *et al.,* 2016). Thus, maintaining mitochondrial homeostasis is important for neuronal health. *PARIS* is a transcriptional repressor which regulates the expression of *PGC-1α* by binding to its promoter (Castillo-Quan, 2011; Shin *et al*., 2011). Furthermore, levels of PARIS are regulated by Parkin and PINK1. PINK1 mediates the phosphorylation of Paris which primes it for ubiquitylation by Parkin (Lee *et al*., 2017). *Parkin* and *PINK1* themselves are important mitophagy genes. In a similar manner, PINK1 will accumulate on the OMM of damaged mitochondria and recruit Parkin to the mitochondria. The subsequent ubiquitylation of the OMM triggers selective autophagy (Pickrell and Youle, 2015). Maintaining optimal levels of mitochondrial biogenesis and mitophagy are vital for cellular health. *PARIS* is one gene which links these pathways, and further characterizing altered expression may give insight into ageing and the onset of PD.

Bioinformatic analysis reveals conserved domains that are shared between Paris homologues in *D. melanogaster* and *H. sapiens*. Both proteins contain several C2H2-type zinc finger domains towards the C-terminus, which are relatively small with finger-like projections. These may possess the ability to bind DNA, RNA, protein and lipid substrates (Hall, 2005). PARIS in *H. sapiens* contains a KRAB domain at the N-terminus which likely functions in the ability of PARIS as a transcriptional repressor (Urrutia, 2003). While *D. melanogaster* Paris does not contain a KRAB domain, it does possess an N-terminal ZAD domain. The ZAD domain functions in controlling nuclear localization of transcription factors and likely acts in a similar fashion to the KRAB domain (Zolotarev *et al*., 2016). ZAD C2H2 proteins were shown to block

interactions between enhancers and promotors in transgenic *D. melanogaster* (Fedotova *et al*., 2017). Models of altered *Paris* expression in *D. melanogaster* can therefore be applied to *PARIS* function in humans.

Altered expression of *Paris* significantly affected longevity and locomotor ability over time in *D. melanogaster*. Overexpression of *Paris* increased longevity significantly, particularly when expressed in the motor neurons. However, locomotor ability seems to be decreased with *Paris* expression. Expression through the motor neuron-specific driver *D42-Gal4* is particularly interesting, as initial climbing ability appears poorer than the control. This contrasts locomotor ability which had a much slower decline over time. Similar results have been found in previous research done by the Staveley research group (Baker, 2018). In other *Drosophila* and mice models, accumulations of *Paris* have been shown to cause progressive dopaminergic neuron loss due to the repression of *PGC-1α*, and thus defective mitochondrial biogenesis. In both these models, the diseased phenotypes could be rescued by overexpression of *PGC-1α*, *Parkin*, or *PINK1* (Pirooznia *et al*., 2020; Stevens *et al*., 2015). Alternatively, conditions of Parkin or PINK1 deficiency see an increase in levels of Paris. Pirooznia *et al* (2020) found that both ubiquitous and DA neuron-specific expression of *Paris* leads to the selective loss of dopaminergic neurons and decreased locomotor ability over time. Stevens *et al* (2015) and Lee *et al* (2017) both obtained similar results when viewed in mice. The locomotor assay results seem to align with this previous literature, as increased levels of Paris are expected to repress *PGC-1α* expression. This would decrease mitochondrial biogenesis, affecting neuronal viability and affecting locomotor ability. While the longevity results of this study seem contradictory, this may allude to a more complex affect that altered *Paris* expression has on lifespan.

The inhibition of *Paris* through *RNAi* decreases longevity and locomotor ability when expressed through specific *Gal4* transgenes. When expressed in motor neurons and DA neurons, longevity decreases with *Paris* inhibition. This effect is particularly severe when *UAS-Paris-RNAi* is expressed using the motor neuron-specific driver *C380-Gal4*. Locomotor ability is moderately decreased specifically when expression is driven in the motor neurons. Previous research from the Staveley research group found a decrease in both longevity and climbing ability (Baker, 2018). These results are interesting, as one would expect the inhibition of *Paris* to increase *PGC-1α*-mediated mitochondrial biogenesis. However, like in the case of *srl,* this may indicate a more complex influence that *Paris* has on lifespan and locomotor ability.

Chapter 5 – Further Characterization of *Vps13*

5.1 Introduction

5.1.1 Lysosomal and Mitochondrial Crosstalk

Lysosomes are small organelles which contain many hydrolytic enzymes responsible for the degradation and recycling of macromolecules and/or whole organelles. Often, these functions act as the endpoint in many trafficking pathways, including the endocytic, phagocytic, and autophagic pathways (Bartel *et al*., 2019). Given the convergence of several pathways at the lysosome, it has an important role in the coordination of sorting and delivery of lipids to membrane compartments. Lysosomes rely heavily on trafficking routes to transport lipids, with these routes often connecting the function of lysosomes to the endoplasmic reticulum (ER) and Golgi apparatus (Thelen and Zoncu, 2017). Specifically, non-vesicular lipid transport is of special interest, as it is mediated by various lipid-binding proteins which localize at contact sites between organelles.

Both mitochondria and lysosomes form contact sites with the ER which are important for the transfer of phospholipids and cholesterol. These contact sites are mediated by the Vps13 lipid transfer protein family. Vps13A links the mitochondria to the ER, while Vps13C links lysosomes/late endosomes to the ER. Although it is unclear how these various contact sites aid in maintaining both mitochondrial and lysosomal function, evidence shows that defects in one organelle may disturb the contact sites between others (Deus *et al*., 2019). In some cases, lysosomal defects cause the accumulation of undigested ganglioside GM1 which can affect membrane composition at mitochondria-ER contact sites. This may result in excessive Ca^{2+}

uptake by mitochondria, cell death, and neurodegeneration (Sano *et al*., 2009). In addition, several transcription factors can influence both mitochondrial and lysosomal autophagy/biogenesis, such as CREB, FoxO, and E2F1. It is, however, unclear if these transcription factors can act on these pathways simultaneously (Deus *et al*., 2019). While not well characterized, there is mounting evidence for the crosstalk between lysosomes and mitochondria.

Mitophagy is an important process in mitochondrial homeostasis and links the activities of lysosomes and mitochondria. Depolarization of mitochondria is a trigger for mitophagy, which is coordinated by *PINK1* and *Parkin*, two well established Parkinson Disease (PD)-related genes. PINK1 accumulates on the OMM of damaged or depolarized mitochondria. Parkin is phosphorylated by PINK1 into its active form, where this E3 ubiquitin ligase ubiquitinates protein substrates present on the OMM. Mitophagy receptors are then recruited to promote the interaction of autophagosomes and damaged mitochondria, and the targeted degradation by lysosomes (Deus *et al*., 2019; Navarro-Romeo *et al*., 2020). This provides further evidence of the link between these two organelles.

5.1.2 VPS13C

Vacuolar protein sorting 13C (VPS13C) is a large and evolutionarily conserved protein. In humans, it contains 86 exons which span a 208-kb genomic region and is part of the VPS13 protein family, along with three other human proteins (VPS13A, VPS13B, and VPS13D). There are two main transcriptional variants, 1A and 2A. Variant 1A lacks exons 6 and 7, and is expressed in most tissues, while variant 2A encodes a longer protein which is expressed strictly in the brain (Lesage *et al*., 2016). Notably, variant 2A may be expressed in the brain at higher

levels than variant 1A. All four proteins in the VPS13 family have been implicated in neurodegenerative or neurodevelopmental disease.

VPS13C transports lipids by acting at contact sites between intracellular organelles. A key feature is the presence of domains that possess hydrophobic cavities which shield lipids from the aqueous environment of the cytoplasm (Thelen and Zoncu, 2017; Ugur *et al*., 2020). Various animal models have shown that loss of function in VPS13 family proteins can affect membrane traffic at Golgi-endosome interfaces, autophagy, cytoskeletal organization, calcium signalling, and mitochondrial homeostasis (Kumar *et al*., 2018; Ugur *et al*., 2020). Such is the case of *VPS13C* which has been found to be a contributory factor in the development of early-onset PD (Reinisch and Prinz, 2021). Yeast models have shown that VPS13 can accumulate at contacts between the yeast vacuole (resembling mammalian lysosomes) and the nuclear envelope of the endoplasmic reticulum. This demonstrates that VPS13 is likely involved in lipid transport between the endoplasmic reticulum and mitochondria, via an indirect route that may involve the lysosome (Ugur *et al.,* 2020; Kumar *et al*., 2018). In mammals, VPS13C is localized at contacts between the ER and late endosomes/lysosomes. Mutations in *VPS13C* have been linked to rare cases of early-onset, autosomal recessive PD, while common variants are a risk factor for sporadic PD (Lesage *et al.,* 2016; Abeliovich and Gitler, 2016; Kumar *et al*., 2018). Knockdown of *VPS13C* has been shown to increase mitochondrial recruitment of PINK1 and Parkin, upregulate parkin transcripts, and increase PINK1/Parkin mediated mitophagy. Lower mitochondrial membrane potential, mitochondrial fragmentation, and increased respiration rates have been observed (Lesage *et al*., 2016; Ugur *et al*., 2020). Studies suggest that there is "crosstalk" between lysosomes and mitochondria, as well as mitochondrial dysfunction in response to lysosome dysfunction (Ugur *et al*., 2020). Thus, VPS13C seems to act in pathways

that run parallel to those indirectly involved in mitochondrial health (Benson and Huntley, 2019). Dysfunction in lysosomes could cause PD through impairment of mitochondrial homeostasis and quality control. A single *VPS13C* homologue (*Vps13*) exists in *D. melanogaster,* which makes studying gene interactions easier.

5.2 Materials and Methods

5.2.1 Bioinformatic Analysis

A bioinformatic analysis was conducted to determine the similarity between protein sequences of VPS13C homologues in *D. melanogaster* (NP_001260781.1) and *H. sapiens* (EAW77607.1). See Chapter 2, section 2.1 for detail on Bioinformatic analysis.

5.2.2 *Drosophila melanogaster* stocks and crosses

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* stock centre at Indiana University (Indiana, USA) and the Vienna *Drosophila* Resource Centre as part of the Vienna Biocentre Core Facilities (Vienna, Austria). See Table 5.1 for list of genotypes used. See Chapter 2, section 2.3 for detail on *D. melanogaster* crosses.

5.2.3 Longevity Assay

An analysis on the survival of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in median lifespan. See Chapter 2, section 2.4 for full longevity assay methods.

5.2.4 Locomotor Assay

An analysis on the locomotor ability of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in locomotor ability over time. See Chapter 2, section 2.5 for full locomotor assay methods.

Table 5.1: Genotypes and location of expression patterns used in the analysis of altered expression of *Vps13*

Abbreviated Genotype	**Location of Expression**	**Insertion Chromosome**	**Reference**
Control Lines			
UAS-lacZ	---	2	Brand *et al*., 1993
UAS-LUC-RNAi	---	3	Perkins *et al*., 2015
Driver Lines			
C380-Gal4	Motor neuron	X	Sanyal, 2009
D42-Gal4	Motor neuron	3	Parkes *et al*., 1998
OK6-Gal4	Motor neuron	2	RRID:BDSC_64199
TH-Gal4	Dopaminergic neuron	3	Inamdar *et al*., 2014
Repo-Gal4	Glial cell	3	RRID:BDSC_7415
Responder Lines			
UAS-Vps13-EY	---	2	Bellen *et al*, 2004
UAS-Vps13-RNAi^{42625}	---	2	Perkins *et al,* 2015
UAS-Vps13-RNAi^{29972}	---	3	Dietzl *et al*., 2007

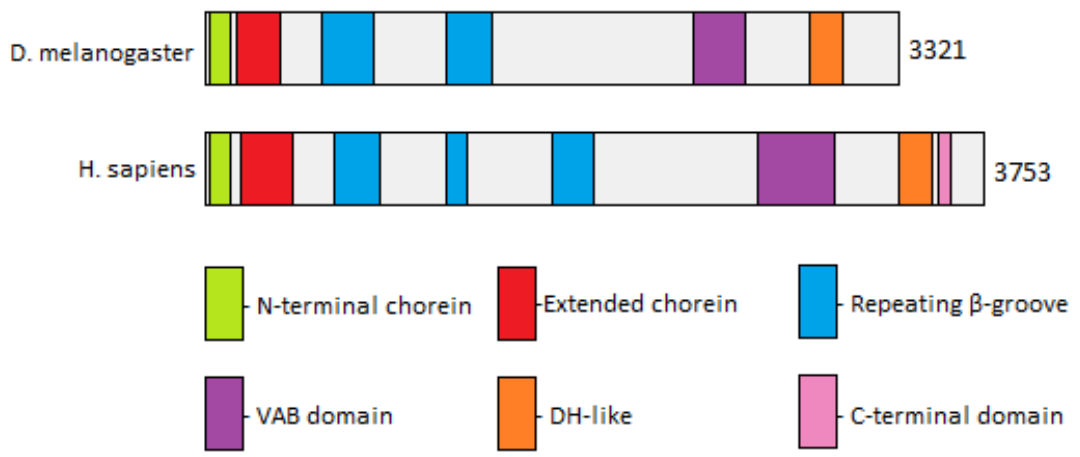
5.3 Results

5.3.1 Bioinformatic Analysis of Vps13C

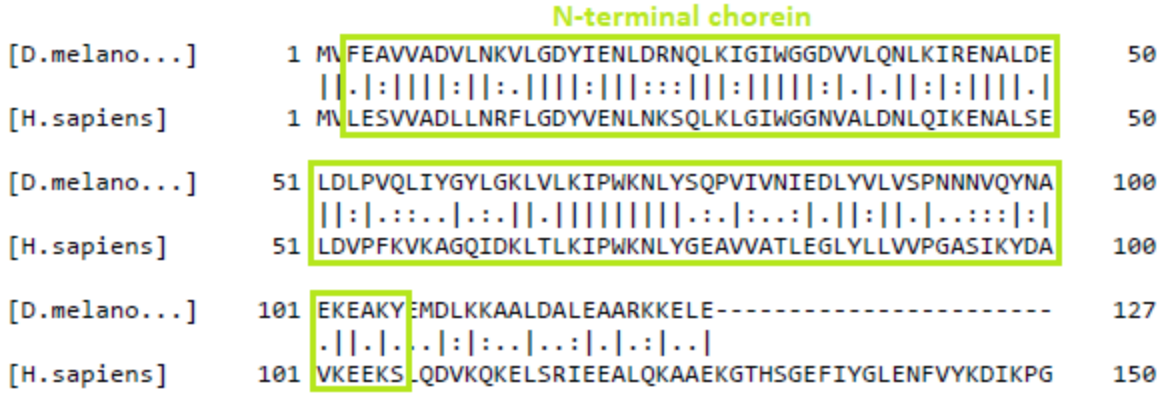
A pairwise alignment between *D. melanogaster* Vps13 (NP_001260781.1) and *H. sapiens* Vps13C (EAW77607.1) shows a high degree of conservation with 45% similarity between proteins (Figure 5.1). The use of Interpro to identify the presence of domains revealed that each protein contains a highly conserved N-terminal chorein domain, which contains a scooped shape lined with hydrophobic residues that can bind the tails of glycerophospholipids (Leonzino *et al*., 2021). Downstream from the N-terminal chorein domain is an extended chorein region. Both *D. melanogaster* Vps13 and *H. sapiens* Vps13C have repeating β-groove regions (also known as the repeating region of Vps13), though *H. sapiens* have one additional repeating region. These regions contain an FFAT motif which interacts with the VAMP-associated protein (VAP), facilitating the tethering of the ER (Kumar *et* al., 2018; Cai *et al*., 2022). Further downstream is the Vps13 adaptor binding (VAB) domain which allows Vps13 to interact with membrane-specific adaptor proteins. In the case of Vps13C, the VAB domain interacts with the Ypt35 protein which recruits Vps13C to endosomal membranes (Bean *et al*., 2018). Lastly, both *D. melanogaster* and *H. sapiens* proteins share a DH-like domain which appears to be the lipid-binding region of Vps13 (Kumar *et al*., 2018). The Vps13C protein in *H. sapiens* contains a C-terminal domain which was not reported for *D. melanogaster.* However, the pairwise alignment shows that there is a high degree of conservation between *D. melanogaster* and *H. sapiens* in the area where this C-terminal domain is found. This domain is also found in autophagy-related protein 2 (ATG2) proteins, which may function in lipid transport and facilitates the anchoring of Vps13/ATG2 to mitochondria, late endosomes, or lipid droplets (Dziurdzik and Conibear, 2021). Given the degree of domain conservation between the *D. melanogaster* and *H. sapiens* proteins,

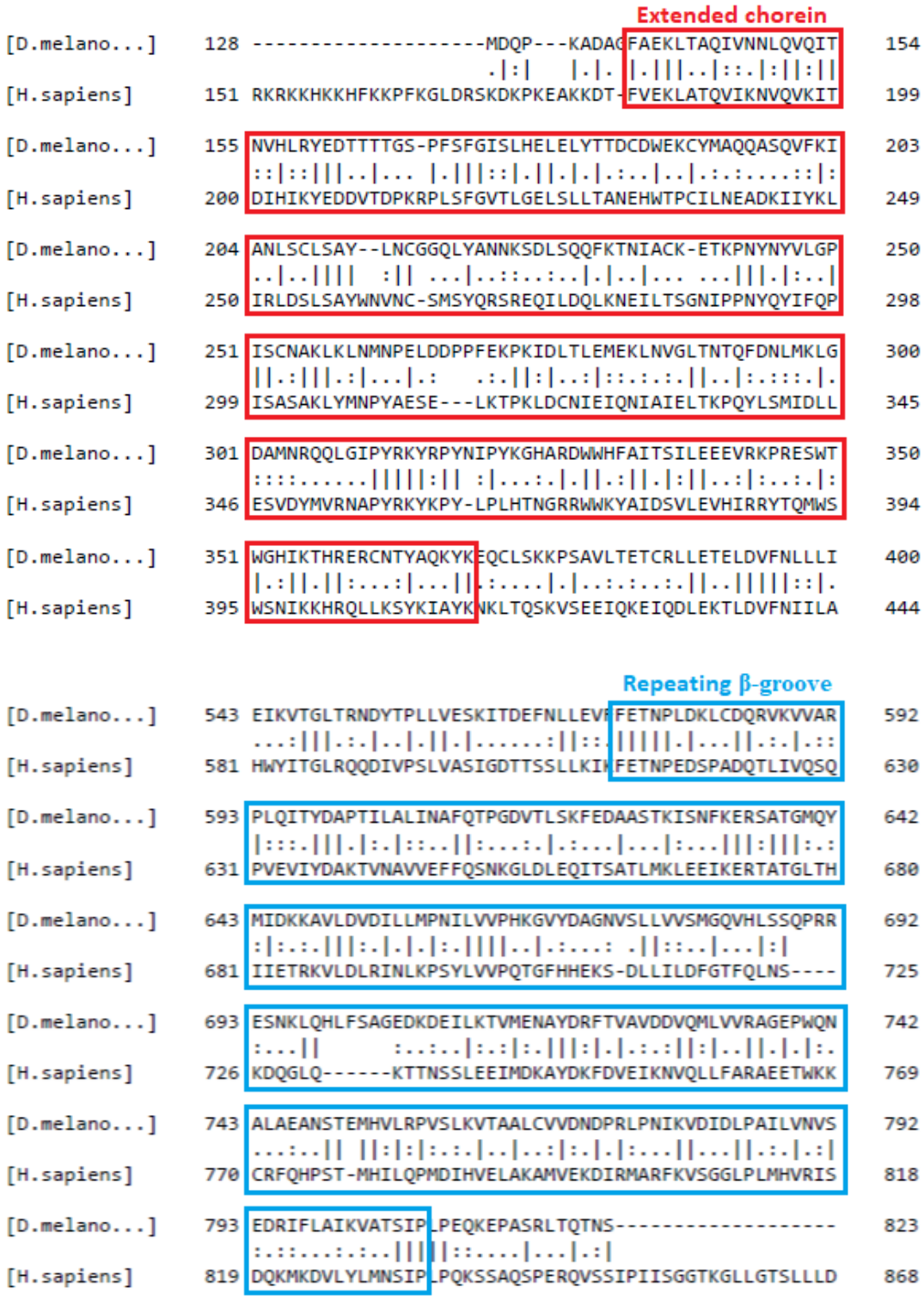
Vps13 is ideal for studying *VPS13C* function without the redundancy found within the *Vps13* family in humans.

