Characterization of *Paris* in *Drosophila melanogaster*

by

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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

2018

St. John's Newfoundland and Labrador

Abstract

Parkinson disease (PD) is a neurodegenerative movement disorder that affects 1 to 2% of the human population over the age of 65. This prevalent disease has characteristics including resting tremor, rigidity, bradykinesia and postural instability due to the loss of dopaminergic neurons in the *substantia nigra pars compacta* in the brain. Mitochondrial dynamics play a significant role in PD with a balance between surveillance and biogenesis being key. The newly discovered gene *Paris* has been proposed to be central in the coordination of mitochondrial processes. These processes seem to be controlled by a number of PD associated genes. We have identified *CG15436* as an excellent candidate to carry out studies on the *Paris* function in *Drosophila melanogaster*. Knockdown of *CG15436* reduces longevity and locomotor ability overtime to produce a *Paris* dependent *Drosophila* model of PD. As well, *CG15436* RNA interference negatively influences neurodevelopment when expressed in the eye. Interestingly, overexpression of *CG15436* produces similar results to knockdown of *CG15436* in longevity and locomotor assays as well as eye phenotypic expression. Alterations to the expression of the *Paris* candidate, either through ectopic expression or knockdown seem to result in a suboptimal set of conditions that lead to potential models of PD. As such, *CG15436* seems to fulfill the conditions to indicate that it functions as *Paris* in the *Drosophila* model system.

Acknowledgements

Firstly, I would like to express sincere gratitude to my supervisor, Dr. Brian E. Staveley for his advice, patience and guidance throughout my degree. I would also like to thank my supervisory committee, Dr. Dawn Marshall and Dr. Helene Volkoff, for providing positive criticism and feedback throughout my project. I would like to thank Dylan Goudie for all his assistance with SEM imaging. To past and present Staveley lab members, I wish to express sincere gratitude for all their guidance and providing such a positive, encouraging environment to work. Special thanks must be extended to Frankie Slade for passing along your extensive knowledge and skillset which was crucial to my success as a new Masters student in the Staveley lab. As well, special thanks to Kayla Petten, who was not only my labmate, but also my friend, who I could always count on to make me laugh until I cried, especially on days when I was overwhelmed with research and writing. I would like to express my deepest gratitude to my family and friends. You have supported me and believed in me unconditionally throughout this entire journey and for that I am forever grateful.

Research for this thesis has been supported by graduate student fellowships from the Memorial University School of Graduate Studies. Dr. Brian Staveley's laboratory is supported by grants from the Natural Sciences and Engineering Research Council.

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List of Abbreviations

°C – Degree Celsius

BLAST – Basic Local Alignment Search Tool

tBLASTn – Translated Nucleotide Basic Local Alignment Search Tool

cm – Centimeter

D. melanogaster – Drosophila melanogaster

g/L – grams per liter

g/ml – grams per milliliter

GTP – guanosine triphosphate

PD – Parkinson Disease

ml/L – milliliters per liter

ml - milliliters

N/A – Not Applicable

NCBI – National Center for Biotechnology Information

Paris - parkin interacting substrate

RISC – RNA-induced silencing complex

RNA – Ribonucleic acid

RNAi – RNA interference

SE – Standard error

SEM – Standard Error of the Mean

UAS – Upstream Activation Sequence

Introduction

Purpose

Parkinson disease (PD) is a neurodegenerative disease that has a significant influence upon our society today. Although common, and despite the fact that the symptoms of this debilitating disease are generally not present until later in life, the consequences of this disease are great and are observed all around the world. The aim of this study is to characterize the gene *Paris* and determine its influence upon the control of locomotor and survivorship abilities and therefore its relationship with PD through a *Drosophila melanogaster* model. The relationship between mitochondrial function and the effect that Parkinson disease has on these important organelles will be of importance in this study.

Parkinson Disease

Parkinson disease (PD) is a neurodegenerative movement disorder that affects 1 to 2% of the human population over the age of 65. This makes this disease one of the most prevalent in our world today (Weintraub et al., 2008). PD is the second most common neurodegenerative disorder, with Alzheimer disease being the most common (de Lau and Breteler, 2006). A pathological sign of PD is the loss of dopaminergic neurons in the *substantia nigra pars compacta* in the brain of the patient (Zhou et al., 2017). PD has characteristics including resting tremor, rigidity, bradykinesia and postural instability. As life expectancy increases, so too does the prevalence of PD with 4 to 5% of the population over the age of 85 affected (Trinh et al., 2014). Patients with PD have a progression of neuronal degeneration and Lewy bodies (numerous protein aggregations) present in the cerebral cortex and limbic structures. This may

eventually lead to dementia in 25 to 40% of PD patients (de Lau and Breteler, 2006). As explained, this progressive neurodegenerative disease leads to many detrimental characteristics.

Genetic mutations explain only a small portion of the cases of PD since approximately 90% of PD cases are sporadic (de Lau and Breteler, 2006). It is important to understand the mechanisms involved in PD and this came with the identification of the forms of PD that are inherited, which are not distinguishable from the sporadic forms (Schielsing et al., 2008). These rare familial forms account for approximately 15% of PD cases, and clinical, pathological and biochemical features are common between sporadic and familial forms of PD which allows for insight into the genes involved in PD (Ammal Kaidery and Thomas, 2018). There is no cure for this progressive neurodegenerative disease. There have been some treatments developed although all only providing temporary relief from the symptoms.

Mitochondria and Parkinson disease

Mitochondria are vital organelles in cells of eukaryotic organisms that are needed to maintain homeostasis (Bingol and Sheng, 2016; Franz et al., 2015). These double membrane-bound organelles are very important in the metabolism of the cell and are necessary for cellular survival (Baker et al., 2013). The number and dynamic morphology of mitochondria in the cell are balanced with the regulation of biogenesis and mitophagy (mitochondrial autophagy) during the ageing of the organism as well as its conditional stress response (Franz et al., 2015). There are many different cellular pathways that help to keep mitochondria functional and healthy (Bingol and Sheng, 2016). Cell signalling pathways are an important role of mitochondria (Franz et al., 2015). Since mitochondria provide a large portion of the energy that is needed for proper cellular function, its decline plays a key role in ageing (Weinrich et al., 2017). Mitochondria

have internal mechanisms, which are used to refold or degrade proteins that are rendered non-functional or damaged (Franz et al., 2015). Mitophagy is important in metabolism as it adjusts the mass of mitochondria and removes mitochondria during certain processes of differentiation. Autophagy as well as the ubiquitin-proteasome system support mitochondrial surveillance. The failure of the process of mitochondrial surveillance and autophagy is linked to PD. (Franz et al., 2015). When whole mitochondrial segments are damaged, selective removal of these mitochondria occurs due to mitophagy (Baker et al., 2013).