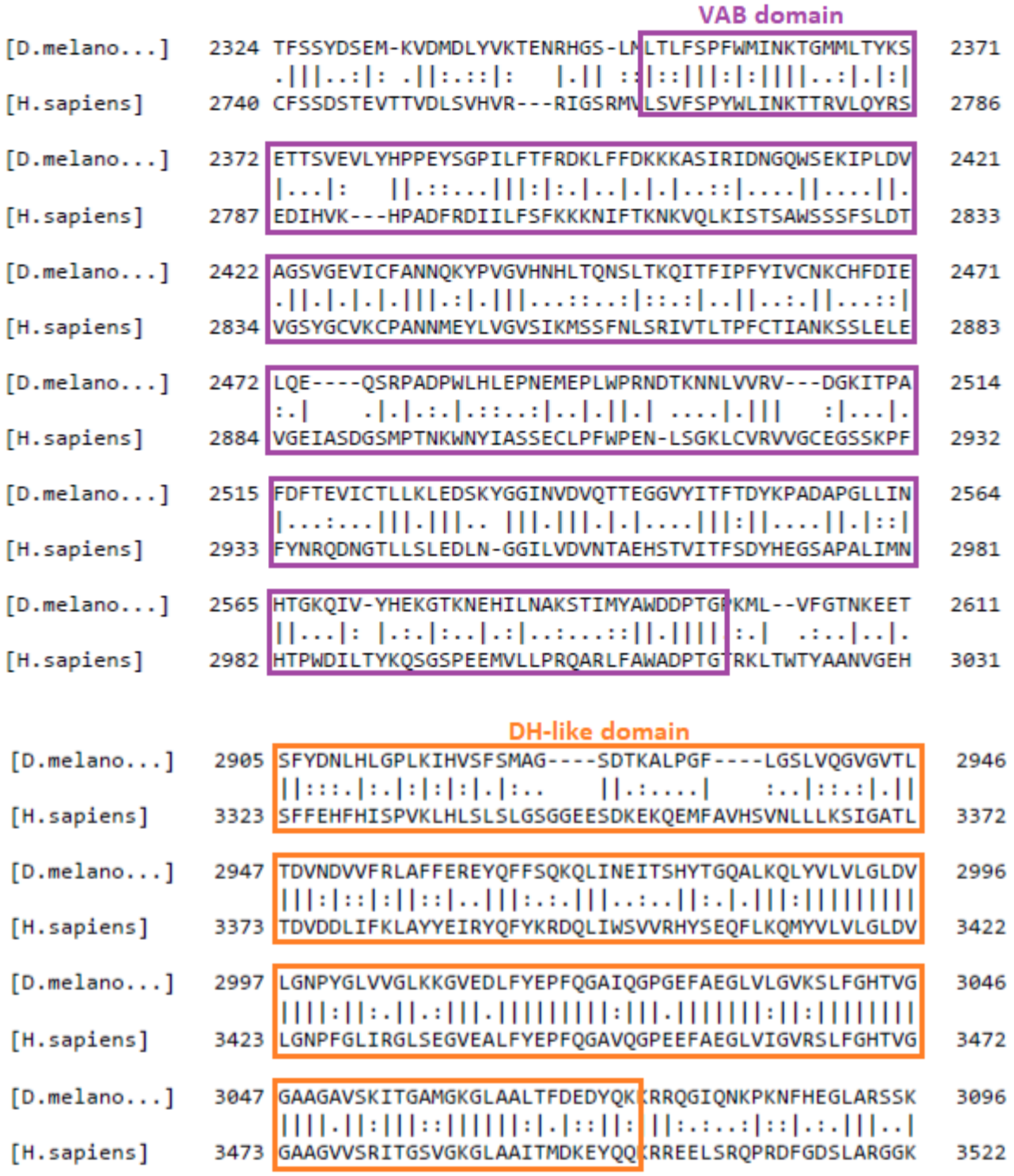
A)


D. melanogaster
3321
H. sapiens
3753
N-terminal chorein
Extended chorein
Repeating β-groove
VAB domain
DH-like
C-terminal domain

B)


N-terminal chorein
[D.melano...] 1 MVFEAVVADVLNKVLGDYIENLDRNQLKIGIWGGDVVLQNLKIRENALDE 50
[H.sapiens] 1 MVLESVVADLLNRFLGDYVENLNKSQLKLGIWGGNVALDNLQIKENALSE 50
[D.melano...] 51 LDLPVQLIYGYLGKLVLKIPWKNLYSQPVIVNIEDLYVLVSPNNNVQYNA 100
[H.sapiens] 51 LDVPFKVKAGQIDKLTLKIPWKNLYGEAVVATLEGLYLLVVPGASIKYDA 100
[D.melano...] 101 EKEAKYEMDLKKAALDALEAARKKELE---------- 127
[H.sapiens] 101 VKEEKSLQDVKQKELSRIEEALQKAAEKGTHSGEFIYGLENFVYKDIKPG 150


Extended chorein
Repeating β-groove


VAB domain
[D.melano...] 2324 TFSSYDSEM-KVDMDLYVKTENRHGS-LMLTLFSPFWMINKTGMMLTYKS 2371
[H.sapiens] 2740 CFSSDSTEVTTVDLSVHVR---RIGSRMVLSVFSPYWLINKTTRVLQYRS 2786
[D.melano...] 2372 ETTSVEVLYHPPEYSGPILFTFRDKLFFDKKKASIRIDNGQWSEKIPLDV 2421
[H.sapiens] 2787 EDIHVK---HPADFRDIILFSFKKKNIFTKNKVQLKISTSAWSSSFSLDT 2833
[D.melano...] 2422 AGSVGEVICFANNQKYPVGVHNHLTQNSLTKQITFIPFYIVCNKCHFDIE 2471
[H.sapiens] 2834 VGSYGCVKCPANNMEYLVGVSIKMSSFNLSRIVTLTPFCTIANKSSLELE 2883
[D.melano...] 2472 LQE----QSRPADPWLHLEPNEMEPLWPRNDTKNNLVVRV---DGKITPA 2514
[H.sapiens] 2884 VGEIASDGSMPTNKWNYIASSECLPFWPEN-LSGKLCVRVVGCEGSSKPF 2932
[D.melano...] 2515 FDFTEVICTLLKLEDSKYGGINVDVQTTEGGVYITFTDYKPADAPGLLIN 2564
[H.sapiens] 2933 FYNRQDNGTLLSLEDLN-GGILVDVNTAEHSTVITFSDYHEGSAPALIMN 2981
[D.melano...] 2565 HTGKQIV-YHEKGTKNEHILNAKSTIMYAWDDPTGPKML--VFGTNKEET 2611
[H.sapiens] 2982 HTPWDILTYKQSGSPEEMVLLPRQARLFAWADPTGTRKLTWTYAANVGEH 3031
DH-like domain
[D.melano...] 2905 SFYDNLHLGPLKIHVSFSMAG----SDTKALPGF----LGSLVQGVGVTL 2946
[H.sapiens] 3323 SFFEHFHISPVKLHLSLSLGSGGEESDKEKQEMFAVHSVNLLLKSIGATL 3372
[D.melano...] 2947 TDVNDVVFRLAFFEREYQFFSQKQLINEITSHYTGQALKQLYVLVLGLDV 2996
[H.sapiens] 3373 TDVDDLIFKLAYYEIRYQFYKRDQLIWSVVRHYSEQFLKQMYVLVLGLDV 3422
[D.melano...] 2997 LGNPYGLVVGLKKGVEDLFYEPFQGAIQGPGEFAEGLVLGVKSLFGHTVG 3046
[H.sapiens] 3423 LGNPFGLIRGLSEGVEALFYEPFQGAVQGPEEFAEGLVIGVRSLFGHTVG 3472
[D.melano...] 3047 GAAGAVSKITGAMGKGLAALTFDEDYQKCRRQGIQNKPKNFHEGLARSSK 3096
[H.sapiens] 3473 GAAGVVSRITGSVGKGLAAITMDKEYQQCRREELSRQPRDFGDSLARGGK 3522

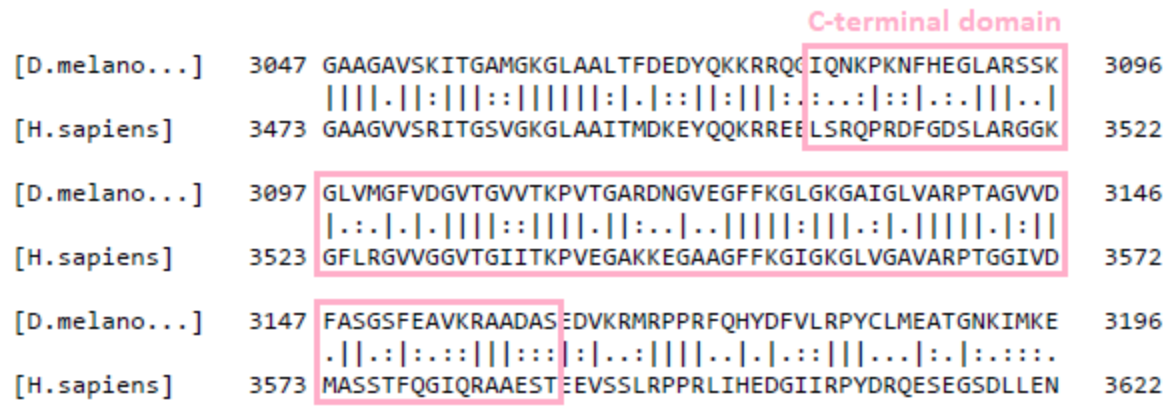


Figure 5.1: The Vps13 protein in *D. melanogaster* and Vps13C protein in *H. sapiens* are highly conserved. A) Aligned sequences show the position of each domain in *D. melanogaster* Vps1 and *H. sapiens* Vps13C. Green represents the N-terminal chorein, red represents the extended chorein region, blue represents the repeating β-groove region, purple represents the Vps13 adaptor binding (VAB) domain, orange represents the Dbl homology (DH)-like domain. **B)** A multiple alignment between Vps13 and Vps13C shows a high degree of sequence conservation within several shared domains. "|" indicates identical amino acids in all sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions.

5.3.2 Overexpression of *Vps13* affects median lifespan in a *Gal4*-dependent manner but does not significantly alter locomotor ability.

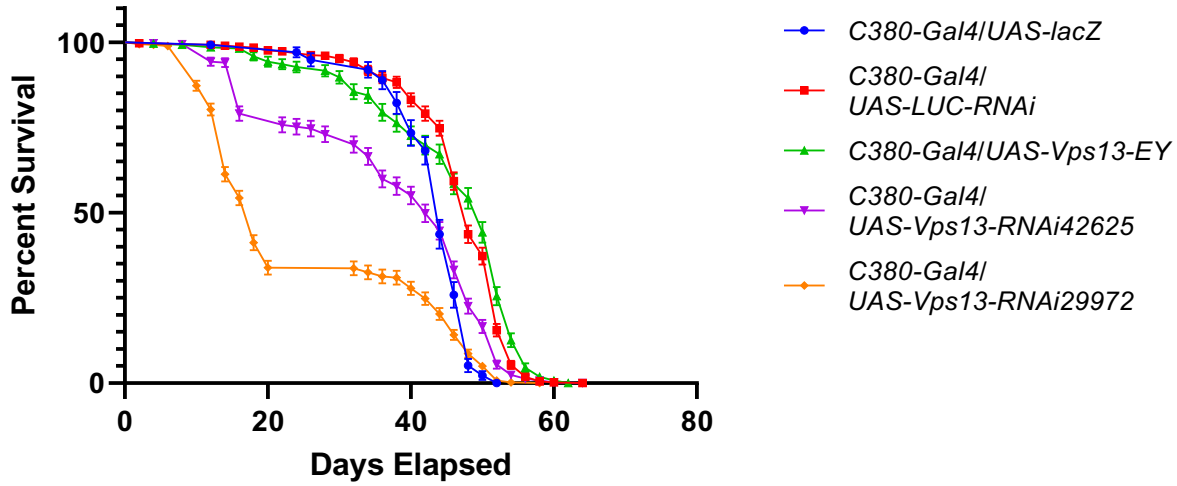
An analysis of the consequences that *Vps13* overexpression has on lifespan and locomotor ability of *D. melanogaster* shows that overexpression affects median lifespan depending on the expression pattern of the *Gal4* transgene when compared to a control (*UAS-lacZ*). Expression of *Vps13* using the motor neuron-specific *C380-Gal4* transgene resulted in an increase in median lifespan (Figure 5.2A). When expressed with the glial cell-specific *Repo-Gal4* transgene (Figure 5.5A) and dopaminergic neuron-specific *TH-Gal4* (Figure 5.6) transgene, *Vps13* overexpression significantly decreased median lifespan. A more severe decrease in longevity is exhibited when *Vps13* is expressed with *OK6-Gal4* (Figure 5.4). There was no statistically significant influence on locomotor ability when *Vps13* is overexpressed.

5.3.2 Inhibition of *Vps13* affects lifespan in a *Gal4*-dependent manner but does not significantly alter locomotor ability.

The consequence of *Vps13* inhibition on *D. melanogaster* lifespan and locomotor ability overtime were carried out using two *Vps13* inhibition responder transgenes: *UAS-Vps13-RNAi*^{42625} and *UAS-Vps13-RNAi*^{29972}. When *Vps13-RNAi* are expressed, the effect on *D. melanogaster* lifespan is dependent on the expression pattern of the transgene. Expression of both *Vps13* inhibition transgenes decreases median lifespan when driven by the *C380-Gal4* transgene (Figure 5.2A), though the reduction in lifespan is more severe with *UAS-Vps13-RNAi*^{29972}. When *Vps13* is inhibited using the *D42-Gal4* (Figure 5.3A) and the *TH-Gal4* (Figure 5.6A), a slight increase in median lifespan is seen with the *UAS-Vps13-RNAi*^{42625} line, while a

slight decrease in median lifespan is seen with the *UAS-Vps13-RNAi*^{29972} line. Inhibition of *Vps13* through both the *OK6-Gal4* (Figure 5.4A) and *Repo-Gal4* (Figure 5.5A) transgenes increases median lifespan in both *Vps13* inhibition lines. There is no statistically significant influence of *Vps13* inhibition on locomotor ability when compared to a *UAS-LUC-RNAi* control.

A)



B)

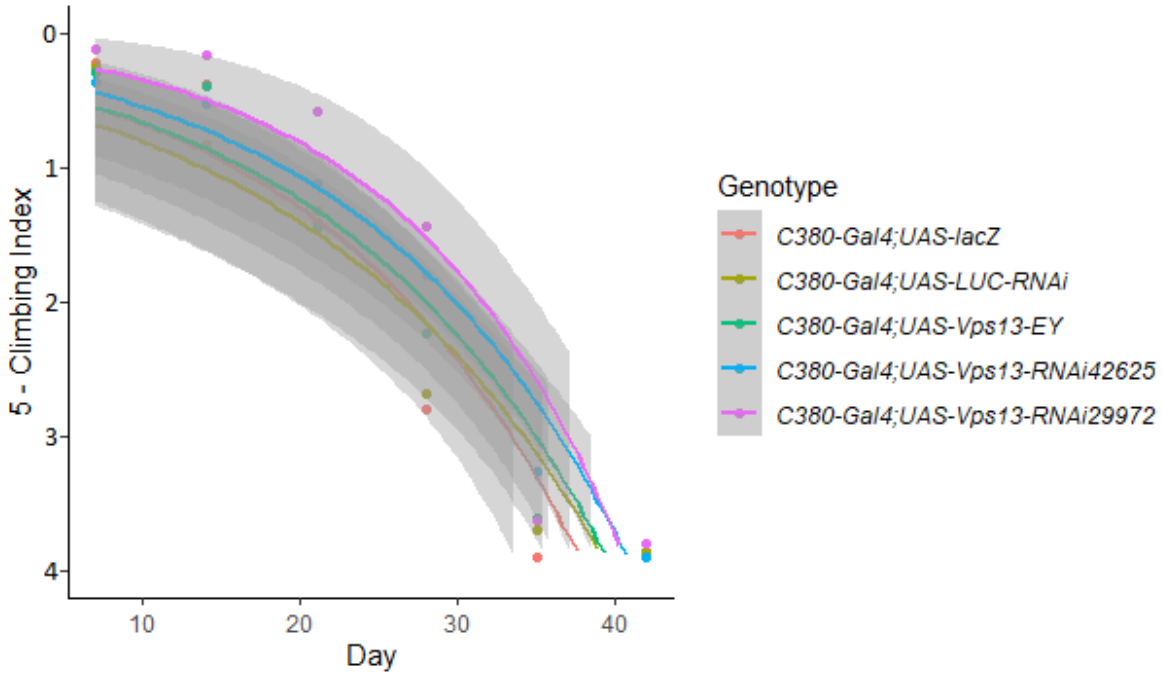
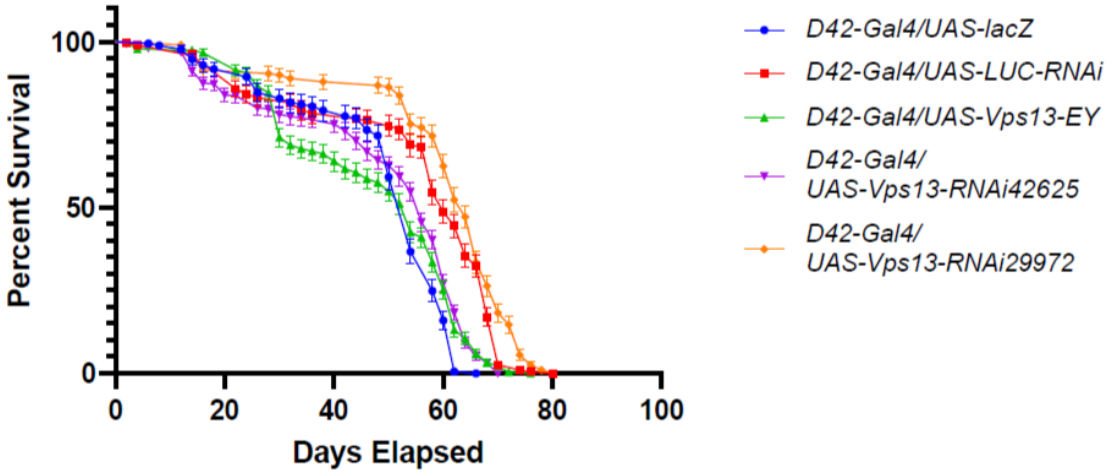


Figure 5.2: Altered expression of *Vps13* directed through the C380-*Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *C380-Gal4/UAS-lacZ* (n=135), *C380-Gal4/UAS-LUC-RNAi* (n=373), *C380-Gal4/UAS-Vps13-EY* (n=262), *C380-Gal4/UAS-Vps13-RNAi*^{42625} (n=367), *C380-Gal4/UAS-Vps13-RNAi*^{29972} (n=517). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

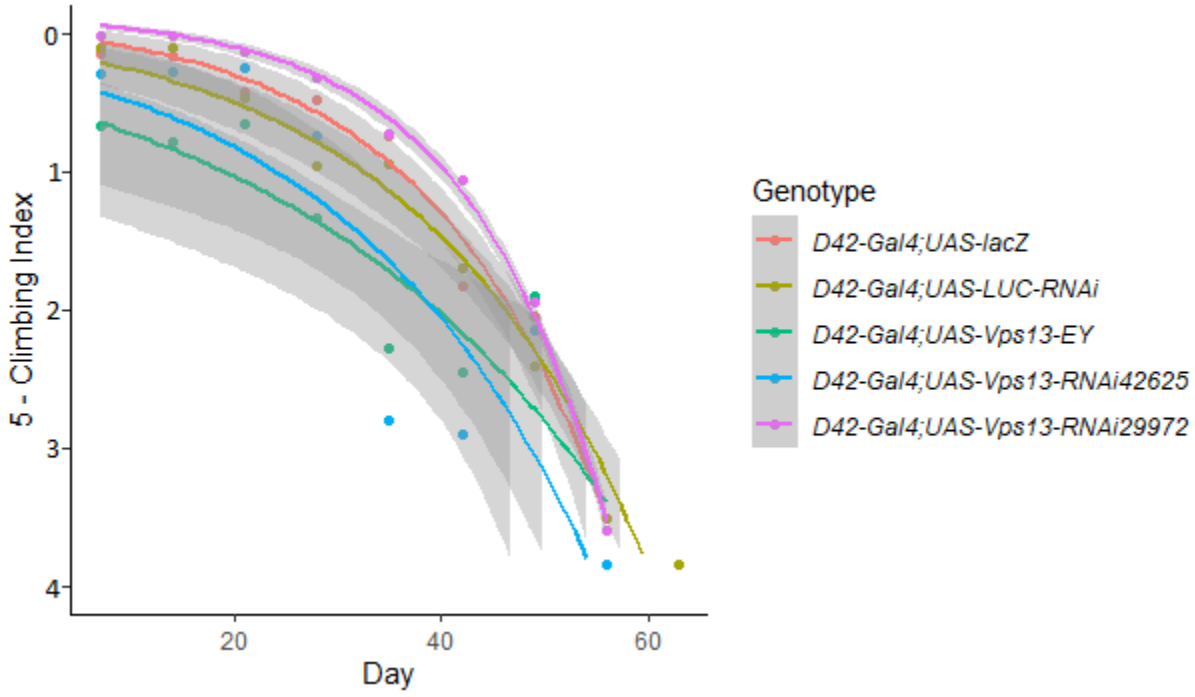
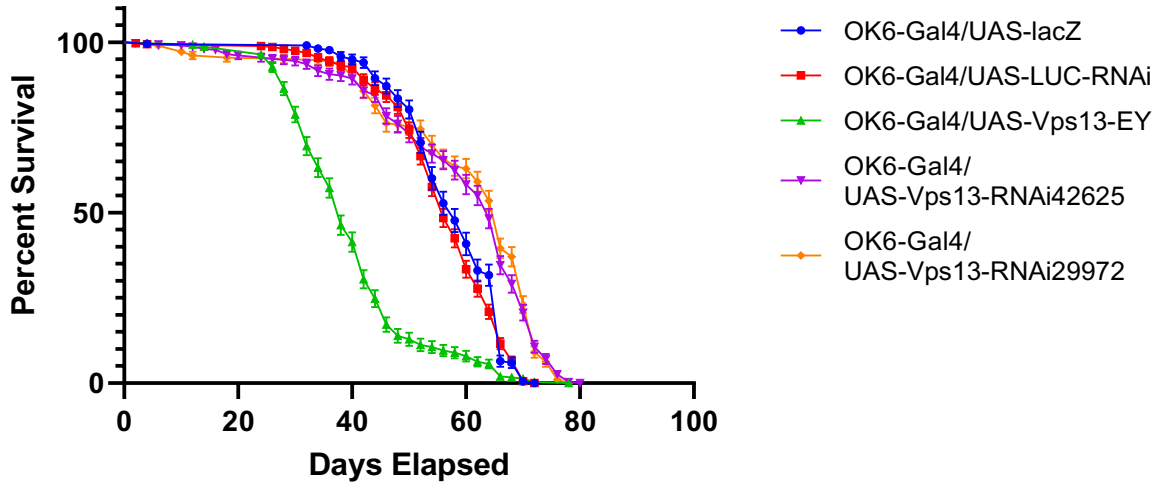


Figure 5.3: Altered expression of *Vps13* directed through the *D42-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *D42-Gal4/UAS-lacZ* (n=169)*, D42-Gal4/UAS-LUC-RNAi* (n=189), *D42-Gal4/UAS-Vps13-EY* (n=266), *D42-Gal4/UAS-Vps13-RNAi*^{42625} (n=305), *D42-Gal4/UAS-Vps13-RNAi*^{29972} (n=197). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

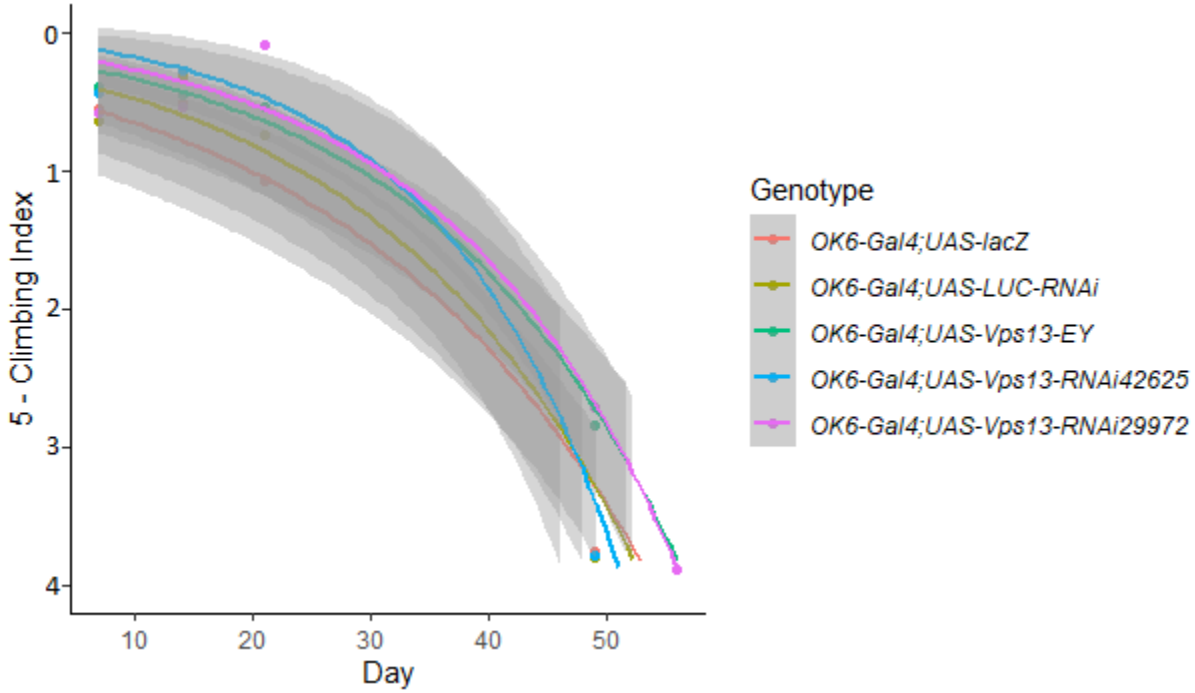
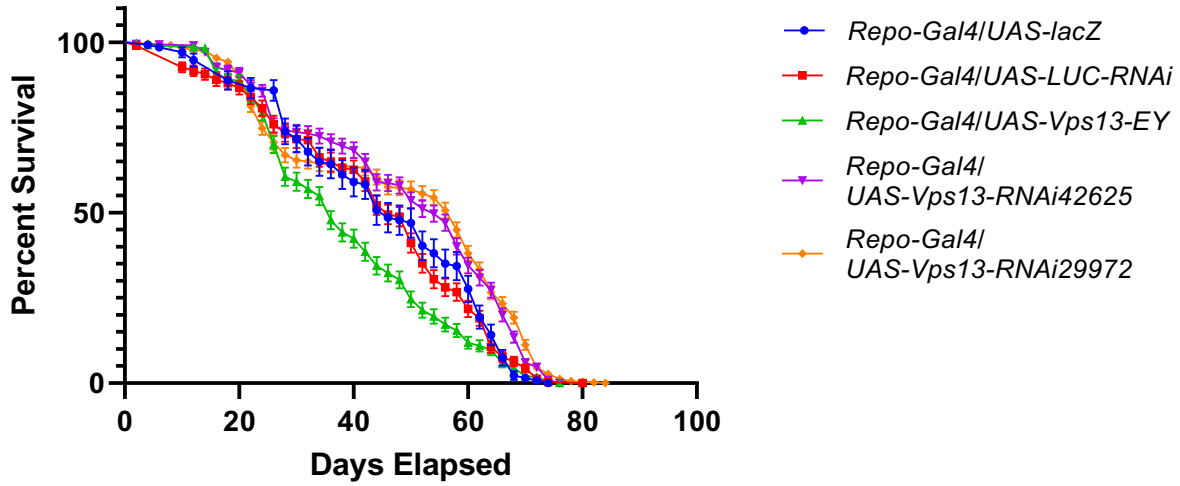


Figure 5.4: Altered expression of *Vps13* directed through the *OK6-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *OK6-Gal4/UAS-lacZ* (n=218), *OK6-Gal4/UAS-LUC-RNAi* (n=353), *OK6-Gal4/UAS-Vps13-EY* (n=302), *OK6-Gal4/UAS-Vps13-RNAi*^{42625} (n=309), *OK6-Gal4/UAS-Vps13-RNAi*^{29972} (n=286). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Vps13* expression in the motor neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

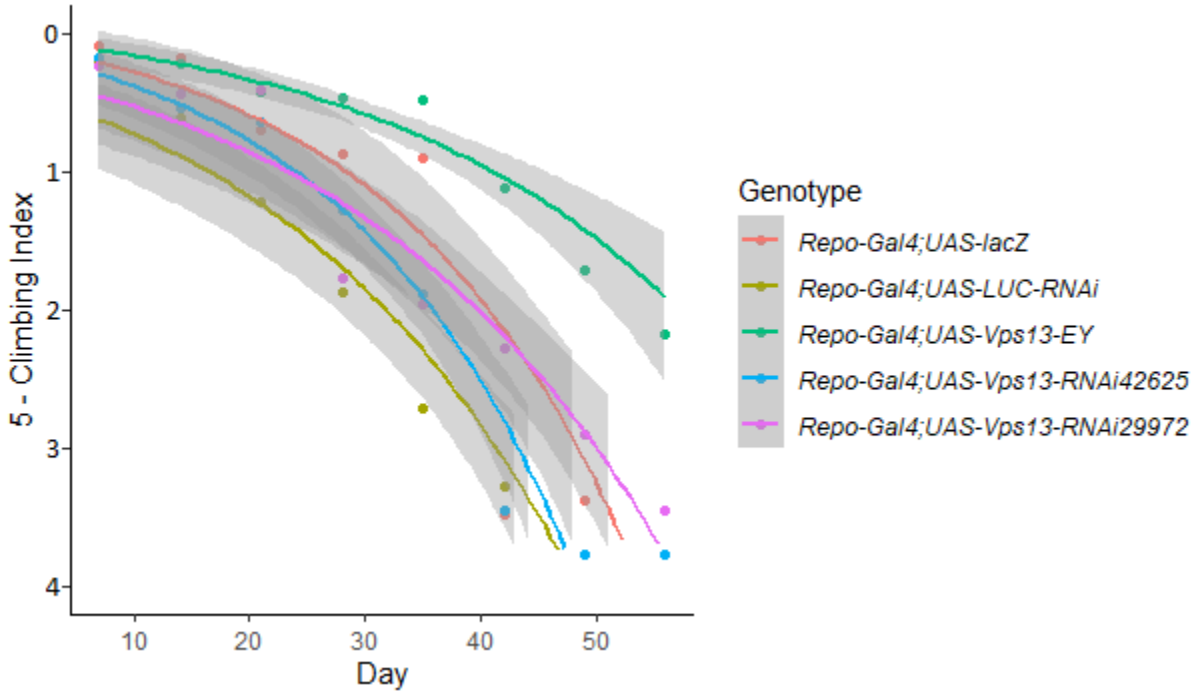
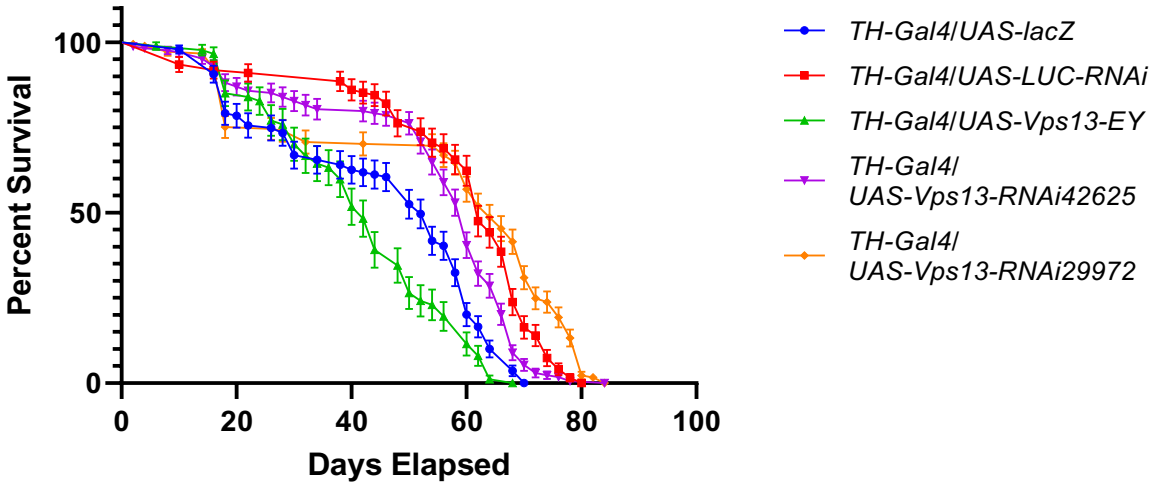


Figure 5.5: Altered expression of *Vps13* directed through the Repo-*Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Vps13* expression in glial cells. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *Repo-Gal4/UAS-lacZ* (n=134), *Repo-Gal4/UAS-LUC-RNAi* (n=299), *Repo-Gal4/UAS-Vps13-EY* (n=337), *Repo-Gal4/UAS-Vps13-RNAi*^{42625} (n=414), *Repo-Gal4/UAS-Vps13-RNAi*^{29972} (n=480). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Vps13* expression in the glial cells. Locomotor ability was determined by a generalized linear model (CI=95%).