A functional decline in mitochondrial autophagy of dopaminergic neurons is a characteristic of PD and these neurons present in the *substantia nigra* are under a much higher mitochondrial stress. Due to this higher stress, they are much more vulnerable to defects that can occur due to genetic mutations related to Parkinson disease (Bingol and Sheng, 2016). Gene mutations in genes such as *PINK1* and *Parkin* are related to PD through involvement with mitochondrial maintenance and turnover (Wang et al., 2016). Parkin-mediated proteasomal turnover of mitofusin (outer membrane GTPases of the mitochondria) is vital to the fission-driven separation of parts of the mitochondria that get damaged, which can lead to selective ubiquitination of non-functional mitochondria (Franz et al., 2015). Neurological diseases can be the result of mitochondrial dysfunction at any level in the hierarchy of quality control. This mitochondrial dysfunction is related to a large quantity of diseases due to the importance of mitochondrial function in cell survival (Baker et al., 2013). It is important to understand the connection between mitochondria and PD for further research into the disease.

Drosophila melanogaster **as a model organism**

There are only two and a half times more genes present in the human genome than in that of the fly, with most of the additional genes being duplicates. This animal is such a good model organism mainly due to its ease of use. The animal is very easy to maintain in a laboratory setting and its breeding is non-problematic. The generation time for this animal is very short at just two weeks, which makes it very important and useful in genetic studies (Burdette and van den Heuvel, 2004). Much is known about the *Drosophila melanogaster* genome and there is a *D. melanogaster* homologue for 75% of human disease genes (Marsh et al., 2003). Due to the ease of genetic manipulation as well as all of these aspects mentioned, *D. melanogaster* is an ideal model organism for human diseases (Brand and Perrimon, 1993). This makes this organism an ideal organism for my study on PD.

Neurodegenerative disease can be modeled in *D. melanogaster*. The nervous system in *Drosophila* is similar to that of humans. The similarities stretch across the components of the nervous system such as the brain, neurons and the glia (O'Kane, 2011). The nervous system of an adult *D. melanogaster* has 10^5 neurons. The nervous system has a bilaterally symmetrical brain which is connected to a ventral nerve cord. This nerve cord then innervates the thorax and abdomen. The brain of *D. melanogaster* is made up of three lobes which are called the protocerebrum, deutocerebrum and the tritocerebrum. These three lobes have been shown to be homologous to the forebrain, midbrain and hindbrain regions of vertebrates. The human nervous system contains 4 lobes and approximately 100 billion neurons which makes it a much more complex nervous system than that of *D. melanogaster* (Herculano-Houzel, 2012). Although there is a difference in the complexities of the human and *D. melanogaster* nervous systems, the nervous systems have similarities in their shape, biochemical properties and their synaptic

functions (Lee et al., 2010). There are many of similarities between human and *D. melanogaster* neurons in both functional and molecular characteristics. These characteristics include axons, pumps, dentrites, voltage-gated channels, presynaptic vesicles and the manner of synaptic vesicle release (O'Kane, 2011). Due to these similarities between the human and *D. melanogaster* nervous system, *Drosophila* are an excellent model organism for the study of neurodegeneration.

Neurodevelopmental defects can be analysed in the *Drosophila* eye. The eye of *D. melanogaster* is made up of a repetitive pattern of ommatidia (Figure 1). Neurodevelopment can be measured using the eye structure due to its association with neurons. Neuron specific expression can be achieved in the eye cells using a driver, *GMR-GAL4* (Marsh et al., 2003). The differentiation of the specialized cells that will become photoreceptors starts in the eye imaginal disc with clusters of differentiating neurons. The eye is formed as the morphogenetic furrow moves from posterior to anterior. The fully formed adult eye of *D. melanogaster* has 750 to 800 ommatidia which are used for light sensing (Frankfort and Mardon, 2002). Each ommatidium is comprised of 20 cells, which include the photoreceptor neurons, pigment cells, cone cells and bristles (Sarkar et al., 2018). In each ommatidium, eight photoreceptors are present which are photosensitive neurons. This gives a total of over 6000 neurons in the eye of *D. melanogaster* (Frankfort and Mardon, 2002). The bristles, ommatidia and eye surface area can be analyzed, as changes in the structure of the eye can be an indicator of defects in neurodevelopment.

UAS-GAL4 System

There are many different types of genetic manipulation tools to use when conducting *D. melanogaster* genetic experiments. *D. melanogaster* can be used in forward genetic screens as an unbiased method, and used for the discovery of proteins and biochemical pathways. Forward

genetic screens are used to isolate mutants that affect genes of interest in research. Reverse genetic screens can be used and this tool required the knowledge of preexisting genetic information (Celotto and Palladino, 2005). Ectopic expression of a gene can be induced to overexpress or inhibit certain genes in transgenic organisms using the UAS-GAL4 system (Brand and Perrimon, 1993). This system uses a promoter to drive the expression of a transcriptional activator GAL4 which has been derived from yeast, *Saccharomyces cerevisae*, in order to activate a target gene. The GAL4 protein acts as a transcriptional activator for only genes that have the GAL4 binding sites. It binds to the upstream activation sequence (UAS) and drives the gene expression (Figure 2). It is possible to control the gene expression very precisely with this method since GAL4 gets inserted near the gene of interest and UAS is further upstream in opposing parental lines (Phelps and Brand, 1998). The UAS-GAL4 system is an ideal system to use for genetic manipulation of genes for human disease research.