A)



B)

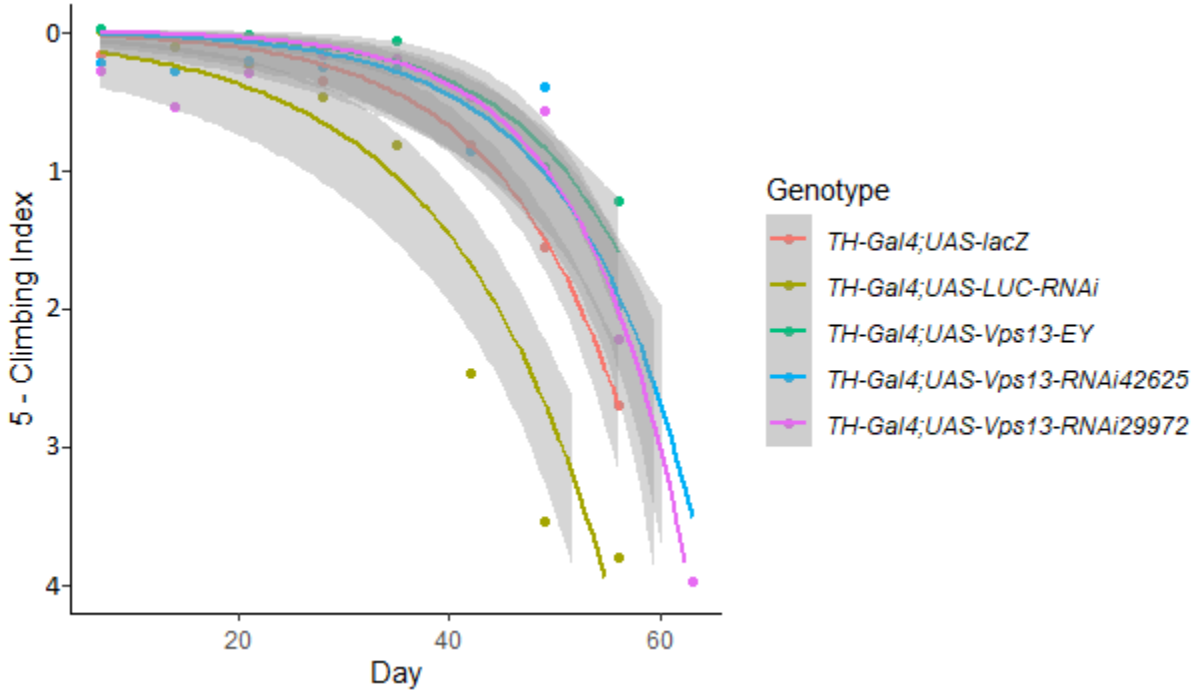


Figure 5.6: Altered expression of *Vps13* directed through the *TH-Gal4* transgene affects lifespan. **A:** Longevity assay of *D. melanogaster* males displaying altered *Vps13* expression in the dopaminergic neurons. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean. Genotypes are as follows: *TH-Gal4/UAS-lacZ* (n=139), *TH-Gal4/UAS-LUC-RNAi* (n=122), *TH-Gal4/UAS-Vps13-EY* (n=87), *TH-Gal4/UAS-Vps13-RNAi*^{42625} (n=168), *TH-Gal4/UAS-Vps13-RNAi*^{29972} (n=181). **B:** Locomotor assay of *D. melanogaster* males displaying altered *Vps13* expression in the dopaminergic neurons. Locomotor ability was determined by a generalized linear model (CI=95%).

5.4 Discussion

Evidence suggests that there exists crosstalk between the lysosomes and mitochondria. Both mitochondria and lysosomes form contact sites with the ER, which are important for facilitating the exchange of lipids. The Vps13 lipid transfer protein family is key in the mediation of this, as VPS13A links the mitochondria to the ER, while VPS13C links lysosomes/late endosomes to the ER. It is unclear how these various contact sites aid in maintaining both mitochondrial and lysosomal function, but evidence shows that defects in one organelle may disturb the contact sites between others (Deus *et al*., 2019). In some cases, lysosomal defects cause the accumulation of undigested ganglioside GM1, which can affect membrane composition at mitochondria-ER contact sites. This may result in excessive Ca^{2+} uptake by mitochondria, cell death, and neurodegeneration (Sano *et al*., 2009). Mitophagy is another important process that links the activities of lysosomes and mitochondria. Depolarization of mitochondria is a trigger for mitophagy, which is coordinated by *PINK1* and *Parkin*. Levels of PINK1 accumulate on the OMM of damaged or depolarized mitochondria. Parkin is phosphorylated by PINK1 into its active form, where this E3 ubiquitin ligase ubiquitinates protein substrates present on the OMM. Mitophagy receptors are then recruited to promote the interaction of autophagosomes and damaged mitochondria, and the targeted degradation by lysosomes (Dues *et* al., 2019; Navarro-Romeo *et al*., 2020). This provides insight into how lipid transport and the endolysosomal pathway may be directly or indirectly linked to mitochondrial biogenesis. *PINK1* and *Parkin* are known to influence mitochondrial biogenesis through mediation by *PARIS*. PARIS binds to and is regulated by Parkin and PINK1. PINK1 does so by mediating phosphorylation of PARIS which primes it for ubiquitylation by Parkin (Lee *et al.,* 2017). In turn, PARIS regulates levels of PGC-1α by binding to its promotor and repressing expression (Castillo-Quan, 2011; Shin *et al*.,

2011). This study aims to better characterize Vps13C and better understand the connection between lipid transport and PD.

Bioinformatic analysis shows a high degree of conservation between *D. melanogaster* Vps13 and *H. sapiens* VPS13C. Both proteins contain a highly conserved N-terminal chorein domain which contains a scooped shape lined with hydrophobic residues which can bind the tails of glycerophospholipids (Leonzino *et al*., 2021). This is important in the function of an LTP. Downstream from the N-terminal chorein domain is an extended chorein region. These proteins too have repeating β-groove regions (also known as the repeating region of Vps13), though *H. sapiens* has one additional repeating region. These regions contain an FFAT motif which interacts with the VAMP-associated protein (VAP), a type IV membrane protein. This interaction likely facilitates the tethering of the ER (Kumar *et al*., 2018; Cai *et al*., 2022). Further downstream is the Vps13 adaptor binding (VAB) domain which allows Vps13 to interact with membrane-specific adaptor proteins. In the case of Vps13C, the VAB domain interacts with the Ypt35 protein which recruits Vps13C to endosomal membranes (Bean *et al*., 2018). Lastly, both *D. melanogaster* and *H. sapiens* proteins share a DH-like domain which appears to be the lipid-binding region of Vps13 (Kumar *et al*., 2018). The Vps13C protein in *H. sapiens* contains a C-terminal domain, also found in autophagy-related protein 2 (ATG2) proteins, which may also function in lipid transport and facilitates the anchoring of Vps13/ATG2 to mitochondria, late endosomes, or lipid droplets (Dziurdzik and Conibear, 2021). Given the degree of conservation between the *D. melanogaster* and *H. sapiens* proteins, *Vps13* is the homologue of *Vps13C.*

The overexpression of *Vps13* influences longevity in a *Gal4* dependent manner. When *C380-Gal4* is used to drive *Vps13* in the motor neurons, median lifespan increases. However, there seems to be an overall decrease in longevity with *Vps13* overexpression. Particularly with

the use of the motor neuron-specific driver *OK6-Gal4*, a severe reduction in longevity (20 days) is seen compared to the control. A less severe result is seen using the glial cell and dopaminergic drivers *Repo-Gal4* and *TH-Gal4,* respectively. Similarly, previous research from the Staveley research group found an increase using a motor neuron-specific driver, and a severe decrease in longevity when overexpression is driven in the dopaminergic neurons (Kasravi, 2019). While this study did not find any significant alteration of locomotor ability when *Vps13* is overexpressed, Kasravi (2019) found a severe decrease in locomotor ability when expression is driven using *D42-Gal4* and a slight decrease when expressed using *TH-Gal4*.

The inhibition of *Vps13* influences longevity in a transgene-specific manner. Inhibition driven through *C380-Gal4* decreases longevity in both *UAS-Vps13-RNAi* lines, with a particularly severe reduction in lifespan (24 days) in the *UAS-Vps13-RNAi*^{29972} line when compared to the control. Reduced longevity is seen in *UAS-Vps13-RNAi*^{42625} when expressed using *D42-Gal4* and *TH-Gal4* transgenes, though these are slight changes. In contrast, *UAS-Vps13-RNAi*^{29972} slightly increases longevity when expressed using *D42-Gal4* and *TH-Gal4.* This may be an indication to the degree of silencing achieved by each *Vps13-RNAi* line. A moderate increase in longevity is seen in both *RNAi* lines when inhibition is driven using *OK6-Gal4* and *Repo-Gal4*. Similarly, Kasravi (2018) found an increase in longevity with *D42-Gal4;UAS-Vps13-RNAi*^{29972} and *TH-Gal4;UAS-Vps13-RNAi*^{29972} flies. Other research shows that loss of function mutations in Vps13C causes mitochondrial dysfunction and increases PINK1/Parkin mediated mitophagy, as well as the upregulation of the *Parkin* gene (Lesage *et al*., 2016; Smolders *et al*., 2021). This was seen in *D. melanogaster* where loss of function in *Vps13* mutants show a decreased lifespan and age-dependent neurodegeneration (Vonk *et al*., 2017). The discrepancy in findings may be attributed to the difference in *Vps13* inhibition. In this study,

RNAi was used to inhibit *Vps13* expression post-transcriptionally rather than using a mutated form of the *Vps13* gene. In this instance, while *Vps13* inhibition is upregulating the *Parkin* gene and PINK1/Parkin driven mitophagy, it is possible that Paris is being inhibited. This may result in an increase in mitochondrial biogenesis and an overall increase in neuroprotection. The proposed relationship that lipid transport, the endolysosomal system, mitophagy, and mitochondrial biogenesis have should be explored further.

Chapter 6 – Evaluating Neural-*Gal4* and *UAS*-responding transgenes

6.1 Introduction

6.1.1 Use of negative controls

The *UAS-Gal4* system is well established as a powerful tool for controlling the expression of transgenes in *Drosophila melanogaster* (Brand and Perrimon, 1993; Busson and Pret, 2007). This system makes use of the yeast *Gal4* transcription factor and its perceived inactivity in *D. melanogaster*. However, the expression of *Gal4* can have effects on the phenotype of *Drosophila*. Such is the case when *GMR-Gal4* is used for targeted expression in the fly compound eye. Homozygotes display a highly disorganized ommatidial array and higher levels of apoptosis compared to heterozygotes (Kramer and Staveley, 2003). It is therefore important to use negative controls, such as *UAS-lacZ*, and examine the role that benign transgenes may play in phenotypic expression. *lacZ* is a gene found in *Escherichia coli* (*E. coli*) which encodes β-galactosidase, an enzyme responsible for cleaving β-glycosidic bonds found in lactose (Silver Key *et al*., 2015). Since *lacZ* is not naturally found in the *D. melanogaster* genome, this makes it a useful control for the presence of *Gal4* transgenes.

RNAi-mediated gene knockdown is used in the *UAS-Gal4* system to determine loss-of-function phenotypes. Expression of such *RNAi* transgenes can silence endogenous genes post-transcriptionally (Perrimon *et al*., 2010). However, non-specific phenotypes (false positives) may result from off target effects (Kondo *et al*., 2009; Langer *et al*., 2010; Jonchere and Bennett, 2013). In the past, our research group has evaluated *UAS-GFP-RNAi* expression in a subset of neurons in *D. melanogaster*. Green fluorescent protein (GFP) is an autofluorescent protein originally isolated from the jellyfish *Aequorea forskalea* and is commonly used as a biological

marker (Grover *et al*., 2009; Prendergast and Mann, 1978). *UAS-GFP-RNAi* was a proposed negative control line for *RNAi* loss-of-function experiments but was found to reduce lifespan in *D. melanogaster* and does not always perform as a benign control (Chavoshi-Jolfaei and Staveley, 2020). Therefore, when using *RNAi*, negative controls should be analyzed. *UAS-LUC-RNAi* is one proposed control that involves *RNAi* specified for the luciferase protein from fireflies. Here we demonstrate that it appears to be a suitable control responder transgene for longevity assays.

6.1.2 Potential for enhancing longevity with *Gal4* transgenes

The identification of *Gal4* directing transgenes (or "drivers"), which have potential to increase lifespan under certain conditions, is ideal for enhanced longevity assays. A key feature of Parkinson Disease (PD) is the loss of dopaminergic (DA) neurons and impaired movement (Antony *et al*., 2013). Thus, *TH-Gal4* and *D42-Gal4* are widely used drivers which express target genes in the DA and motor neurons, respectively. Previous research done by our lab shows that *TH-Gal4* when driving *PINK1* overexpression, as well as *Ref(2)P* inhibition leads to an increased median lifespan in *D. melanogaster* (Todd and Staveley, 2012; Hurley and Staveley, 2021). The *D42-Gal4* transgene has been shown to increase lifespan in many instances such as driving overexpression of *G6PD*, *dTOR*, and *hSOD1* (Parkes *et al.*, 1998; Legan *et al.*, 2008; Mockett and Nobles, 2013).

Some potential *Gal4* drivers which are emerging in *Drosophila* research include the motor neuron-specific drivers *OK6-Gal4* and *C380-Gal4*, as well as the glial cell-specific driver *Repo-Gal4*. A *Gal4* transgene which drives expression in the glial cells is of particular interest

given the protective and supportive role they provide for neurons (Jäkel and Dimou, 2017). Particularly, astrocytes and microglial cells have been implicated in PD. Post-mortem brain samples show that dopaminergic neurons in areas of the brain which are poorly populated with astrocytes may be more prone to degeneration. In addition, α-synuclein inclusions in the *SNpc* astrocytes seem to correlate with the severity of dopaminergic neuron loss. Activated microglial cells are observed abundantly in the *SNpc* (Vila *et al*., 2001; Miyazaki and Asanuma, 2020). Here we identify if *OK6-Gal4*, *C380-Gal4* and *Repo-Gal4* appear to be suitable conditionally directing transgenes for enhanced longevity assays.

6.2 Methods

6.2.1 *Drosophila melanogaster* stocks and crosses

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* stock centre at Indiana University (Indiana, USA) and the Vienna *Drosophila* Resource Centre as part of the Vienna Biocentre Core Facilities (Vienna, Austria). See Table 6.1 for list of genotypes used. See Chapter 2, section 2.3 for detail on *D. melanogaster* crosses.

6.2.2 Longevity Assay

An analysis on the survival of *D. melanogaster* was conducted, comparing experimental fly lines to control lines, to determine differences in median lifespan. See Chapter 2, section 2.4 for full longevity assay methods.

Table 6.1: Genotypes and location of expression patterns used in the evaluation of neural-*Gal4* and *UAS*-responding transgenes.

Abbreviated Genotype	**Location of Expression**	**Insertion Chromosome**	**Reference**
Responder Lines			
UAS-lacZ	---	2	Brand *et al*., 1993
UAS-LUC-RNAi	---	3	Perkins *et al*., 2015
Driver Lines			
C380-Gal4	Motor neuron	X	Sanyal, 2009
D42-Gal4	Motor neuron	3	Parkes *et al*., 1998
OK6-Gal4	Motor neuron	2	RRID:BDSC_64199
TH-Gal4	Dopaminergic neuron	3	Inamdar *et al*., 2014
Repo-Gal4	Glial cell	3	RRID:BDSC_7415

6.3 Results

6.3.1 *UAS-LUC-RNAi* does not diminish longevity

The expression of *UAS-LUC-RNAi* under the direction of *C380-Gal4* (motor neuron-specific), *OK6-Gal4* (motor neuron-specific), and *Repo-Gal4* (glial-specific) yielded no significant difference in longevity when compared to the previously established *UAS-lacZ* control. When crossed to *TH-Gal4* (dopaminergic neuron-specific) and *D42-Gal4* (motor neuron-specific), expression of *UAS-LUC-RNAi* showed an increase in longevity. Therefore, this may be a suitable *RNAi* control for *D. melanogaster* longevity assays.

6.3.2 *C380-Gal4* does not contribute to enhanced longevity

To assess if the *C380-Gal4* transgene is suitable for *D. melanogaster* longevity assays, the median lifespan of *UAS-lacZ* expression under different *Gal4* transgenes is compared. The expression of *UAS-lacZ* under the direction of *C380-Gal4* appears to sensitize flies to degeneration. When comparing the median lifespan of *C380-Gal4* flies (44 days) to the other motor neuron-specific drivers, *D42-Gal4* (54 days) and *OK6-Gal4* (58 days), there was a statistically significant decrease in median lifespan. This result is not unlike those seen for previous studies which have used *C380-Gal4* to drive gene expression (Wilkinson *et al*., 2021). This suggests that *C380-Gal4* not be used in longevity assays. However, there may be use for *C380-Gal4* if attempting to model severe cases of neurodegeneration.

6.3.3 *Repo-Gal4* does not seem to contribute to enhanced longevity

To assess if the *Repo-Gal4* transgene is suitable for *D. melanogaster* longevity assays, the median lifespan of *UAS-lacZ* expression under different *Gal4* transgenes is compared. The median lifespan of *UAS-lacZ* expression through *Repo-Gal4* was significantly lower (46 days) when compared to *D42-Gal4* (54 days), *TH-Gal4* (52 days), and *OK6-Gal4* (58 days). Thus, *Repo-Gal4* may not be a suitable driver for longevity assays.

6.3.4 *OK6-Gal4* median lifespan is similar to *TH-Gal4* and *D42-Gal4* when driving *lacZ* expression

When crossed to *UAS-lacZ*, the drivers *D42-Gal4* (54 days), *TH-Gal4* (52 days), and *OK6-Gal4* (58 days) had similar median lifespans. There was no statistical difference between that of *D42-Gal4* and *TH-Gal4*. However, *OK6-Gal4* showed a small but statistically significant increase in lifespan compared to both *D42-Gal4* and *TH-Gal4*. Given that the *OK6-Gal4* transgene did not impact median lifespan negatively, it may be a suitable driver for *D. melanogaster* longevity assays.

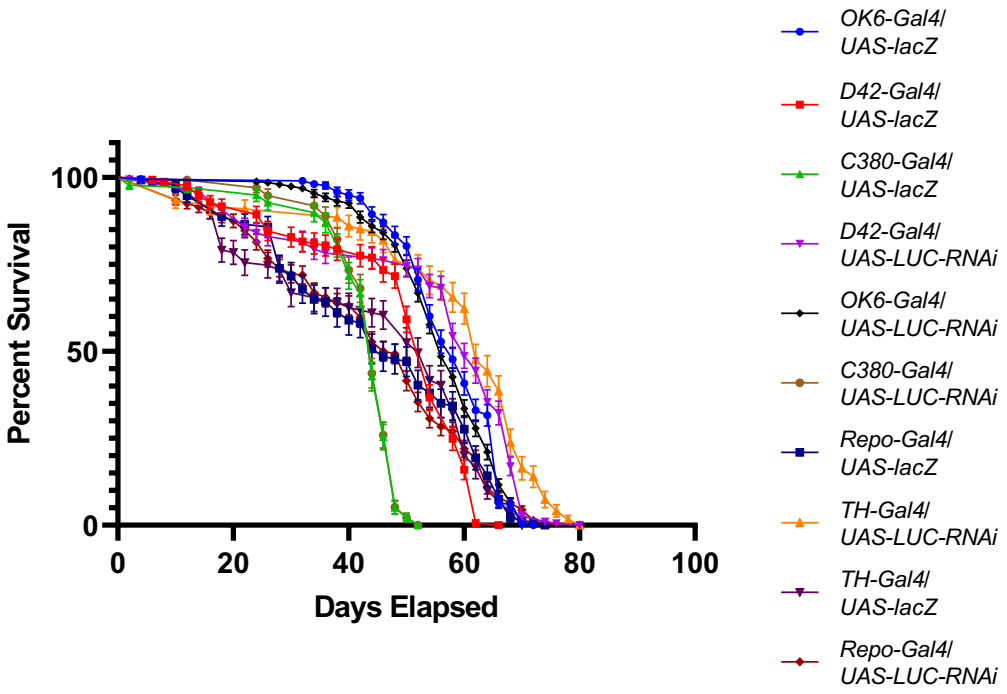


Figure 6.1: Longevity of critical class males when responder genes *UAS-lacZ*$^{4\text{-}1\text{-}2}$ and *UAS-LUC-RNAi* are placed under the control of several tissue-specific directing transgenes. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean.

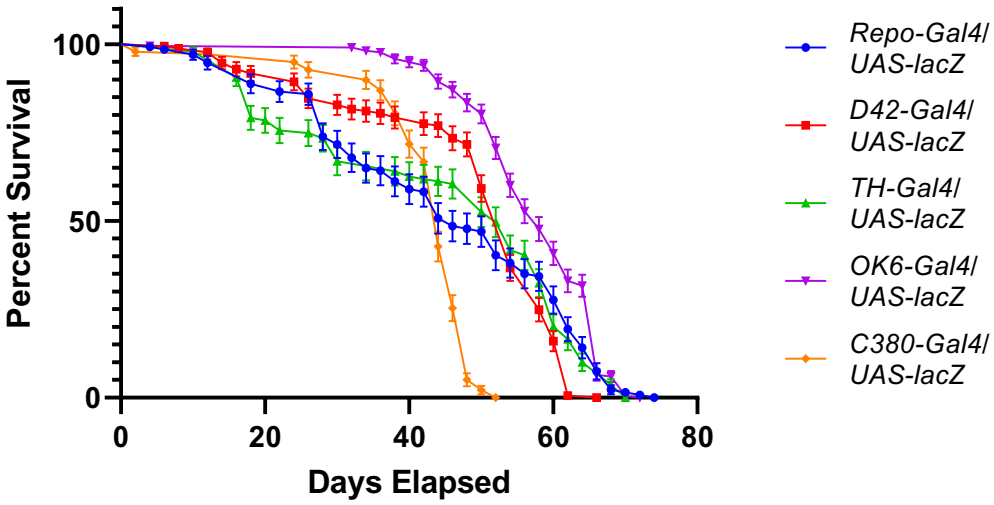


Figure 6.2: Longevity of critical class males when *UAS-lacZ* is placed under the control of several tissue-specific directing transgenes. Longevity is depicted by percent survival. Significance is P <0.05 using the log-rank test. Error bars represent standard error of the mean.

6.4 Discussion

The *UAS-Gal4* system first described by Brand and Perrimon (1993) is a powerful tool for altering gene expression in *D. melanogaster*. This system makes use of the yeast *Gal4* transcription factor and its perceived inactivity in *D. melanogaster*. *Gal4* expression, however, has been shown to influence phenotype in some instances. When *GMR-Gal4* is used for targeted expression in the fly compound eye, homozygotes display a highly disorganized ommatidial array and higher levels of apoptosis compared to heterozygotes (Kramer and Staveley, 2003). This highlights the importance of negative controls, such as *UAS-lacZ.* Since *lacZ* is not naturally found in the *D. melanogaster* genome, this makes it a useful control for the presence of *Gal4* transgenes. *RNAi*-mediated gene knockdown is used in the *UAS-Gal4* system to determine loss-of-function phenotypes (Perrimon *et al*., 2010). Expression of such *RNAi* transgenes can

silence endogenous genes post-transcriptionally, though it is possible for off target effects to produce non-specific phenotypes (Kondo *et al*., 2009; Langer *et al*., 2010; Jonchere and Bennett, 2013). Therefore, when using *RNAi*, negative controls should be analyzed. *UAS-LUC-RNAi* is a proposed control which involves *RNAi* specified for the luciferase protein from fireflies. This experiment found that when driven in the motor neurons, dopaminergic neurons and glial cells, *UAS-LUC-RNAi* expression does not diminish longevity when compared to the previously established *UAS-lacZ* control. The expression of *UAS-LUC-RNAi* under the direction of *C380-Gal4* (motor neuron-specific), *OK6-Gal4* (motor neuron-specific), and *Repo-Gal4* (glial-specific) yielded no significant difference in longevity. When crossed to *TH-Gal4* (dopaminergic neuron-specific) and *D42-Gal4* (motor neuron-specific), expression of *UAS-LUC-RNAi* showed an increase in longevity. While expression of *UAS-LUC-RNAi* with these drivers differs from the *UAS-lacZ* control, given that *UAS-LUC-RNAi* expression does not diminish longevity means it may be a suitable *RNAi* control.

The identification of *Gal4* directing transgenes which have potential to increase lifespan under certain conditions is ideal for enhanced longevity assays. Previous research done by the Staveley research group shows that *TH-Gal4* when driving *PINK1* overexpression, as well as *Ref(2)P* inhibition leads to an increased median lifespan in *D. melanogaster* (Todd and Staveley, 2012; Hurley and Staveley, 2021). The *D42-Gal4* transgene has also been shown to increase lifespan in many other instances such as driving overexpression of *G6PD*, *dTOR*, and *hSOD1* (Parkes *et al.*, 1998; Legan *et al*., 2008; Mockett and Nobles, 2013). This study aimed to evaluate the motor neuron-specific drivers *C380-Gal4* and *OK6-Gal4*, as well as the glial cell-specific driver *Repo-Gal4* which have not previously been used by this lab. This was done by expressing *UAS-lacZ* in each *Gal4* transgene and comparing median lifespan. The expression of *UAS-lacZ*

under the direction of *C380-Gal4* appears to sensitize flies to degeneration. When comparing the median lifespan of *C380-Gal4* flies (44 days) to the other motor neuron-specific drivers, *D42-Gal4* (54 days) and *OK6-Gal4* (58 days), there was a statistically significant decrease in median lifespan. This result is not unlike those seen for previous studies which have used *C380-Gal4* to drive gene expression (Wilkinson *et al*., 2021). This suggests that *C380-Gal4* not be used in longevity assays. However, there may be use for *C380-Gal4* if attempting to model severe cases of neurodegeneration. When crossed to *UAS-lacZ*, the drivers *D42-Gal4* (54 days), *TH-Gal4* (52 days), and *OK6-Gal4* (58 days) had similar median lifespans. There was no statistical difference between that of *D42-Gal4* and *TH-Gal4*. However, *OK6-Gal4* showed a small but statistically significant increase in lifespan compared to both *D42-Gal4* and *TH-Gal4*. Given that the *OK6-Gal4* transgene did not impact median lifespan negatively, it may be a suitable driver for *D. melanogaster* longevity assays. The median lifespan of *UAS-lacZ* expression through *Repo-Gal4* was significantly lower (46 days) when compared to *D42-Gal4* (54 days), *TH-Gal4* (52 days), and *OK6-Gal4* (58 days). Thus, *Repo-Gal4* may not be a suitable driver for longevity assays.

Chapter 7 – Summary

7.1 Summary

As global life expectancy increases so does the prevalence of age-related neurodegenerative disease. Such an example is Parkinson Disease (PD), which significantly impacts the quality of life of those afflicted. Current forms of treatment do not cure or stop disease progression, but rather manage symptoms. Understanding the complex underlying cellular and molecular pathways involved in PD onset is imperative for developing new and better treatments.

Drosophila melanogaster is a model organism, and when combined with the *UAS-Gal4* system creates a powerful tool for the study of altered gene expression. *PGC-1α*, *PARIS*, and *VPS13C* have been implicated in neurodegeneration, particularly in PD. Evaluating the effect of altered *srl* expression in *D. melanogaster* shows that overexpression alters lifespan and locomotor ability in a tissue-specific manner. Interestingly, overexpression can lead to a decreased lifespan and locomotor ability. The inhibition of *srl* also influences *D. melanogaster* in a tissue-specific manner. It is unexpected that in most instances *srl* inhibition can lead to increased lifespan. Further research into understanding if there are 'optimal' levels of *srl* expression, and how inhibition of *srl* could increase lifespan is required.

Investigation into the effect of altered expression of *Paris* on *D. melanogaster* yielded unexpected results. Overexpression of *Paris* led to an increase in lifespan, while inhibition of *Paris* led to a decrease in lifespan. One would expect the overexpression of *Paris* to inhibit mitochondrial biogenesis via *srl* inhibition, and vice versa when *Paris* is inhibited. Further research should be done to better characterize the interactions between *Paris* and *srl* in fruit flies.

Investigation into the effect of altered *Vps13* expression yielded varying results which were dependent on both the tissue-specific expression of the *Gal4* transgene, as well as the *UAS-Vps13-RNAi* line used. Overexpression of *Vps13* seems to decrease lifespan in most instances, except for enhanced expression using the *C380-Gal4* transgene. This seems to be a good model for neurodegeneration, as this decrease in lifespan could be attributed to upregulated *parkin/PINK1* mediated mitophagy, and downregulated levels of *srl*. However, the interplay between these proteins is not well understood, and further research is required to understand the influence that altered *Vps13* dynamics have on mitochondrial health.

This study made use of *RNAi* transgenes which have been previously used by the Staveley research group. Expression of the *RNAi* transgenes is intended to produce critical class progeny which have reduced or inhibited expression of the target gene. However, one way to validate this could be to coexpress both an overexpression and inhibition transgene and determine if the longevity or locomotor phenotype is intermediate to that of the overexpression and inhibition lines on their own. This would be particularly useful for understanding the difference in the two *UAS-Vps13-RNAi* lines, as in some instances each has an 'opposite' effect on longevity.

7.2 Future directions

This study further characterizes altered *PGC-1α*, *PARIS*, and *VPS13C*. Future research should, however, investigate the interactions these genes and their protein products may have. There is mounting evidence for lysosomal/mitochondrial crosstalk which is still not well understood. *VPS13C* may be indirectly linked to the mitochondrial biogenesis pathway, through influencing *Parkin* and *PINK1*. Insight may be gained by the coexpression of *Vps13* and *Paris* or

srl, particularly if enhanced longevity or rescued phenotypes result from such experiments. Any future studies should also include female *D. melanogaster* to eliminate sex bias.

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Appendix A – Supplemental Data for Chapter 3

```
[D.melano...]    399 SVKNTQCTGLGLGGKNLSLLK--------------QERDAALSVCAAK--    432
                     :...|:         .|||              :.|:::...|.:|
[H.sapiens]      203 --QRRPCS---------ELLKYLTTNDDPPHTKPTENRNSSRDKCTSKKK    241

[D.melano...]    433 ----------PIAPPTLSTTTPTLTP---------PAKRKLNLEEYKQRR    463
                             ...|.|||.   .|||         |.:.| .:|......
[H.sapiens]      242 SHTQSQSQHLQAKPTTLSL---PLTPESPNDPKGSPFENK-TIERTLSVE    287

[D.melano...]    464 CGGVGAVYPKPTPPKQA----------KIEVAAKRIAPTPVSKPTPSPKK    503
                     ..|...:.|..|||.:|          |::.:.|.:.|.|..||..|...
[H.sapiens]      288 LSGTAGLTPPTTPPHKANQDNPFRASPKLKSSCKTVVPPPSKKPRYSESS    337

[D.melano...]    504 AEIVNIVNSLKCPRTEQSSLPMDPITMAKNKVLRMLEMKRAQQLKIIDSR    553
                     ....|  ||.| ...|||.|...   ::|:.||
[H.sapiens]      338 GTQGN--NSTK-KGPEQSELYAQ---LSKSSVL----------------    364

[D.melano...]    554 VSAKVPRVTKLPPLKDIVKDTYCMETEDPGTEI-----------APLSNK    592
                     ......|.||.|.|:......|| ::.:..|||           ..|.||
[H.sapiens]      365 TGGHEERKTKRPSLRLFGDHDYC-QSINSKTEILINISQELQDSRQLENK    413

[D.melano...]    593 -LHPDYEEIIIVSA---SCNTDITIPPN-QLSKASPRSLLKSSVLLYNIS    637
                      :..|::..|..|.   .|....|:..: |:|..|.|..|:..
[H.sapiens]      414 DVSSDWQGQICSSTDSDQCYLRETLEASKQVSPCSTRKQLQDQ-------    456

[D.melano...]    638 NGQDANKNMSNSLIASIQSEVARQTSNTTLSTIQSNATKVMSSADKNCQH    687
                                .|::|:.:...:.:.:.....|.|.....|.:..:
[H.sapiens]      457 --------------EIRAELNKHFGHPSQAVFDDEADKTGELRDSDFSN    491

[D.melano...]    688 GEDMVIMHLPKDRVRKTLVSIATQTDLQPEFPLLTLPPKRQSRERTRRNY    737
                     .:   ...||. .:...|.......|.:.|...|:.|
[H.sapiens]      492 EQ---FSKLPM-FINSGLAMDGLFDDSEDESDKLSYP-------------    524

[D.melano...]    738 RRRRTQGSGS----NMSTSSSDFSSDCSSLVS----------HRSRSQYD    773
                         ..|:.|    |:|.|.|.|:|.|...||          .|.||:..
[H.sapiens]      525 ----WDGTQSYSLFNVSPSCSSFNSPCRDSVSPPKSLFSQRPQRMRSRSR    570

[D.melano...]    774 SIDQLRNLDVAATCGGSIGNGGYSSRSTQR---HRSSVSSSSYSENGQYR    820
                     |..:.|          |.....| |||..|   .|||..|..|.|:..||
[H.sapiens]      571 SFSRHR----------SCSRSPY-SRSRSRSPGSRSSSRSCYYYESSHYR    609

[D.melano...]    821 RRQRRTSYNKRRSRNQKRGSTSSSCSGSEKSDRER-SRSPHRKLRHSRSR    869
                     .|..|.|....|||::...|........|:...|| .|..:|:....|..
[H.sapiens]      610 HRTHRNSPLYVRSRSRSPYSRRPRYDSYEEYQHERLKREEYRREYEKRES    659

[D.melano...]    870 SRSKSDTRYPNNNSSSNNNNRRGFFDRNVSQPAVEERRIVYVGRIEQETT    919
                     .|:|...|.                    .|.|:||||::|||:|..:||
[H.sapiens]      660 ERAKQRERQ-------------------RQKAIEERRVIYVGKIRPDTT    689

[D.melano...]    920 KEILRRKFLPYGSIKQITIHYKENGMKYGFVTYERAQDAFTAIDTSH--R    967
                     :..||.:|..:|.|::.|::.:::|..|||:||....|||.|::..:  |
[H.sapiens]      690 RTELRDRFEVFGEIEECTVNLRDDGDSYGFITYRYTCDAFAALENGYTLR    739

[D.melano...]    968 DSQISMYDISFGGRRAFCRSSYADLDNAGINNYNSYVFPKEAPAPNVVED   1017
                     .|..:.:::.|.||:.|.:|:|||||:      ||..|...:........
[H.sapiens]      740 RSNETDFELYFCGRKQFFKSNYADLDS------NSDDFDPASTKSKYDSL    783

[D.melano...]   1018 SFEALLLQVKAKLNAGKSPVGSSTLEAPSASGTVLQGHSQM   1058
                     .|::||.:.:..|..
[H.sapiens]      784 DFDSLLKEAQRSLRR------------------------    798
```

Figure A1: The srl protein shows highly conserved protein domains. EMBOSS Needle pairwise sequence alignment of *D. melanogaster* srl and *H. sapiens* PGC-1α protein. "|" indicates identical amino acids in both sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions. The names of each species in the alignment appear in brackets on the right-hand side of the alignment. Numbers on the left and right-hand side of the alignment indicate the first and last amino acid of the sequence in that row.

Table A1. Completed list of genotypes used in the analysis of altered expression of *srl*.

Genotype	Abbreviation	Reference
Control Lines		
w; UAS-lacZ^{4-1-2}	*UAS-lacZ*	Brand *et al*., 1994
y[1] v[1] ;P{y[+t7.7] v[+t.8]=TRIP.JF01355}attP2	*UAS-LUC-RNAi*	Perkins *et al*., 2015
Driver Lines		
P{w[+m]=GAL4}C380, w[*]*	*C380-Gal4*	Sanyal, 2009
w[*]; P{w[+mW.hs]=GawB}D42	*D42-Gal4*	Parkes *et al.,* 1998
P{w[+mW.hs]=GawB}OK6	*OK6-Gal4*	RRID:BDSC_64199
w[1118]; P{w[+m]= GAL4}repo/TM3, Sb[1]*	*Repo-Gal4*	RRID:BDSC_7415
w[*]; P{w[+mC]=ple-GAL4.F}3	*TH-Gal4*	Inamdar *et al.,* 2014
Responder Lines		
y[1] w[67c23]; P{y[+mDint2] w[+mC]=EPgy2}srl[EY05931]	*UAS-srl-EY*	Bellen *et al,* 2004
y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00857}attP2	*UAS-srl-RNAi^{33914}*	Perkins *et al.,* 2015
y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00858}attP2	*UAS-srl-RNAi^{33915}*	Perkins *et al.,* 2015

Table A2. Log-rank statistical analysis of fly longevity with altered expression of *srl* through the *C380-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
C380-Gal4/ UAS-lacZ	135	44	N/A	N/A	N/A
C380-Gal4/ UAS-srl-EY	432	48	60.48	<0.0001	Yes
C380-Gal4/ UAS-LUC-RNAi	373	48	N/A	N/A	N/A
C380-Gal4/ UAS-srl-RNAi^{33914}	394	54	276.9	<0.0001	Yes
C380-Gal4/ UAS-srl-RNAi^{33915}	404	54	298.3	<0.0001	Yes

Table A3. Log-rank statistical analysis of fly longevity with altered expression of *srl* through the *D42-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
D42-Gal4/ UAS-lacZ	169	54	N/A	N/A	N/A
D42-Gal4/ UAS-srl-EY	460	50	0.01721	0.8956	No
D42-Gal4/ UAS-LUC-RNAi	189	60	N/A	N/A	N/A
D42-Gal4/ UAS-srl-RNAi^{33914}	297	50	20.87	<0.0001	Yes
D42-Gal4/ UAS-srl-RNAi^{33915}	220	56	16.15	<0.0001	Yes

Table A4. Log-rank statistical analysis of fly longevity with altered expression of *srl* through the *OK6-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
OK6-Gal4/ UAS-lacZ	218	58	N/A	N/A	N/A
OK6-Gal4/ UAS-srl-EY	414	42	169.3	<0.0001	Yes
OK6-Gal4/ UAS-LUC-RNAi	353	56	N/A	N/A	N/A
OK6-Gal4/ UAS-srl-RNAi^{33914}	181	66	70.64	<0.0001	Yes
OK6-Gal4/ UAS-srl-RNAi^{33915}	181	66	61.29	<0.0001	Yes