RNA Interference (RNAi) and its Function

Over the last few years, an inducible RNA interference (RNAi) system has been developed. This system can be coupled with the UAS-GAL4 system in *Drosophila* (Dietzl et al., 2007). The "knock down" effects of transcriptional genes can be studied with the use of this system. RNAi is a regulatory method to silence genes post-transcriptionally. RNAi recruits a naturally occurring RNA-degrading mechanism which destroys the activity of a selected endogenous gene. This is done without altering the selected endogenous gene. A ribonuclease III enzyme called Dicer splits the double stranded DNA into pieces of 21 to 23 lengths. These pieces then unwind into single stranded short interfering DNA. The single stranded short interfering RNA is then incorporated into the RNA-induced silencing complex (RISC). This

RISC complex is a riboprotein complex. RISC has a nuclease component called either Argonaute or Slicer which degrades the mRNA depending upon the exact complementarity of the short interfering RNA. The RISC component is able to travel through the cytoplasm and will only break up the particular RNA with which it compliments. Due to the degradation of the mRNA generated from a gene, the expression of that gene is silenced. The function of that gene is therefore lost and the effects of loss-of-function can be studied. With the function of a gene being lost, its effect in certain biological pathways can be observed as well as its role in these pathways.

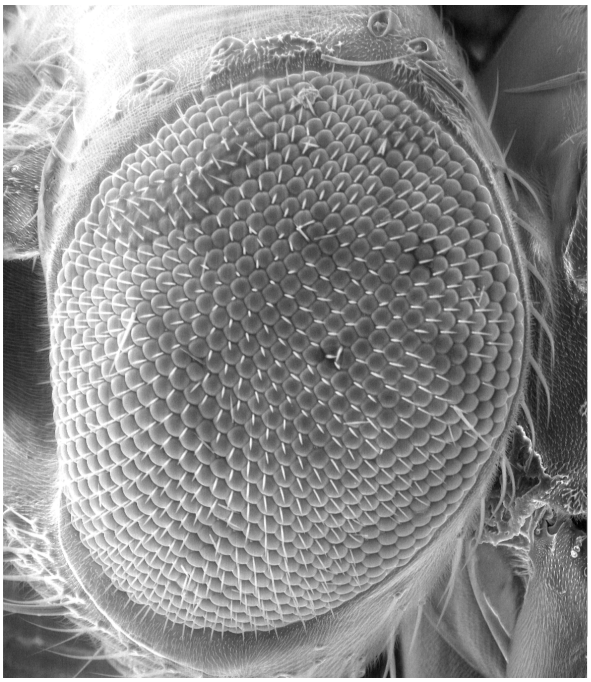


Figure 1. Scanning electron micrograph of *Drosophila melanogaster* eye of the genotype *GMR-GAL4; UAS-lacZ*. The presence of ommatidia and bristles are evident in this image taken with the FEI MLA 650F scanning electron microscope (500x magnification).

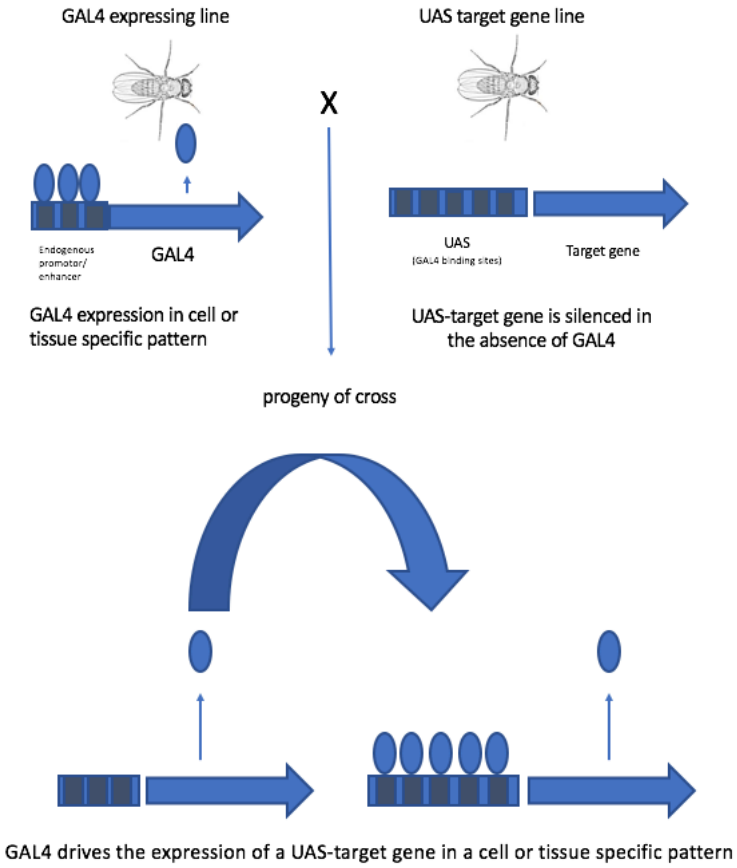


Figure 2. *UAS-GAL4* system in *Drosophila melanogaster.*

In this system, the maternal *UAS* line is crossed to the paternal *GAL4* line in order to produce progeny with target genes expressed through the binding of *UAS* to *GAL4*. Redrawn using Microsoft Powerpoint 365 adapted from Brand and Perrimon (1993).

Gene of interest

Paris is known as the "parkin interacting substrate" as well as the "zinc finger protein 746 (ZN746)". Paris has been shown in previous studies to be required for the loss of dopaminergic neurons in adult conditional parkin knockout mice (Stevens et al., 2015). *Paris* has been shown to be involved in a pathway including *parkin* and *PGC-1α* (known as *spargel* in studies involving *Drosophila melanogaster*). *Parkin* and *PGC-1α* have been shown to be associated with PD by their mutation or inactivation. Previous studies completed on *Paris* make this gene of great interest in PD research.

Parkin is an ubiquitin E3 ligase encoded by the *PARK2* gene (Siddiqui et al., 2016). Paris undergoes polyubiquitination by parkin which targets it though lysine-48 for ubiquitin proteasomal degradation (Stevens et al., 2015). This ability of parkin to regulate Paris is done through the ubiquitin proteasome system (Shin et al., 2011). *Parkin* is associated with autosomal recessive Parkinsonism as well as having a very important role in the quality control of mitochondria. This quality control is done through PINK1/parkin signalling (Zhou et al. 2017). A mutation in *parkin* has effects such as function loss which is linked to autosomal recessive PD (Stevens et al., 2015). *Parkin* has a role in increasing the PINK1 generated phosphoubiquitin signal. This role subsequently induces fast and stable mitophagy (Zhou et al., 2017). A loss of *parkin* leads to an increase in the size of mitochondria (Stevens et al., 2015). This gene is important to study due to its involvement with *Paris* and mitochondria.

PGC-1α is involved in mitochondrial function and it is known that mitochondrial defects are a characteristic of Parkinson disease (Stevens et al., 2015). *PGC-1α* is a co-activating transcription factor that controls the transcription of many genes involved in cellular metabolism, as well as mitochondrial biogenesis and respiration (Shin et al., 2011). As such, it is a major

regulator of mitochondrial size, number and function. *PGC-1α* is a transcriptional regulator of numerous bioenergetic and antioxidant pathways. Based on other PD literature, studies have focused on defects in oxidative phosphorylation and the importance of energy production in neurons by *PGC-1α* (Stevens et al., 2015). The interaction between *Paris, PGC-1α* and *parkin* needs further research to understand their role.

Paris is a transcriptional repressor and is involved in the expression regulation of *PGC-1α* (Stevens et al., 2015). It has been shown that a decrease in *parkin* is related to a subsequent increase in the Paris levels and then a reduction in the expression of *PGC-1α* (Siddiqui et al, 2016). The identification of the new *parkin* interacting substrate may provide insight into the molecular mechanisms that are involved in neurodegeneration because of the relation to the inactivation of *parkin* in PD (Shin et al., 2011). A major driver in the degeneration of dopaminergic neurons and the defects in mitochondrial biogenesis is the Paris-mediated downregulation of *PGC-1α* that is due to the absence of *parkin*. The repression of the *PGC-1α* promotor due to the accumulation of *Paris* is a likely hindrance to mitochondrial protein production. This can lead to a decrease in the total number of mitochondria found in cells. The loss of *parkin*'s ubiquitin E3 ligase activity is the major cause for the loss of dopaminergic neurons. The increased *Paris* levels in PD, which are a result of the inactivation of *parkin*, are likely contributing to the pathogenesis of the neurodegenerative disease. This contribution is through the down-regulation of *PGC-1α* and some other target genes (Stevens et al., 2015). It is necessary to investigate these genes and their interactions to further understand their functions and association with PD.

Goals and Objectives

This aim of this study is to answer important questions in the field of neurobiological research using a variety of techniques. The effects of gene alteration examined in the model organism, *D. melanogaster* should provide insight into the following questions: Can the alteration of gene expression, overexpression or knockdown, of *Paris* provide a model for PD? What is the characterization, in terms of function, of the gene *Paris* and its link to PD? If one, what is the relationship among genes *parkin, Paris* and *spargel* and how do they interact and affect each other in their known pathway?

Materials and Methods

Bioinformatics Analysis

Identification of the *Drosophila* homologue of Paris from human sequence

The *Drosophila melanogaster* homologue of *Paris* was identified using the National Centre for Biotechnology Information's (NCBI) tBLASTn search tool. The *D. melanogaster* genomic sequences were searched using the amino acid sequence of the human zinc finger protein 746 (accession number XP_005250012.1). Accession numbers were retrieved from NCBI to be used in the alignment. The closest *Drosophila* homologue was identified as *CG15436* (accession number NM_134996.4) with 32% identical residues in the tBLASTn search.

Identification of additional homologues, multiple alignment and domain identification

Homologues of *Drosophila melanogaster Paris* were identified using the NCBI's Basic Local Alignment Search Tool (BLAST) with the tBLASTn function. The *D. melanogaster* CG15436 sequence was queried against the BLAST database. Sequences were aligned using Clustal Omega to show similarity. Domains were identified using Pfam (Sanger Institute) and NCBI Conserved Domains Database. A phylogenetic tree was constructed using Clustal Omega. The accession numbers for the alignment including vertebrates and invertebrates include *Drosophila melanogaster CG15436* (accession number NM_134996.4), *Culex quinquefasciatus* zinc finger protein (accession number XM_001866910.1), *Homo sapiens* zinc finger protein 746 (accession number XP_005250012.1) and *Mus musculus* zinc finger protein 746 (accession number XM_006506557.3).

Two additional alignments were constructed. The vertebrate alignment included *Homo sapiens* zinc finger protein 746 (accession number XP_005250012.1), *Mus musculus* zinc finger protein 746 (accession number XM_006506557.3), *Danio rerio* zinc finger protein (XM_017355354.1) and *Gallus gallus* zinc finger protein 398-like (accession number XM_015281107.1). The invertebrate alignment included *Drosophila melanogaster CG15436* (accession number NM_134996.4), *Copidosoma floridanum* zinc finger protein 501-like (accession number XM_023390610.1), *Culex quinquefasciatus* zinc finger protein (accession number ZM_001846764.1) and *Bombus impatiens* zinc finger protein 271 (XM_012387283.2).

Drosophila **Culturing and Crosses**

Drosophila **media**

Drosophila melanogaster stocks were maintained on a standard media composed of 65 g/L cornmeal, 15 g/L yeast, 5.5 g/L agar and 50 ml/L fancy grade molasses in water with 5 ml of 0.1 g/ml methyl paraben in ethanol and 2.5 ml of propionic acid. Approximately 7 ml of medium was allowed to solidify per vial. The medium was prepared by Dr. Brian E. Staveley approximately twice a month and stored at 4 to 6°C until use.

Drosophila **stocks**

All *Drosophila* stocks were obtained from the Bloomington *Drosophila* Stock Centre (Indiana University, IN, USA) unless otherwise noted. The overexpression line of *CG15436* was obtained from FlyORF (University of Zurich, Switzerland). This line was not obtained until all crosses with knockdown lines of *CG15436* were completed. These experiments were therefore completed at a later date as a separate set of experiments. The recombinant lines *GMR*-

GAL4;UAS-park^{RNAi} and *ddc-GAL4-UAS;park*^{RNAi} were prepared by Dr. Brian E. Staveley. See Table 1 for a full list of all genotypes used.