Table A5. Log-rank statistical analysis of fly longevity with altered expression of *srl* through the *Repo-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
Repo-Gal4/ UAS-lacZ	134	46	N/A	N/A	N/A
Repo-Gal4/ UAS-srl-EY	504	50	2.804	0.0940	No
Repo-Gal4/ UAS-LUC-RNAi	299	46	N/A	N/A	N/A
Repo-Gal4/ UAS-srl-RNAi^{33914}	416	50	23.18	<0.0001	Yes
Repo-Gal4/ UAS-srl-RNAi^{33915}	360	64	104.1	<0.0001	Yes

Table A6. Log-rank statistical analysis of fly longevity with altered expression of *srl* through the *TH-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
TH-Gal4/ UAS-lacZ	139	52	N/A	N/A	N/A
TH-Gal4/ UAS-srl-EY	149	54	0.6478	0.4209	No
TH-Gal4/ UAS-LUC-RNAi	122	62	N/A	N/A	N/A
TH-Gal4/ UAS-srl-RNAi^{33914}	130	54	0.2535	0.6146	No
TH-Gal4/ UAS-srl-RNAi^{33915}	279	62	0.4386	0.5078	No

Table A7. Tukey statistical analysis of locomotor ability with altered expression of *srl* through the *C380-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
C380-Gal4;UAS-lacZ/ C380-Gal4;UAS-srl-EY	0.1082	0.5295	0.204	1.000	No
C380-Gal4;UAS-LUC-RNAi/ C380-Gal4;UAS-srl-RNAi^{33914}	-1.3953	0.8089	-1.725	0.408	No
C380-Gal4;UAS-LUC-RNAi/ C380-Gal4;UAS-srl-RNAi^{33915}	-0.6490	0.6244	-1.039	0.831	No

Table A8. Tukey statistical analysis of locomotor ability with altered expression of *srl* through the *D42-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
D42-Gal4;UAS-lacZ/ D42-Gal4;UAS-srl-EY	2.09007	0.35972	5.810	<0.001	Yes (***)
D42-Gal4;UAS-LUC-RNAi/ D42-Gal4;UAS-srl-RNAi^{33914}	0.33553	0.29335	1.144	0.772	No
D42-Gal4;UAS-LUC-RNAi/ D42-Gal4;UAS-srl-RNAi^{33915}	0.30747	0.28706	1.071	0.812	No

Table A9. Tukey statistical analysis of locomotor ability with altered expression of *srl* through the *OK6-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
OK6-Gal4;UAS-lacZ/ OK6-Gal4;UAS-srl-EY	-0.01843	0.37796	-0.049	1.000	No
OK6-Gal4;UAS-LUC-RNAi/ OK6-Gal4;UAS-srl-RNAi^{33914}	0.81307	0.35328	2.302	0.141	No
OK6-Gal4;UAS-LUC-RNAi/ OK6-Gal4;UAS-srl-RNAi^{33915}	0.77633	0.35134	2.210	0.172	No

Table A10. Tukey statistical analysis of locomotor ability with altered expression of *srl* through the *Repo-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
Repo-Gal4;UAS-lacZ/ Repo-Gal4;UAS-srl-EY	0.30150	0.46532	0.648	0.9663	No
Repo-Gal4;UAS-LUC-RNAi/ Repo-Gal4;UAS-srl-RNAi^{33914}	0.16224	0.30618	0.530	0.9839	No
Repo-Gal4;UAS-LUC-RNAi/ Repo-Gal4;UAS-srl-RNAi^{33915}	0.11150	0.31908	0.349	0.9967	No

Table A11. Tukey statistical analysis of locomotor ability with altered expression of *srl* through the *TH-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
TH-Gal4;UAS-lacZ/ TH-Gal4;UAS-srl-EY	1.4740686	1.1616481	1.269	0.698	No
TH-Gal4;UAS-LUC-RNAi/ TH-Gal4;UAS-srl-RNAi^{33914}	-0.2135018	0.6216593	-0.343	0.997	No
TH-Gal4;UAS-LUC-RNAi/ TH-Gal4;UAS-srl-RNAi^{33915}	-0.2143957	0.5601338	-0.383	0.995	No

Appendix B – Supplemental Data for Chapter 4

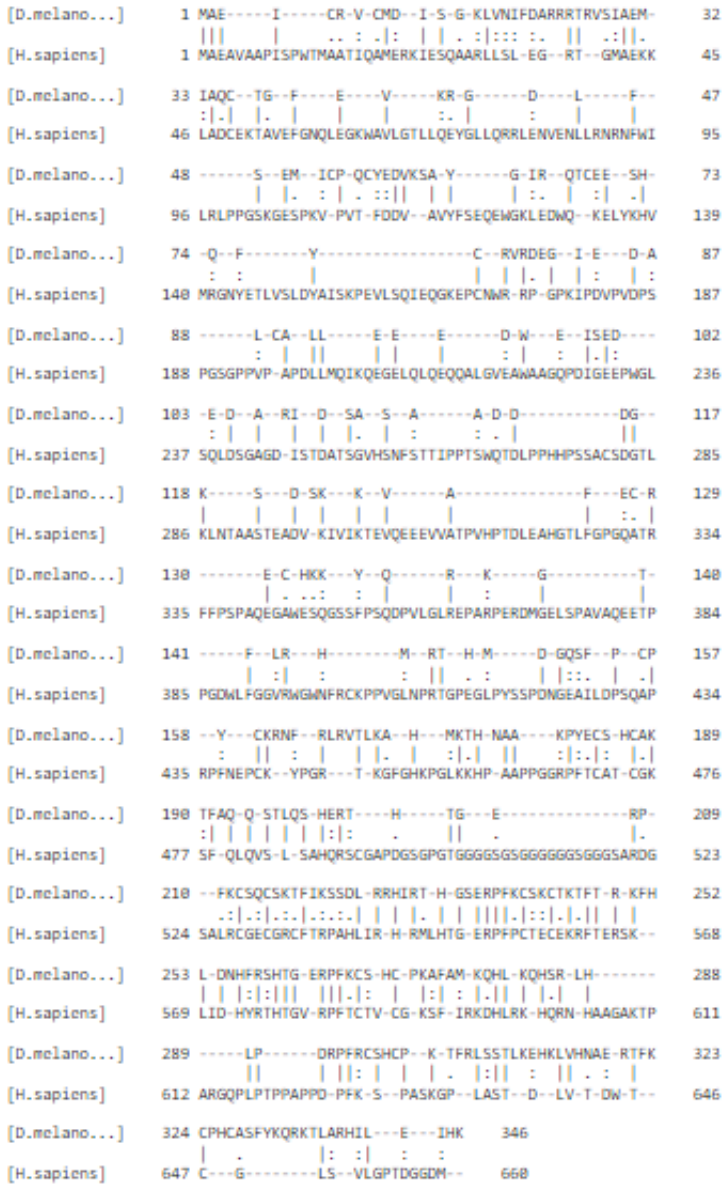


Figure B1: The PARIS protein shows highly conserved protein domains. EMBOSS Needle pairwise sequence alignment of *D. melanogaster* and *H. sapiens* PARIS. "|" indicates identical amino acids in both sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions. The names of each species in the alignment appear in brackets on the right-hand side of the alignment. Numbers on the left and right-hand side of the alignment indicate the first and last amino acid of the sequence in that row.

Table B1. Completed list of genotypes used in the analysis of altered expression of *Paris*.

Genotype	Abbreviation	Reference
Control Lines		
w; UAS-lacZ^{4-1-2}	*UAS-lacZ*	Brand *et. al*., 1994
y[1] v[1] ;P{y[+t7.7] v[+t.8]=TRIP.JF01355}attP2	*UAS-LUC-RNAi*	Perkins *et al*., 2015
Driver Lines		
P{w[+m]=GAL4}C380, w[*]*	*C380-Gal4*	Sanyal, 2009
w[*]; P{w[+mW.hs]=GawB}D42	*D42-Gal4*	Parkes *et al.,* 1998
P{w[+mW.hs]=GawB}OK6	*OK6-Gal4*	RRID:BDSC_64199
w[1118]; P{w[+m]= GAL4}repo/TM3, Sb[1]*	*Repo-Gal4*	RRID:BDSC_7415
w[*]; P{w[+mC]=ple-GAL4.F}3	*TH-Gal4*	Inamdar *et al.,* 2014
Responder Lines		
M{UAS-CG15436. ORF.3xHA.GW}ZH-86Fb	*UAS-Paris-ORF*	Bischof *et al*., 2014
w[1118]; P{GD9020}v39986	*UAS-Paris-RNAi*	Dietzl *et al*., 2007

Table B2. Log-rank statistical analysis of fly longevity with altered expression of *Paris* through the *C380-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
C380-Gal4/ UAS-lacZ	135	44	N/A	N/A	N/A
C380-Gal4/ UAS-LUC-RNAi	373	48	N/A	N/A	N/A
C380-Gal4/ UAS-Paris-RNAi	298	20	404.2	<0.0001	Yes

Table B3. Log-rank statistical analysis of fly longevity with altered expression of *Paris* through the *D42-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
D42-Gal4/ UAS-lacZ	169	54	N/A	N/A	N/A
D42-Gal4/ UAS-Paris-ORF	212	60	42.83	<0.0001	Yes
D42-Gal4/ UAS-LUC-RNAi	189	60	N/A	N/A	N/A
D42-Gal4/ UAS-Paris-RNAi	305	52	61.17	<0.0001	Yes

Table B4. Log-rank statistical analysis of fly longevity with altered expression of *Paris* through the *OK6-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
OK6-Gal4/ UAS-lacZ	218	58	N/A	N/A	N/A
OK6-Gal4/ UAS-Paris-ORF	277	62	36.58	<0.0001	Yes
OK6-Gal4/ UAS-LUC-RNAi	353	56	N/A	N/A	N/A
OK6-Gal4/ UAS-Paris-RNAi	304	58	1.071	0.3007	No

Table B5. Log-rank statistical analysis of fly longevity with altered expression of *Paris* through the *Repo-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
Repo-Gal4/ UAS-lacZ	134	46	N/A	N/A	N/A
Repo-Gal4/ UAS-Paris-ORF	300	54	2.058	0.1514	No
Repo-Gal4/ UAS-LUC-RNAi	299	46	N/A	N/A	N/A
Repo-Gal4/ UAS-Paris-RNAi	321	44	2.212	0.1370	No

Table B6. Log-rank statistical analysis of fly longevity with altered expression of *Paris* through the *TH-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
TH-Gal4/ UAS-lacZ	139	52	N/A	N/A	N/A
TH-Gal4/ UAS-Paris-ORF	80	58	0.9437	0.3313	No
TH-Gal4/ UAS-LUC-RNAi	122	62	N/A	N/A	N/A
TH-Gal4/ UAS-Paris-RNAi	144	56	16.11	<0.0001	Yes

Table B7. Tukey statistical analysis of locomotor ability with altered expression of *Paris* through the *C380-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
C380-Gal4;UAS-lacZ/ C380-Gal4;UAS-Paris-ORF	-0.063066	0.599578	-0.105	1.0	No
C380-Gal4;UAS-LUC-RNAi/ C380-Gal4;UAS-Paris-RNAi	-0.318691	0.559824	-0.569	0.941	No

Table B8. Tukey statistical analysis of locomotor ability with altered expression of *Paris* through the *D42-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
D42-Gal4;UAS-lacZ/ D42-Gal4;UAS-Paris-ORF	1.0859	0.4396	2.470	0.0623	Yes (.)
D42-Gal4;UAS-LUC-RNAi/ D42-Gal4;UAS-Paris-RNAi	0.8396	0.2718	3.089	0.0105	Yes (*)

Table B9. Tukey statistical analysis of locomotor ability with altered expression of *Paris* through the *OK6-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
OK6-Gal4;UAS-lacZ/ OK6-Gal4;UAS-Paris-ORF	0.1946	0.2364	0.823	0.843	No
OK6-Gal4;UAS-LUC-RNAi/ OK6-Gal4;UAS-Paris-RNAi	0.7844	0.2607	3.008	0.014	Yes (*)

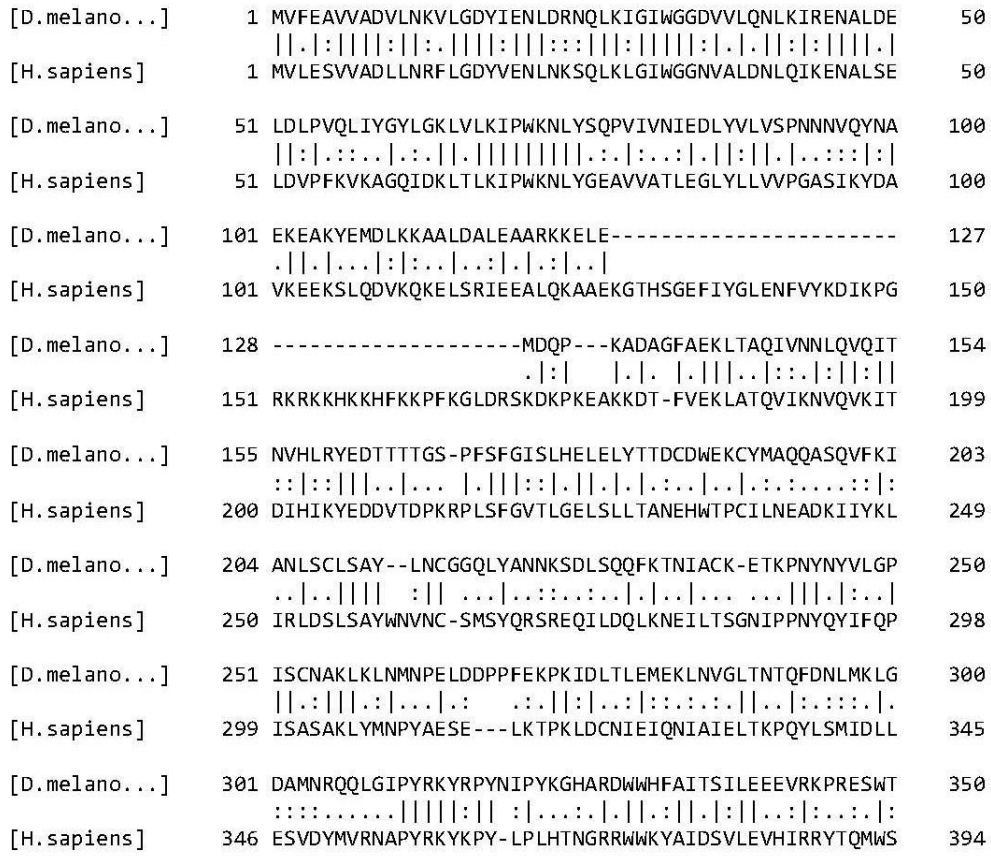
Table B10. Tukey statistical analysis of locomotor ability with altered expression of *Paris* through the *Repo-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
Repo-Gal4;UAS-lacZ/ Repo-Gal4;UAS-Paris-ORF	0.90891	0.38362	2.369	0.0819	Yes (.)
Repo-Gal4;UAS-LUC-RNAi/ Repo-Gal4;UAS-Paris-RNAi	-0.57276	0.40385	-1.418	0.4844	No

Table B11. Tukey statistical analysis of locomotor ability with altered expression of *Paris* through the *TH-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
TH-Gal4;UAS-lacZ/ TH-Gal4;UAS-Paris-EY	1.9677	1.0575	1.861	0.237	No
TH-Gal4;UAS-LUC-RNAi/ TH-Gal4;UAS-Paris-RNAi	-0.4729	0.7750	-0.610	0.926	No