Table 1: Genotypes of all stocks used to characterize *CG15436* in this study

Genotype	Abbreviation	Expression Pattern	Balancer	Reference
Control Lines				
w; UAS-lacZ^{4-1-2}	*UAS-lacZ*	---	---	(Brand et *al.,* 1994)
Driver Lines				
w;GMR-GAL4^{12}	*GMR-GAL4*	Eye	---	(Freeman, 1996)
w^{1118}; P{Ddc-GAL4.L}4.3D	*ddc-GAL4*	Neuron		(Li et al., 2000)
w*; P{ple-GAL4.F}3	*TH-GAL4*	Dopaminergic neuron		(Inamdar et al., 2014)
w[*]; P{w[+mW.hs]=GawB}D42	*D42-GAL4*	Motorneuron-specific		(Parkes et al., 1998)
UAS Lines				
y^{1} sc* v^{1}; P{TRiP.HMC04637}attP40	*UAS-CG15436^{RNAi}*	---	---	(Merzetti and Staveley, 2016)
y^{1}; P{SUPor-P}srl^{KG08646}ry^{506}/TM3, Sb^{1} Ser^{1}	*UAS-spargel^{RNAi}*	---	---	(Benedyk et al., 1994)
M{UAS-*CG15436.ORF.3xHA.GW}ZH-86Fb*	*UAS-CG15436^{ORF}*	---	---	(Staveley, unpublished)
Recombinant Lines				
w; ddc-GAL4/CyO; UAS-parkin^{RNAi}/TM3	*GMR-GAL4; UAS-park^{RNAi}*	Eye		Staveley, unpublished
w; GMR-GAL4^{12}/CyO; UAS-parkin^{RNAi}/TM3	*ddc-GAL4; UAS-park^{RNAi}*	Neuron		Staveley, unpublished

Drosophila crosses

The stocks were stored at room temperature (~ 21°C). For crosses, males and females of desired phenotypes that contain *UAS* or *GAL4* were mated. For the maternal genotype, virgin females were isolated every 8 to 12 hours. They were then mated with males of the appropriate genotype that had been isolated for 24 hours. For this mating, 3 to 5 females and 2 to 3 males of the chosen genotypes were placed on fresh media for breeding. In order to increase the productivity of the breeding, the flies were flipped onto new media 3 separate times every 2 to 3 days. The parental flies were then discarded and the male progeny of the critical class were collected once eclosure occured. In order to drive gene expression, neuronal transgenes were used. An example of these neuronal transgenes is *ddc-Gal4* (Li et al, 2000). The target genes were either knocked down by RNAi or overexpressed. The critical class males are those that do not express dominant mutant phenotypes associated with balancer chromosomes. Examples of these balancer chromosome phenotypes include *Curly* wings (*CyO*), *Stubble* bristles (*TM3*), or *Tubby* body and *Humeral* bristles (*TM6B*).

Biometric Analysis of the Compound Eye

Eye analysis of *D. melanogaster* was used to determine the effects of gene manipulation on ommatidia and bristle numbers. Critical class males were collected by setting up crosses as previously described. The critical class male progeny which result from the individual crosses were collected when eclosure occurs and were matured for 3 to 5 days in groups of no more than 20 on standard *Drosophila* medium at 25°C. They were then frozen and stored at a temperature of -80°C until use. These flies were thawed when ready to be mounted onto metal stubs onto their right side (left eye facing upwards) using forceps so that all eyes photographed were the left

eye. The sample size used for each cross made was ten flies. These stubs mounted with flies were desiccated for at least 24 hours before imaging. Scanning electron micrographs were taken of each male fly's left eye using the Mineral Liberation Analyzer FEI 650F or the FEI Quanta 400 Scanning Electron Microscope. The images were analyzed using the software program ImageJ (Abramoff et al., 2004). Total number of ommatidia and total number of bristles were determined. Data was analyzed using Graphpad Prism 7 (Graphpad Software Inc.) where mean ± standard error of the mean was calculated. Unpaired t-tests were used to determine significance. Results were deemed statistically significant when $p < 0.05$.

Behavioural Assays

Longevity assay

An analysis of survival of *Drosophila melanogaster* was completed to examine the lifespan of affected flies and the comparison to control flies. Male progeny of the critical class were collected from crosses as described. The males were collected daily and placed in vials containing fresh medium. Males were placed in groups of up to 20 individuals on fresh media to prevent overcrowding and stored at 25°C until a sample size of 300 individuals for each cross had been collected. Every 48 hours the flies were scored and media was changed whenever there was a death scored in a vial and twice per 7 day cycle. Flies were considered dead when no movement was observed when agitated (Staveley et al., 1990). Data was analyzed using the software Graphpad Prism 7 (Graphpad Software Inc.) using the log-rank test with significance considered at $p < 0.05$.

Locomotor assay

A locomotor analysis was used to examine the motor control of flies throughout their lifespan. For this analysis, seventy male progeny were collected from each cross as described. Critical class males were collected on the day of eclosion and maintained in vials of ten flies per vial and kept at 25°C. Ideal conditions were maintained as for the survival analysis. These flies were transferred to new medium once per week throughout the experimentation. One week after collection and every seven days after, the climbing ability of five cohorts of flies per genotype was assessed. Ten trials were conducted for each cohort of ten flies per genotype. This provides a total of 500 trials per genotype per week. The flies were scored every seven days based on their ability to climb inside a 30 cm glass tube with a 1.5 cm diameter that was marked with five 2 cm sections along a buffer zone (Todd and Staveley, 2004). The scoring was based upon the height reached for two cm intervals of the tube. This climbing tube is described in Todd and Staveley (2008). A climbing index was calculated as: Climbing index = Σ nm/N where n is the number of flies at a given level, m is the score of the level which is between 1 and 5 and N is the total number of flies climbed in that trial. Data was analyzed using the software GraphPad Prism 7 (GraphPad Software Inc.). A nonlinear regression curve was then generated with a 95% confidence interval to analyze the graphs of 5-climbing index as a function of time in days for each genotype. The slope for each graph represents the rate of decline in climbing ability and the Y-intercept represents the initial climbing ability and both of these parameters are calculated for each curve (Merzetti and Staveley, 2015). Slopes of the curves were compared using a 95% confidence interval. Curves were deemed significantly different if no overlap was found by the 95% confidence interval.

Results

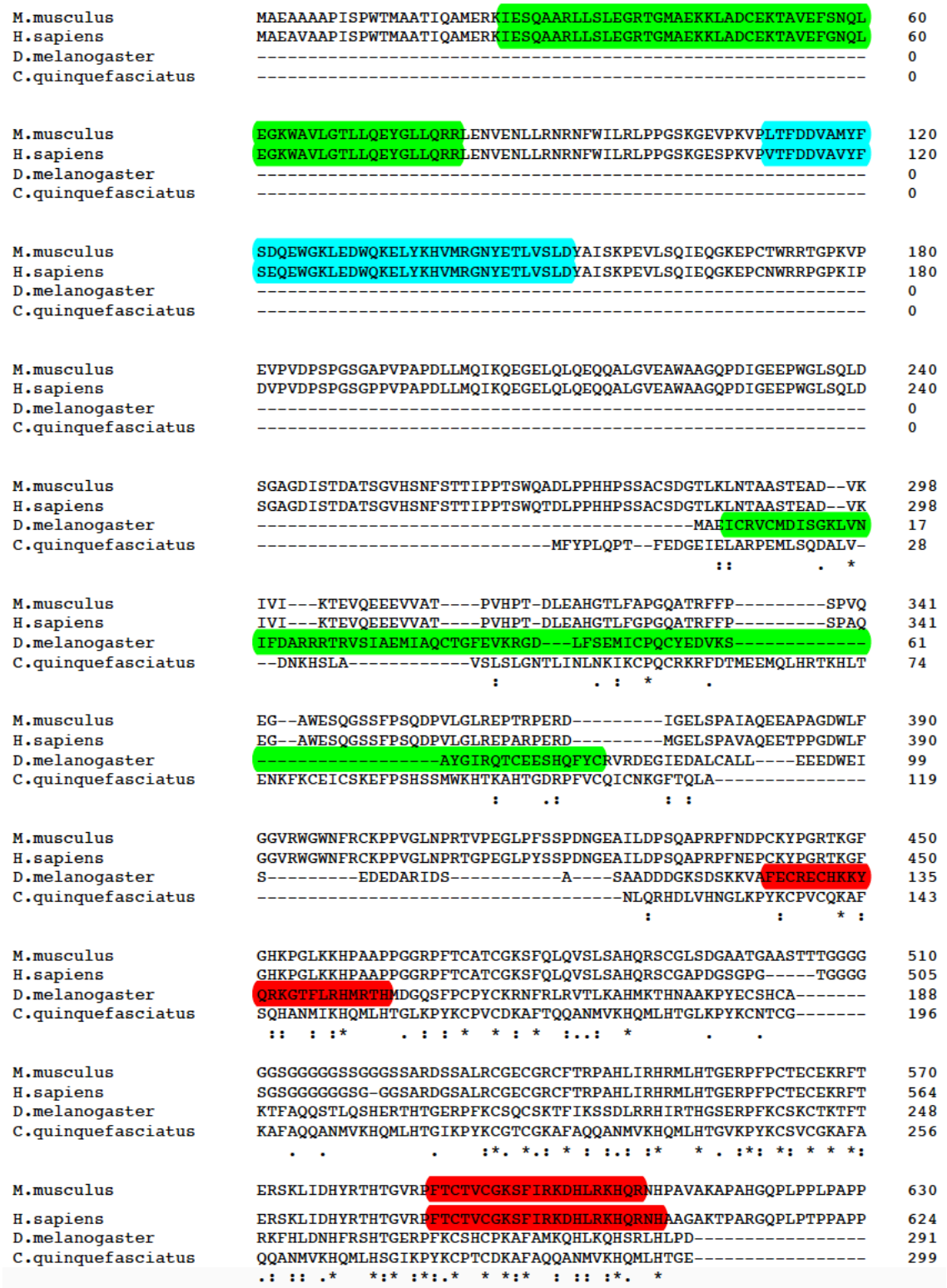
Bioinformatic Analysis of Paris

The Paris protein is conserved between vertebrates and invertebrates

The multiple alignment of vertebrate and invertebrate versions of the Paris protein was conducted using sequences from *D. melanogaster* (NM_134996.4), *C. quinquefasciatus* (XM_001866910.1), *M. musculus* (XM_006506557.3) and *H. sapiens* (XP_005250012.1) (Figure 3). When comparing these four species, the Paris proteins show some similarities in residues among the species. The alignment showed common zinc finger associated domain, zinc finger C2H2 type domain and KRAB box. There is sufficient similarity in the *D. melanogaster CG15436* to determine that it was the homologue for human zinc finger protein 746 (Paris).

The phylogenetic tree (Figure 4) shows *D. melanogaster* CG15436 as having the highest distance value across all species used in the multiple alignment. These numbers represent the amount of genetic change through evolution. *H. sapiens* and *M. musculus* have the lowest amount of genetic change with values of 0.0309 and 0.03274, respectively. *D. melanogaster* has the highest amount of genetic change with a value of 0.36262.

The vertebrate alignment (Figure 5B) included *Homo sapiens* zinc finger protein 746 (accession number XP_005250012.1), *Mus musculus* zinc finger protein 746 (accession number XM_006506557.3), *Danio rerio* zinc finger protein (XM_017355354.1) and *Gallus gallus* zinc finger protein 398-like (accession number XM_015281107.1). The invertebrate alignment (Figure 5A) included *Drosophila melanogaster CG15436* (accession number NM_134996.4), *Copidosoma floridanum* zinc finger protein 501-like (accession number XM_023390610.1), *Culex quinquefasciatus* zinc finger protein (accession number ZM_001846764.1) and *Bombus impatiens* zinc finger protein 271 (XM_012387283.2).