Appendix C – Supplemental Data for Chapter 5



```
[D.melano...]    351 WGHIKTHRERCNTYAQKYKEQCLSKKPSAVLTETCRLLETELDVFNLLLI    400
                     |.:||.||:...:|...||.:....|.|..:.:..:.||..|||||::|.
[H.sapiens]      395 WSNIKKHRQLLKSYKIAYKNKLTQSKVSEEIQKEIQDLEKTLDVFNIILA    444

[D.melano...]    401 RQRVNIEI-------AKQREAVPEQKSGWFSG-WGWGGGAKKDDQTTSQK    442
                     ||:..:|:       .|:.....|::.||||| ||.....|||:::.
[H.sapiens]      445 RQQAQVEVIRSGQKLRKKSADTGEKRGGWFSGLWGKKESKKKDEESL---    491

[D.melano...]    443 LVEKFEAAMTSEEKEKMYRAIGYQENAKPTDLPESYEAIRMNFKLIALEV    492
                     :.|..:..||.|||:|::.||||.|:.....||:.|.|..|..||::..|
[H.sapiens]      492 IPETIDDLMTPEEKDKLFTAIGYSESTHNLTLPKQYVAHIMTLKLVSTSV    541

[D.melano...]    493 GLYKDERNSSAATKDFHELPSLVLLNFSMATALITQRPAAEAISIIAGMR    542
                     .: ::.:|          :|.::.:........::|||.|:|:.:.|.:.
[H.sapiens]      542 TI-RENKN----------IPEILKIQIIGLGTQVSQRPGAQALKVEAKLE    580

[D.melano...]    543 EIKVTGLTRNDYTPLLVESKITDEFNLLEVFFETNPLDKLCDQRVKVVAR    592
                     ...:|||.:.|..|.||.|......:||::.|||||.|...||.:.|.::
[H.sapiens]      581 HWYITGLRQQDIVPSLVASIGDTTSSLLKIKFETNPEDSPADQTLIVQSQ    630

[D.melano...]    593 PLQITYDAPTILALINAFQTPGDVTLSKFEDAASTKISNFKERSATGMQY    642
                     |:::.|||.|:.|::..||:...:.|.:...|...|:...|||:|||:.:
[H.sapiens]      631 PVEVIYDAKTVNAVVEFFQSNKGLDLEQITSATLMKLEEIKERTATGLTH    680

[D.melano...]    643 MIDKKAVLDVDILLMPNILVVPHKGVYDAGNVSLLVVSMGQVHLSSQPRR    692
                     :|:.:.|||:.|.|.|:.||||..|.:...: .||::..|...|:|
[H.sapiens]      681 IIETRKVLDLRINLKPSYLVVPQTGFHHEKS-DLLILDFGTFQLNS----    725

[D.melano...]    693 ESNKLQHLFSAGEDKDEILKTVMENAYDRFTVAVDDVQMLVVRAGEPWQN    742
                     :...||      :..:..|:.:|:.|||:|.|.:.:||:|..||.|.|:.
[H.sapiens]      726 KDQGLQ------KTTNSSLEEIMDKAYDKFDVEIKNVQLLFARAEETWKK    769

[D.melano...]    743 ALAEANSTEMHVLRPVSLKVTAALCVVDNDPRLPNIKVDIDLPAILVNVS    792
                     ...:..|| ||:|:|:.:.|..|..:|:.|.|:...||...||.:.|.:|
[H.sapiens]      770 CRFQHPST-MHILQPMDIHVELAKAMVEKDIRMARFKVSGGLPLMHVRIS    818

[D.melano...]    793 EDRIFLAIKVATSIPLPEQKEPASRLTQTNS-------------------    823
                     :.:::...:.:..|||||::....|...|.:|
[H.sapiens]      819 DQKMKDVLYLMNSIPLPQKSSAQSPERQVSSIPIISGGTKGLLGTSLLLD    868

[D.melano...]    824 -----------------------------RSSMSISN--FIN-------K    835
                                                 :.:..:.|  .||       |
[H.sapiens]      869 TVESESDDEYFDAEDGEPQTCKSMKGSELKKAAEVPNEELINLLLKFEIK    918

[D.melano...]    836 E-------------------VKKIGPSAS----------------------    845
                     |                   |.::|..|:
[H.sapiens]      919 EVILEFTKQQKEEDTILVFNVTQLGTEATMRTFDLTVVSYLKKISLDYHE    968

[D.melano...]    846 --GS--------SASKDPLLDEI-IQYTSLDVN-----------------    867
                       ||        |:|..|.||.: ::|...|.|
[H.sapiens]      969 IEGSKRKPLHLISSSDKPGLDLLKVEYIKADKNGPSFQTAFGKTEQTVKV   1018

[D.melano...]    868 -FS-----------LGEINFV-----------------------------    876
                      ||           :..||::
[H.sapiens]     1019 AFSSLNLLLQTQALVASINYLTTIIPSDDQSISVAKEVQISTEKQQKNST   1068

[D.melano...]    877 -----------------LF-------------------------------    878
                                     ||
[H.sapiens]     1069 LPKAIVSSRDSDIIDFRLFAKLNAFCVIVCNEKNNIAEIKIQGLDSSLSL   1118

[D.melano...]    879 --------------------------------------------------    878

[H.sapiens]     1119 QSRKQSLFARLENIIVTDVDPKTVHKKAVSIMGNEVFRFNLDLYPDATEG   1168

[D.melano...]    879 --------------------------------------------------    878

[H.sapiens]     1169 DLYTDMSKVDGVLSLNVGCIQIVYLHKFLMSLLNFLNNFQTAKESLSAAT   1218

[D.melano...]    879 -QSSRKCETS-------------------------------------------    887
                      |::.:..||
[H.sapiens]     1219 AQAAERAATSVKDLAQRSFRVSINIDLKAPVIVIPQSSISTNAVVVDLGL   1268
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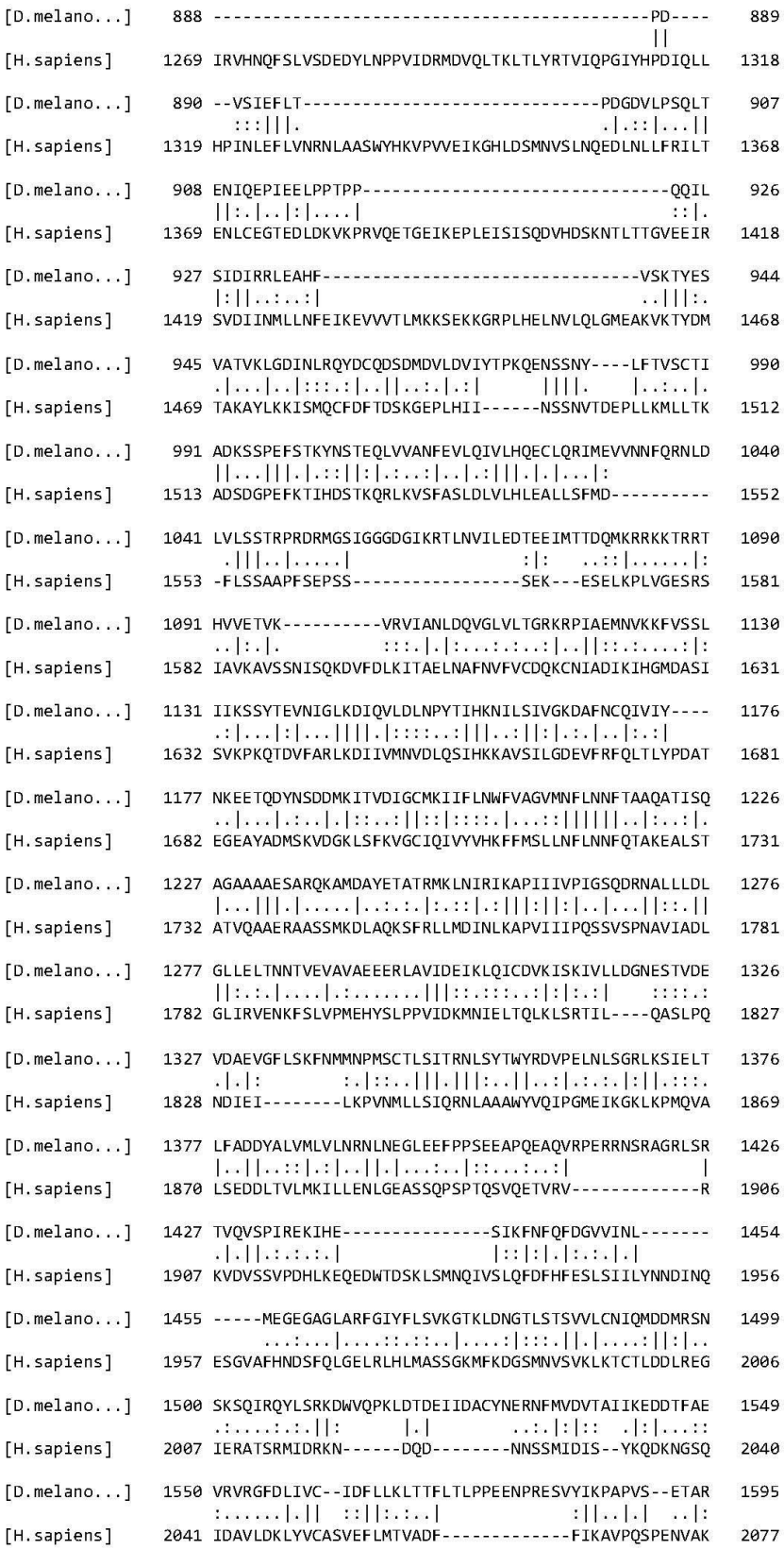
[D.melano...] 888 ----------PD---- 889
[H.sapiens] 1269 IRVHNQFSLVSDEDYLNPPVIDRMDVQLTKLTLYRTVIQPGIYHPDIQLL 1318
[D.melano...] 890 --VSIEFLT----------PDGDVLPSQLT 907
[H.sapiens] 1319 HPINLEFLVNRNLAASWYHKVPVVEIKGHLDSMNVSLNQEDLNLLFRILT 1368
[D.melano...] 908 ENIQEPIEELPPTPP----------QQIL 926
[H.sapiens] 1369 ENLCEGTEDLDKVKPRVQETGEIKEPLEISISQDVHDSKNTLTTGVEEIR 1418
[D.melano...] 927 SIDIRRLEAHF----------VSKTYES 944
[H.sapiens] 1419 SVDIINMLLNFEIKEVVVTLMKKSEKKGRPLHELNVLQLGMEAKVKTYDM 1468
[D.melano...] 945 VATVKLGDINLRQYDCQDSDMDVLDVIYTPKQENSSNY----LFTVSCTI 990
[H.sapiens] 1469 TAKAYLKKISMQCFDFTDSKGEPLHII------NSSNVTDEPLLKMLLTK 1512
[D.melano...] 991 ADKSSPEFSTKYNSTEQLVVANFEVLQIVLHQECLQRIMEVVNNFQRNLD 1040
[H.sapiens] 1513 ADSDGPEFKTIHDSTKQRLKVSFASLDLVLHLEALLSFMD---------- 1552
[D.melano...] 1041 LVLSSTRPRDRMGSIGGGDGIKRTLNVILEDTEEIMTTDQMKRRKKTRRT 1090
[H.sapiens] 1553 -FLSSAAPFSEPSS----------SEK---ESELKPLVGESRS 1581
[D.melano...] 1091 HVVETVK----------VRVIANLDQVGLVLTGRKRPIAEMNVKKFVSSL 1130
[H.sapiens] 1582 IAVKAVSSNISQKDVFDLKITAELNAFNVFVCDQKCNIADIKIHGMDASI 1631
[D.melano...] 1131 IIKSSYTEVNIGLKDIQVLDLNPYTIHKNILSIVGKDAFNCQIVIY---- 1176
[H.sapiens] 1632 SVKPKQTDVFARLKDIIVMNVDLQSIHKKAVSILGDEVFRFQLTLYPDAT 1681
[D.melano...] 1177 NKEETQDYNSDDMKITVDIGCMKIIFLNWFVAGVMNFLNNFTAAQATISQ 1226
[H.sapiens] 1682 EGEAYADMSKVDGKLSFKVGCIQIVYVHKFFMSLLNFLNNFQTAKEALST 1731
[D.melano...] 1227 AGAAAAAESARQKAMDAYETATRMKLNIRIKAPIIIVPIGSQDRNALLLDL 1276
[H.sapiens] 1732 ATVQAAERAASSMKDLAQKSFRLLMDINLKAPVIIIPQSSVSPNAVIADL 1781
[D.melano...] 1277 GLLELTNNTVEVAVAEEERLAVIDEIKLQICDVKISKIVLLDGNESTVDE 1326
[H.sapiens] 1782 GLIRVENKFSLVPMEHYSLPPVIDKMNIELTQLKLSRTIL----QASLPQ 1827
[D.melano...] 1327 VDAEVGFLSKFNMMNPMSCTLSITRNLSYTWYRDVPELNLSGRLKSIELT 1376
[H.sapiens] 1828 NDIEI--------LKPVNMLLSIQRNLAAAWYVQIPGMEIKGKLKPMQVA 1869
[D.melano...] 1377 LFADDYALVMLVLNRNLNEGLEEFPPSEEAPQEAQVRPERRNSRAGRLSR 1426
[H.sapiens] 1870 LSEDDLTVLMKILLENLGEASSQPSPTQSVQETVRV----------R 1906
[D.melano...] 1427 TVQVSPIREKIHE----------SIKFNFQFDGVVINL------- 1454
[H.sapiens] 1907 KVDVSSVPDHLKEQEDWTDSKLSMNQIVSLQFDFHFESLSIILYNNDINQ 1956
[D.melano...] 1455 -----MEGEGAGLARFGIYFLSVKGTKLDNGTLSTSVVLCNIQMDDMRSN 1499
[H.sapiens] 1957 ESGVAFHNDSFQLGELRLHLMASSGKMFKDGSMNVSVKLKTCTLDDLREG 2006
[D.melano...] 1500 SKSQIRQYLSRKDWVQPKLDTDEIIDACYNERNFMVDVTAIIKEDDTFAE 1549
[H.sapiens] 2007 IERATSRMIDRKN------DQD--------NNSSMIDIS--YKQDKNGSQ 2040
[D.melano...] 1550 VRVRGFDLIVC--IDFLLKLTTFLTLPPEENPRESVYIKPAPVS--ETAR 1595
[H.sapiens] 2041 IDAVLDKLYVCASVEFLMTVADF----------FIKAVPQSPENVAK 2077

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[D.melano...]   1596 DTKHSIRSSAILAAQELVPVESSSHEVPNRKMNLILHIDEPDIILVENLE   1645
                     :|:...|.:    |...|.:|......||  |.|...|.:|:::.|.:|.
[H.sapiens]     2078 ETQILPRQT----ATGKVKIEKDDSVRPN--MTLKAMITDPEVVFVASLT   2121

[D.melano...]   1646 DLNTSCIIFNAQVHLNYRSINDKQIVNGQIDALKMYMCAFLPERREMTRH   1695
                     ..:...:..:.|.:|:..:...:|::...:..||:..|.||.|:|.....
[H.sapiens]     2122 KADAPALTASFQCNLSLSTSKLEQMMEASVRDLKVLACPFLREKRGKNIT   2171

[D.melano...]   1696 YILHPCVISLQGSTPEEEGMHISLKLSDIIINVSPATIELLNKAMLSVSS   1745
                     .:|.||.:.::..|......:|::.:.:.||.:||..:..:...|.::|.
[H.sapiens]     2172 TVLQPCSLFMEKCTWASGKQNINIMVKEFIIKISPIILNTVLTIMAALSP   2221

[D.melano...]   1746 GTMTKCAIAEESRNYSNLWHQHHFHSRTYWFTKVEQGVDALEAEQRSVST   1795
                     .| .:....:.|:...|||.....:....||.    |||.......|...
[H.sapiens]     2222 KT-KEDGSKDTSKEMENLWGIKSINDYNTWFL----GVDTATEITESFKG   2266

[D.melano...]   1796 DNEKQKTEKCVIEIPSITLVIESGVGYYTKPLISLDTRITAVFNNWSRSL   1845
                     .......|.|.:.:.||.:.:|.|:|:.|.||:..:::.:....||:..:
[H.sapiens]     2267 IEHSLIEENCGVVVESIQVTLECGLGHRTVPLLLAESKFSGNIKNWTSLM   2316

[D.melano...]   1846 TAHGSLTLNMNYYNQALAEWEPIIELNEVIGRNGVREYTPWELKFEMGME   1895
                     .|...:||.::|||:..|.|||:||..|     |.|:   |.|:.:.:...
[H.sapiens]     2317 AAVADVTLQVHYYNEIHAVWEPLIERVE-----GKRQ---WNLRLDVKKN   2358

[D.melano...]   1896 KVQSEL----EDDAEQQAMHMNIHSAETLEITLSKTCLGLLSELAEAFSQ   1941
                     .||.:.    :|...:..|.::|.|..|:.||:||:||.:.:.||:.||:
[H.sapiens]     2359 PVQDKSLLPGDDFIPEPQMAIHISSGNTMNITISKSCLNVFNNLAKGFSE   2408

[D.melano...]   1942 AIDQNGLTKPDIVAPYVLENDTGFDV----NLNLRKGIFTLHEVHRGGTP   1987
                     ............||:.::|..|..:    |.|||.          .|.|
[H.sapiens]     2409 GTASTFDYSLKDRAPFTVKNAVGVPIKVKPNCNLRV----------MGFP   2448

[D.melano...]   1988 VGANSTLLMVAQSEEVDPSVIKTCTISTGGRAYLQTKDLSTLSEEDSEDY   2037
                       ..|.:..|...:.::.........|.|        :||.||.::|..:
[H.sapiens]     2449 --EKSDIFDVDAGQNLELEYASMVPSSQG--------NLSILSRQESSFF   2488

[D.melano...]   2038 TLYVTIGDINKEIALPVSKSDTRFFNLMRSTSHEPWGIISEVKQEYGTTK   2087
                     ||.:.....:...:||::...|.:|:....:.....::.::....|...
[H.sapiens]     2489 TLTIVPHGYTEVANIPVARPGRRLYNVRNPNASHSDSVLVQIDATEGNKV   2538

[D.melano...]   2088 VNIHGVVSVHNHFTTGLNIYR--RNPAPTAQCFEDIFVGRVRPGEVFHVP   2135
                     :.:...:.:.|||:....||:  :|    .:..|.|  |..||.|.||||
[H.sapiens]     2539 ITLRSPLQIKNHFSIAFIIYKFVKN----VKLLERI--GIARPEEEFHVP   2582

[D.melano...]   2136 LHAIYAESKDLFFSMRG-----YRRSVQGISWASNPSDLNYSHQLHC---   2177
                     |.:...:   ||....|     |:.|...|||   ..:|:.|.::.|
[H.sapiens]     2583 LDSYRCQ---LFIQPAGILEHQYKESTTYISW---KEELHRSREVRCMLQ   2626

[D.melano...]   2178 --DPTNTFEPLIMNARRSKSEVYFENTNKYTLLSAFYTIHLRPPLYLRNS   2225
                       ....:|.|||:|......|:.:..|:... ....|.|||.|.|.|||.
[H.sapiens]     2627 CPSVEVSFLPLIVNTVALPDELSYICTHGED-WDVAYIIHLYPSLTLRNL   2675

[D.melano...]   2226 LPINIQVSVAGCSVRKE--DGLDAQSSQRFVDRGYRKEDFLDYGEKPVNS   2273
                     ||.:::..:.|.:...|  :|                          ::
[H.sapiens]     2676 LPYSLRYLLEGTAETHELAEG--------------------------ST   2698

[D.melano...]   2274 GDVLHLPTVRLASKGKESKSFLVVRLVQYLEKDWSCATEIWDYTDDVITW   2323
                     .||||         .:.|...:.:.||:|..|:|:....|.|...:....
[H.sapiens]     2699 ADVLH---------SRISGEIMELVLVKYQGKNWNGHFRIRDTLPEFFPV   2739

[D.melano...]   2324 TFSSYDSEM-KVDMDLYVKTENRHGS-LMLTLFSPFWMINKTGMMLTYKS   2371
                     .|||..:|: .||:.::|:   |.|| ::|::|||:|:|||||..:|.|:|
[H.sapiens]     2740 CFSSDSTEVTTVDLSVHVR---RIGSRMVLSVFSPYWLINKTTRVLQYRS   2786

[D.melano...]   2372 ETTSVEVLYHPPEYSGPILFTFRDKLFFDKKKASIRIDNGQWSEKIPLDV   2421
                     |...|:   ||.::...|||:|:.|..|.|.|..::|....||....||.
[H.sapiens]     2787 EDIHVK---HPADFRDIILFSFKKKNIFTKNKVQLKISTSAWSSSFSLDT   2833

[D.melano...]   2422 AGSVGEVICFANNQKYPVGVHNHLTQNSLTKQITFIPFYIVCNKCHFDIE   2471
                     .||.|.|.|.|||.:|.|||...::..:|::.:|..||..:.||...::|
[H.sapiens]     2834 VGSYGCVKCPANNMEYLVGVSIKMSSFNLSRIVTLTPFCTIANKSSLELE   2883
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[D.melano...]   2472 LQE----QSRPADPWLHLEPNEMEPLWPRNDTKNNLVVRV---DGKITPA   2514
                     :.|    .|.|.:.|.::..:|..|.||.| ....|.|||   :|...|.
[H.sapiens]     2884 VGEIASDGSMPTNKWNYIASSECLPFWPEN-LSGKLCVRVVGCEGSSKPF   2932

[D.melano...]   2515 FDFTEVICTLLKLEDSKYGGINVDVQTTEGGVYITFTDYKPADAPGLLIN   2564
                     |...:...|||.|||.. |||.|||.|.|....|||:||....||.|::|
[H.sapiens]     2933 FYNRQDNGTLLSLEDLN-GGILVDVNTAEHSTVITFSDYHEGSAPALIMN   2981

[D.melano...]   2565 HTGKQIV-YHEKGTKNEHILNAKSTIMYAWDDPTGPKML--VFGTNKEET   2611