M.musculus MAEAAAAPISPWTMAATIQAMERKIESQAARLLSLEGRTGMAEKKLADCEKTAVEFSNQL 60
H.sapiens MAEAVAAPISPWTMAATIQAMERKIESQAARLLSLEGRTGMAEKKLADCEKTAVEFGNQL 60
D.melanogaster ---------- 0
C.quinquefasciatus ---------- 0
M.musculus EGKWAVLGTLLQEYGLLQRRLENVENLLRNRNFWILRLPPGSKGEVPKVPLTFDDVAMYF 120
H.sapiens EGKWAVLGTLLQEYGLLQRRLENVENLLRNRNFWILRLPPGSKGESPKVPVTFDDVAVYF 120
D.melanogaster ---------- 0
C.quinquefasciatus ---------- 0
M.musculus SDQEWGKLEDWQKELYKHVMRGNYETLVSLDYAISKPEVLSQIEQGKEPCTWRRTGPKVP 180
H.sapiens SEQEWGKLEDWQKELYKHVMRGNYETLVSLDYAISKPEVLSQIEQGKEPCNWRRPGPKIP 180
D.melanogaster ---------- 0
C.quinquefasciatus ---------- 0
M.musculus EVPVDPSPGSGAPVPAPDLLMQIKQEGELQLQEQQALGVEAWAAGQPDIGEEPWGLSQLD 240
H.sapiens DVPVDPSPGSGPPVPAPDLLMQIKQEGELQLQEQQALGVEAWAAGQPDIGEEPWGLSQLD 240
D.melanogaster ---------- 0
C.quinquefasciatus ---------- 0
M.musculus SGAGDISTDATSGVHSNFSTTIPPTSWQADLPPHHPSSACSDGTLKLNTAASTEAD--VK 298
H.sapiens SGAGDISTDATSGVHSNFSTTIPPTSWQTDLPPHHPSSACSDGTLKLNTAASTEAD--VK 298
D.melanogaster ----------MAHICRVCMDISGKLVN 17
C.quinquefasciatus ----------MFYPLQPT--FEDGEIELARPEMLSQDALV- 28
M.musculus IVI---KTEVQEEEVVAT----PVHPT-DLEAHGTLFAPGQATRFFP---------SPVQ 341
H.sapiens IVI---KTEVQEEEVVAT----PVHPT-DLEAHGTLFGPGQATRFFP---------SPAQ 341
D.melanogaster IFDARRRTRVSIAEMIAQCTGFEVKRGD---LFSEMICPQCYEDVKS---------- 61
C.quinquefasciatus --DNKHSLA----------VSLSLGNTLINLNKIKCPQCRKRFDTMEEMQLHRTKHLT 74
M.musculus EG--AWESQGSSFPSQDPVLGLREPTRPERD---------IGELSPAIAQEEAPAGDWLF 390
H.sapiens EG--AWESQGSSFPSQDPVLGLREPARPERD---------MGELSPAVAQEETPPGDWLF 390
D.melanogaster ----------AYGIRQTCEESHQFYCRVRDEGIEDALCALL----EEEDWEI 99
C.quinquefasciatus ENKFKCEICSKEFPSHSSMWKHTKAHTGDRPFVCQICNKGFTQLA---------- 119
M.musculus GGVRWGWNFRCKPPVGLNPRTVPEGLPFSSPDNGEAILDPSQAPRPFNDPCKYPGRTKGF 450
H.sapiens GGVRWGWNFRCKPPVGLNPRTGPEGLPYSSPDNGEAILDPSQAPRPFNEPCKYPGRTKGF 450
D.melanogaster S---------EDEDARIDS----------A----SAADDDGKSDSKKVAFECRECHKKY 135
C.quinquefasciatus ----------NLQRHDLVHNGLKPYKCPVCQKAF 143
M.musculus GHKPGLKKHPAAPPGGRPFTCATCGKSFQLQVSLSAHQRSCGLSDGAATGAASTTTGGGG 510
H.sapiens GHKPGLKKHPAAPPGGRPFTCATCGKSFQLQVSLSAHQRSCGAPDGSGPG-----TGGGG 505
D.melanogaster QRKGTFLRHMRTHMDGQSFPCPYCKRNFRLRVTLKAHMKTHNAAKPYECSHCA------- 188
C.quinquefasciatus SQHANMIKHQMLHTGLKPYKCPVCDKAFTQQANMVKHQMLHTGLKPYKCNTCG------- 196
M.musculus GGSGGGGGSSGGGSSARDSSALRCGECGRCFTRPAHLIRHRMLHTGERPFPCTECEKRFT 570
H.sapiens SGSGGGGGGSG-GGSARDGSALRCGECGRCFTRPAHLIRHRMLHTGERPFPCTECEKRFT 564
D.melanogaster KTFAQQSTLQSHERTHTGERPFKCSQCSKTFIKSSDLRRHIRTHGSERPFKCSKCTKTFT 248
C.quinquefasciatus KAFAQQANMVKHQMLHTGIKPYKCGTCGKAFAQQANMVKHQMLHTGVKPYKCSVCGKAFA 256
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H.sapiens ERSKLIDHYRTHTGVRPFTCTVCGKSFIRKDHLRKHQRNHAAGAKTPARGQPLPTPPAPP 624
D.melanogaster RKFHLDNHFRSHTGERPFKCSHCPKAFAMKQHLKQHSRLHLPD---------- 291
C.quinquefasciatus QQANMVKHQMLHSGIKPYKCPTCDKAFAQQANMVKHQMLHTGE---------- 299

```
M.musculus              DPFKSPAAKGPMAS------TDL----VTDWTCGLSV----------------------   657
H.sapiens               DPFKSPASKGPLAS------TDL----VTDWTCGLSV----------------------   651
D.melanogaster          RPFRCSHCPKTFRLSSTLKEHKLVHNAERTFKCPHCASFYKQRKTLARHILEIHK-----   346
C.quinquefasciatus      KPFKCKSCDKAFSQRANLKKHEMVHLGIRPHTCPLCSKSYSQYSNLKKHLLVHQKQSLKQ   359
                         **:.  .   :         .:        .*  .

M.musculus              ---------------------------------------LGPSDGGGDL-----------   667
H.sapiens               ---------------------------------------LGPTDGGDM-----------   660
D.melanogaster          ------------------------------------------------------------   346
C.quinquefasciatus      QQQNGQVMVILYNCQTCKMQFENILEFERHTKQCNELNNGAANGGHALKLEHINIKSEID   419

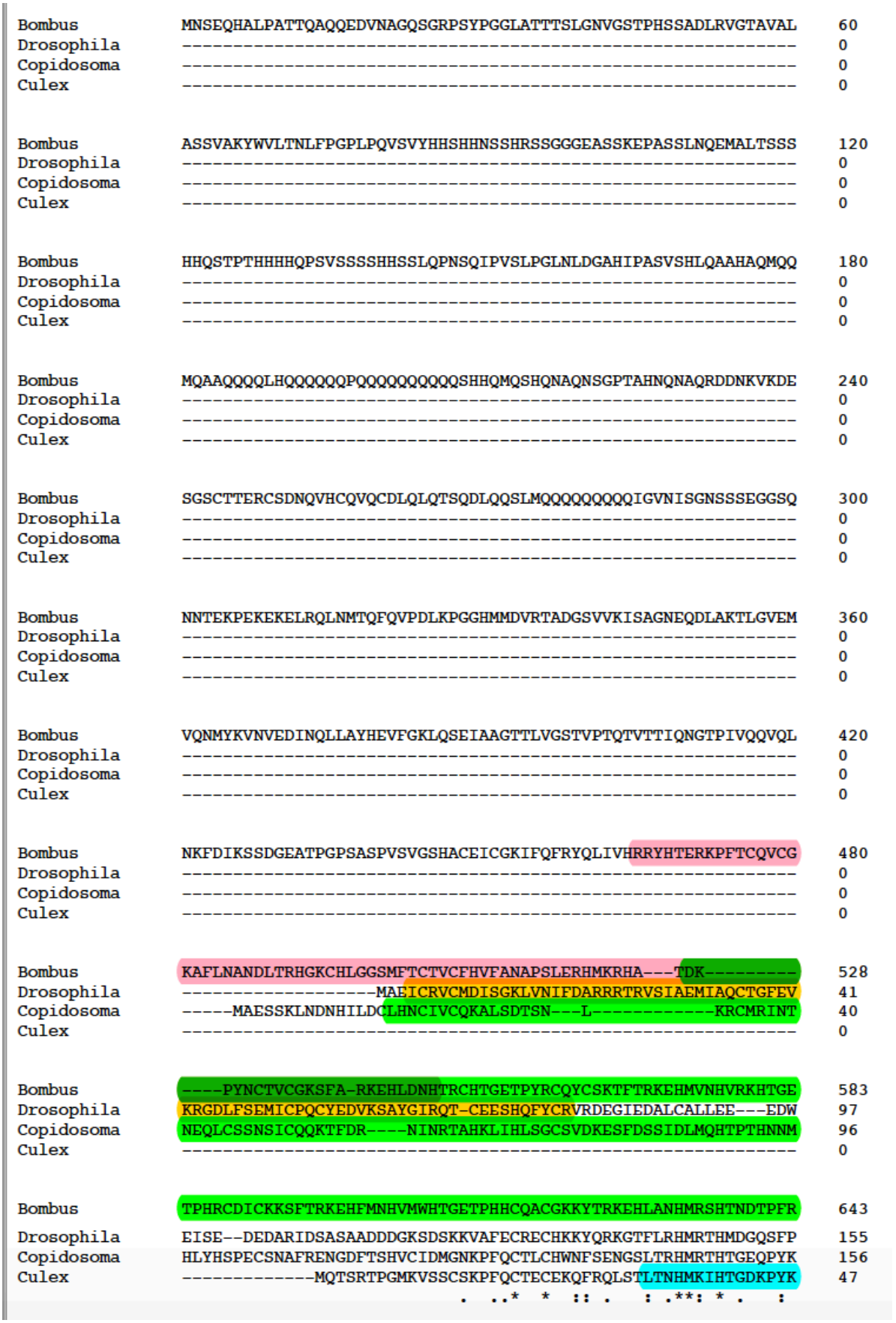
M.musculus              ------------------------------------------------------------   667
H.sapiens               ------------------------------------------------------------   660
D.melanogaster          ------------------------------------------------------------   346
C.quinquefasciatus      IDGSSSSGMTQHITIPHSQSQPPMHIPSAILTSVISSSSASGIHTLSTSHVNHHPGHPQH   479

M.musculus              ------------------------------------------------------------   667
H.sapiens               ------------------------------------------------------------   660
D.melanogaster          ------------------------------------------------------------   346
C.quinquefasciatus      PQQQHTPPAVGQHPQQQQQQQQQQQQQQQAHQQQLPPVSTHQQQHPSLQQQHNGLPIHLQQ   539

M.musculus              ------------------------------------------------------      667
H.sapiens               ------------------------------------------------------      660
D.melanogaster          ------------------------------------------------------      346
C.quinquefasciatus      QLSHHLISSHLPPHQDHQDLHHQVNFHHPHIPHISNLPHKILSPLFHIPPFNNNHST   596
```