                     ||...|: |.:.|:..|.:|..:...::||.||||.:.|  .:..|..|.
[H.sapiens]     2982 HTPWDILTYKQSGSPEEMVLLPRQARLFAWADPTGTRKLTWTYAANVGEH   3031

[D.melano...]   2612 DLKRDGIGEVIMQDGGKVLWVSFLDGLQRVLLFTENESIANRTESTASLQ   2661
                     ||.:||.|:.......::.|||||||.|||||||::.::.::......::
[H.sapiens]     3032 DLLKDGCGQFPYDANIQIHWVSFLDGRQRVLLFTDDVALVSKALQAEEME   3081

[D.melano...]   2662 SITQSIDLRIHGIGLSVINNETGLDILYLGVTSSGIIWESKKVTKNRFKE   2711
                     .....|.|.:|.:|||::|||:..::.|:|:||||::||.|  .|.::|.
[H.sapiens]     3082 QADYEITLSLHSLGLSLVNNESKQEVSYIGITSSGVVWEVK--PKQKWKP   3129

[D.melano...]   2712 LTINENALLEIEYQKYLVHKSVNDVQTYKLDNKFPIDFDL--MILKKTVE   2759
                     .:..:..|||..|||   |:...|....||||.|.::||.  |.::..:.
[H.sapiens]     3130 FSQKQIILLEQSYQK---HQISRDHGWIKLDNNFEVNFDKDPMEMRLPIR   3176

[D.melano...]   2760 RNLRRSFYPAIWLSRKSSPFQSQLHVKINRIQVDNQFLDPIFPVVLAPIP   2809
                     ..::|.|...|.:..|.|..|..|..::..:|||||....:||||..|:.
[H.sapiens]     3177 SPIKRDFLSGIQIEFKQSSHQRSLRARLYWLQVDNQLPGAMFPVVFHPVA   3226

[D.melano...]   2810 PPKSVASTTSLKPFIECSMVQRIMPNSTVRQFKYARILIQEFLFKVDLNF   2859
                     ||||:|..:..||||:.|::.|....|.|.||||..:||||...|:|..|
[H.sapiens]     3227 PPKSIALDSEPKPFIDVSVITRFNEYSKVLQFKYFMVLIQEMALKIDQGF   3276

[D.melano...]   2860 LTAIAEMFAKEVSDEA---AAKQFRQDVESIELPLSAFFEEHSLEEQK--   2904
                     |.||..:|......||   ..|..:||:::    |:|...|.|:.:..
[H.sapiens]     3277 LGAIIALFTPTTDPEAERRRTKLIQQDIDA----LNAELMETSMTDMSIL   3322

[D.melano...]   2905 SFYDNLHLGPLKIHVSFSMAG----SDTKALPGF----LGSLVQGVGVTL   2946
                     ||:::.|:.|:|:|:|.|:..    ||.:....|    :..|::.:|.||
[H.sapiens]     3323 SFFEHFHISPVKLHLSLSLGSGGEESDKEKQEMFAVHSVNLLLKSIGATL   3372

[D.melano...]   2947 TDVNDVVFRLAFFEREYQFFSQKQLINEITSHYTGQALKQLYVLVLGLDV   2996
                     |||:|::|:||::|..|||:.:.|||..:..||:.|.|||:|||||||||
[H.sapiens]     3373 TDVDDLIFKLAYYEIRYQFYKRDQLIWSVVRHYSEQFLKQMYVLVLGLDV   3422

[D.melano...]   2997 LGNPYGLVVGLKKGVEDLFYEPFQGAIQGPGEFAEGLVLGVKSLFGHTVG   3046
                     ||||:||:.||.:|||.|||||||||:|||.|||||||:||:||||||||
[H.sapiens]     3423 LGNPFGLIRGLSEGVEALFYEPFQGAVQGPEEFAEGLVIGVRSLFGHTVG   3472

[D.melano...]   3047 GAAGAVSKITGAMGKGLAALTFDEDYQKKRRQGIQNKPKNFHEGLARSSK   3096
                     ||||.||:|||::||||||:|.|::||:|||:.:..:|::|.:.|||..|
[H.sapiens]     3473 GAAGVVSRITGSVGKGLAAITMDKEYQQKRREELSRQPRDFGDSLARGGK   3522

[D.melano...]   3097 GLVMGFVDGVTGVVTKPVTGARDNGVEGFFKGLGKGAIGLVARPTAGVVD   3146
                     |.:.|.|.||||::||||.||:..|..|||||:|||.:|.|||||.|:||
[H.sapiens]     3523 GFLRGVVGGVTGIITKPVEGAKKEGAAGFFKGIGKGLVGAVARPTGGIVD   3572

[D.melano...]   3147 FASGSFEAVKRAADASEDVKRMRPPRFQHYDFVLRPYCLMEATGNKIMKE   3196
                     .||.:|:.::|||:::|:|..:||||..|.|.::|||...|:.|:.:::.
[H.sapiens]     3573 MASSTFQGIQRAAESTEEVSSLRPPRLIHEDGIIRPYDRQESEGSDLLEN   3622

[D.melano...]   3197 TDKGKFATTDNFIHCEEIIQKSEYLVVTNYRVMYVQRNEMFGVWTSLWSY   3246
                     ..|.....|..: ||.....|...|:|||.||:.::..|:.|:....|..
[H.sapiens]     3623 HIKKLEGETYRY-HCAIPGSKKTILMVTNRRVLCIKEVEILGLMCVDWQC   3671

[D.melano...]   3247 LWNEISSVAATARGV-QFTVKTDGKKVLGLFSSKESP-----RKLVL---   3287
                     .:.:.....:.:..| :.:||..     |||..|:|.     ||:.|
[H.sapiens]     3672 PFEDFVFPPSVSENVLKISVKEQ-----GLFHKKDSANQGCVRKVYLKDT   3716

[D.melano...]   3288 VADERKRDALVDIIESQRSDPNPLRATIA------YPAHN   3321
                     ...||..:|:.| .:|.|.....::.:..      .|:
[H.sapiens]     3717 ATAERACNAIED-AQSTRQQQKLMKQSSVRLLRPQLPS--   3753
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Figure C1: The Vps13 protein shows highly conserved protein domains. EMBOSS Needle pairwise sequence alignment of *D. melanogaster* Vps13 and *H. sapiens* Vps13C protein. "|" indicates identical amino acids in both sequences of the alignment, ":" shows conserved substitutions, and "." indicates semi-conserved substitutions. The names of each species in the alignment appear in brackets on the right-hand side of the alignment. Numbers on the right and left-hand side of the alignment indicate the first and last amino acid of the sequence in that row.

Table C1. Completed list of genotypes used in the analysis of altered expression of *Vps13*.

Genotype	**Abbreviation**	**Reference**
Control Lines		
w; UAS-lacZ^{4-1-2}	*UAS-lacZ*	Brand *et al*., 1994
y[1] v[1] ;P{y[+t7.7] v[+t.8]=TRIP.JF01355}attP2	*UAS-LUC-RNAi*	Perkins *et al*., 2015
Driver Lines		
P{w[+m]=GAL4}C380, w[*]*	*C380-Gal4*	Sanyal, 2009
w[*]; P{w[+mW.hs]=GawB}D42	*D42-Gal4*	Parkes *et al.,* 1998
P{w[+mW.hs]=GawB}OK6	*OK6-Gal4*	RRID:BDSC_64199
w[1118]; P{w[+m]= GAL4}repo/TM3, Sb[1]*	*Repo-Gal4*	RRID:BDSC_7415
w[*]; P{w[+mC]=ple-GAL4.F}3	*TH-Gal4*	Inamdar *et al.,* 2014
Responder Lines		
y[1] w[67c23]; P{y[+mDint2] w[+mC]=EPgy2}EY09640	*UAS-Vps13-EY*	Bellen *et al*, 2004
y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS02460}attP40	*UAS-Vps13-RNAi^{42625}*	Perkins *et al,* 2015
w[1118]; P{GD14789}v29972	*UAS-Vps13-RNAi^{29972}*	Dietzl *et al*., 2007

Table C2. Log-rank statistical analysis of fly longevity with altered expression of *Vps13* through the *C380-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
C380-Gal4/ UAS-lacZ	135	44	N/A	N/A	N/A
C380-Gal4/ UAS-Vps13-EY	262	50	66.66	<0.0001	Yes
C380-Gal4/ UAS-LUC-RNAi	373	48	N/A	N/A	N/A
C380-Gal4/ UAS-Vps13-RNAi^{42625}	367	42	65.69	<0.0001	Yes
C380-Gal4/ UAS-Vps13-RNAi^{29972}	517	18	321.4	<0.0001	Yes

Table C3. Log-rank statistical analysis of fly longevity with altered expression of *Vps13* through the *D42-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
D42-Gal4/ UAS-lacZ	169	54	N/A	N/A	N/A
D42-Gal4/ UAS-Vps13-EY	266	54	3.685	0.0549	No
D42-Gal4/ UAS-LUC-RNAi	189	60	N/A	N/A	N/A
D42-Gal4/ UAS-Vps13-RNAi^{42625}	305	56	46.67	<0.0001	Yes
D42-Gal4/ UAS-Vps13-RNAi^{29972}	197	64	10.97	0.0009	Yes

Table C4. Log-rank statistical analysis of fly longevity with altered expression of *Vps13* through the *OK6-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
OK6-Gal4/ UAS-lacZ	218	58	N/A	N/A	N/A
OK6-Gal4/ UAS-Vps13-EY	302	38	181.9	<0.0001	Yes
OK6-Gal4/ UAS-LUC-RNAi	353	56	N/A	N/A	N/A
OK6-Gal4/ UAS-Vps13-RNAi^{42625}	309	64	69.67	<0.0001	Yes
OK6-Gal4/ UAS-Vps13-RNAi^{29972}	286	66	92.70	<0.0001	Yes

Table C5. Log-rank statistical analysis of fly longevity with altered expression of *Vps13* through the *Repo-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
Repo-Gal4/ UAS-lacZ	134	46	N/A	N/A	N/A
Repo-Gal4/ UAS-Vps13-EY	337	36	8.771	0.0031	Yes
Repo-Gal4/ UAS-LUC-RNAi	299	46	N/A	N/A	N/A
Repo-Gal4/ UAS-Vps13-RNAi^{42625}	414	54	20.42	<0.0001	Yes
Repo-Gal4/ UAS-Vps13-RNAi^{29972}	480	58	27.56	<0.0001	Yes

Table C6. Log-rank statistical analysis of fly longevity with altered expression of *Vps13* through the *TH-Gal4* transgene.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
TH-Gal4/ UAS-lacZ	139	52	N/A	N/A	N/A
TH-Gal4/ UAS-Vps13-EY	87	42	9.016	0.0027	Yes
TH-Gal4/ UAS-LUC-RNAi	122	62	N/A	N/A	N/A
TH-Gal4/ UAS-Vps13-RNAi^{42625}	168	60	12.17	0.0005	Yes
TH-Gal4/ UAS-Vps13-RNAi^{29972}	181	64	8.430	0.0037	Yes

Table C7. Tukey statistical analysis of locomotor ability with altered expression of *Vps13* through the *C380-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
C380-Gal4;UAS-lacZ/ C380-Gal4;UAS-Vps13-EY	0.01324	0.56202	0.024	1.0	No
C380-Gal4;UAS-LUC-RNAi/ C380-Gal4;UAS-Vps13-RNAi^{42625}	0.4862	0.4665	1.042	0.82773	No
C380-Gal4;UAS-LUC-RNAi/ C380-Gal4;UAS-Vps13-RNAi^{29972}	-1.5369	0.7442	-2.065	0.22408	No

Table C8. Tukey statistical analysis of locomotor ability with altered expression of *Vps13* through the *D42-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
D42-Gal4;UAS-lacZ/ D42-Gal4;UAS-Vps13-EY	1.5073	0.6346	2.375	0.11501	No
D42-Gal4;UAS-LUC-RNAi/ D42-Gal4;UAS-Vps13-RNAi^{42625}	0.4862	0.4665	1.042	0.82773	No
D42-Gal4;UAS-LUC-RNAi/ D42-Gal4;UAS-Vps13-RNAi^{29972}	-1.5369	0.7442	-2.065	0.22408	No

Table C9. Tukey statistical analysis of locomotor ability with altered expression of *Vps13* through the *OK6-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
OK6-Gal4;UAS-lacZ/ OK6-Gal4;UAS-Vps13-ORF	-0.6252	0.5162	-1.211	0.730	No
OK6-Gal4;UAS-LUC-RNAi/ OK6-Gal4;UAS-Vps13-RNAi^{42625}	-0.9077	0.7269	-1.249	0.707	No
OK6-Gal4;UAS-LUC-RNAi/ OK6-Gal4;UAS-Vps13-RNAi^{29972}	-0.5080	0.7239	-0.702	0.952	No

Table C10. Tukey statistical analysis of locomotor ability with altered expression of *Vps13* through the *Repo-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
Repo-Gal4;UAS-lacZ/ Repo-Gal4;UAS-Vps13-EY	-0.1624	0.6930	-0.234	0.999	No
Repo-Gal4;UAS-LUC-RNAi/ Repo-Gal4;UAS-Vps13-RNAi^{42625}	-0.5609	0.3814	-1.471	0.565	No
Repo-Gal4;UAS-LUC-RNAi/ Repo-Gal4;UAS-Vps13-RNAi^{29972}	-0.2053	0.3125	-0.657	0.963	No

Table C11. Tukey statistical analysis of locomotor ability with altered expression of *Vps13* through the *TH-Gal4* transgene.

Genotypes	Estimated Coefficient	Standard Error	Z-value	P-value	Significant
TH-Gal4;UAS-lacZ/ TH-Gal4;UAS-Vps13-EY	-0.8932	2.5230	-0.354	0.996	No
TH-Gal4;UAS-LUC-RNAi/ TH-Gal4;UAS-Vps13-RNAi^{42625}	-1.9678	1.0596	-1.857	0.313	No
TH-Gal4;UAS-LUC-RNAi/ TH-Gal4;UAS-Vps13-RNAi^{29972}	-2.7228	1.1865	-2.295	0.130	No

Appendix D – Supplemental Data for Chapter 6

Table D1. Completed list of genotypes used in evaluating neural-Gal4 and UAS-responding transgenes.

Genotype	Abbreviation	Reference
Control Lines		
w; UAS-lacZ^{4-1-2}	*UAS-lacZ*	Brand *et al*., 1994
y[1] v[1] ;P{y[+t7.7] v[+t.8]=TRIP.JF01355}attP2	*UAS-LUC-RNAi*	Perkins *et al*., 2015
Driver Lines		
P{w[+m]=GAL4}C380, w[*]*	*C380-Gal4*	Sanyal, 2009
w[*]; P{w[+mW.hs]=GawB}D42	*D42-Gal4*	Parkes *et al.,* 1998
P{w[+mW.hs]=GawB}OK6	*OK6-Gal4*	RRID:BDSC_64199
w[1118]; P{w[+m]= GAL4}repo/TM3, Sb[1]*	*Repo-Gal4*	RRID:BDSC_7415
w[*]; P{w[+mC]=ple-GAL4.F}3	*TH-Gal4*	Inamdar *et al.,* 2014

Table D2. Log-rank statistical analysis of longevity of flies with both *UAS-lacZ* and *UAS-LUC-RNAi* driven by several tissue specific *Gal4* lines.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance
Repo-Gal4/ UAS-lacZ	134	46	N/A	N/A	N/A
Repo-Gal4/UAS-LUC-RNAi	296	47	0.03041	0.8616	No
D42-Gal4/UAS-lacZ	169	54	N/A	N/A	N/A
D42-Gal4/UAS-LUC-RNAi	189	60	72.20	<0.0001	Yes
TH-Gal4/UAS-lacZ	139	52	N/A	N/A	N/A
TH-Gal4/UAS-LUC-RNAi	122	62	48.64	<0.0001	Yes
OK6-Gal4/UAS-lacZ	218	58	N/A	N/A	N/A
OK6-Gal4/UAS-LUC-RNAi	352	56	1.769	0.1835	No
C380-Gal4/UAS-lacZ	138	44	N/A	N/A	N/A
C380-Gal4/UAS-LUC-RNAi	135	44	0.05181	0.8199	No

Table D3. Log-rank statistical analysis of longevity of flies with *UAS-Gal4* expression driven by several tissue specific *Gal4* lines. Significance was determined with Bonferroni correction.

Genotype	Number of Flies	Median Survival (Days)	Chi-square Value	P-value	Significance (Bonferroni)
Repo-Gal4/UAS-lacZ	134	46	N/A	N/A	N/A
D42-Gal4/UAS-lacZ	169	54	2.188	0.1390	No
TH-Gal4/UAS-lacZ	139	52	0.004968	0.9438	No
OK6-Gal4/UAS-lacZ	218	58	23.26	0.0001	Yes
C380-Gal4/UAS-lacZ	138	44	28.03	0.0001	Yes
D42-Gal4/UAS-lacZ	169	54	N/A	N/A	N/A
TH-Gal4/UAS-lacZ	139	52	2.243	0.1342	No
OK6-Gal4/UAS-lacZ	218	58	53.32	0.0001	Yes
C380-Gal4/UAS-lacZ	138	44	120.6	0.0001	Yes
TH-Gal4/UAS-lacZ	139	52	N/A	N/A	N/A
OK6-Gal4/UAS-lacZ	218	58	22.62	0.0001	Yes
C380-Gal4/UAS-lacZ	138	44	53.67	0.0001	Yes
OK6-Gal4/UAS-lacZ	218	58	N/A	N/A	N/A
C380-Gal4/UAS-lacZ	138	44	269.1	0.0001	Yes