Figure 3: The protein Paris is slightly conserved between vertebrates and invertebrates. Clustal Omega multiple alignment of Paris proteins. Highlighted are the zinc finger associated domain (green), C2H2 type domain (red), and the KRAB box (blue). "*" indicates amino acids that are identical in all sequences in the alignment. ":" indicates conserved substitutions. "." indicates semi-conserved substitutions. BLAST used to obtain protein sequences and Pfam (Sanger Institute) used to obtain conserved domain areas.

Figure 4: Phylogenetic Tree shows a high amount of genetic change between *H. sapiens* Paris and *D. melanogaster* CG15436. Numbers shown are the distance values which represent the number of substitutions as a proportion of the length of the alignment. The numbers are produced as the output of the multiple sequence alignment and represent the "length" of the branches. This is an indication of the evolutionary distance between the sequences.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Culex ---------- 0
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Drosophila ---------- 0
Copidosoma ---------- 0
Culex ---------- 0
Bombus NNTEKPEKEKELRQLNMTQFQVPDLKPGGHMMDVRTADGSVVKISAGNEQDLAKTLGVEM 360
Drosophila ---------- 0
Copidosoma ---------- 0
Culex ---------- 0
Bombus VQNMYKVNVEDINQLLAYHEVFGKLQSEIAAGTTLVGSTVPTQTVTTIQNGTPIVQQVQL 420
Drosophila ---------- 0
Copidosoma ---------- 0
Culex ---------- 0
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Drosophila ---------- 0
Copidosoma ---------- 0
Culex ---------- 0
Bombus KAFLNANDLTRHGKCHLGGSMFTCTVCFHVFANAPSLERHMKRHA---TDK--------- 528
Drosophila ----------MAEICRVCMDISGKLVNIFDARRRTRVSIAEMIAQCTGFEV 41
Copidosoma -----MAESSKLNDNHILDCLHNCIVCQKALSDTSN---L----------KRCMRINT 40
Culex ---------- 0
Bombus ----PYNCTVCGKSFA-RKEHLDNHTRCHTGETPYRCQYCSKTFTRKEHMVNHVRKHTGE 583
Drosophila KRGDLFSEMICPQCYEDVKSAYGIRQT-CEESHQFYCRVRDEGIEDALCALLEE---EDW 97
Copidosoma NEQLCSSNSICQQKTFDR----NINRTAHKLIHLSGCSVDKESFDSSIDLMQHTPTHNNM 96
Culex ---------- 0
Bombus TPHRCDICKKSFTRKEHFMNHVMWHTGETPHHCQACGKKYTRKEHLANHMRSHTNDTPFR 643
Drosophila EISE--DEDARIDSASAADDDGKSDSKKVAFECRECHKKYQRKGTFLRHMRTHMDGQSFP 155
Copidosoma HLYHSPECSNAFRENGDFTSHVCIDMGNKPFQCTLCHWNFSENGSLTRHMRTHTGEQPYK 156
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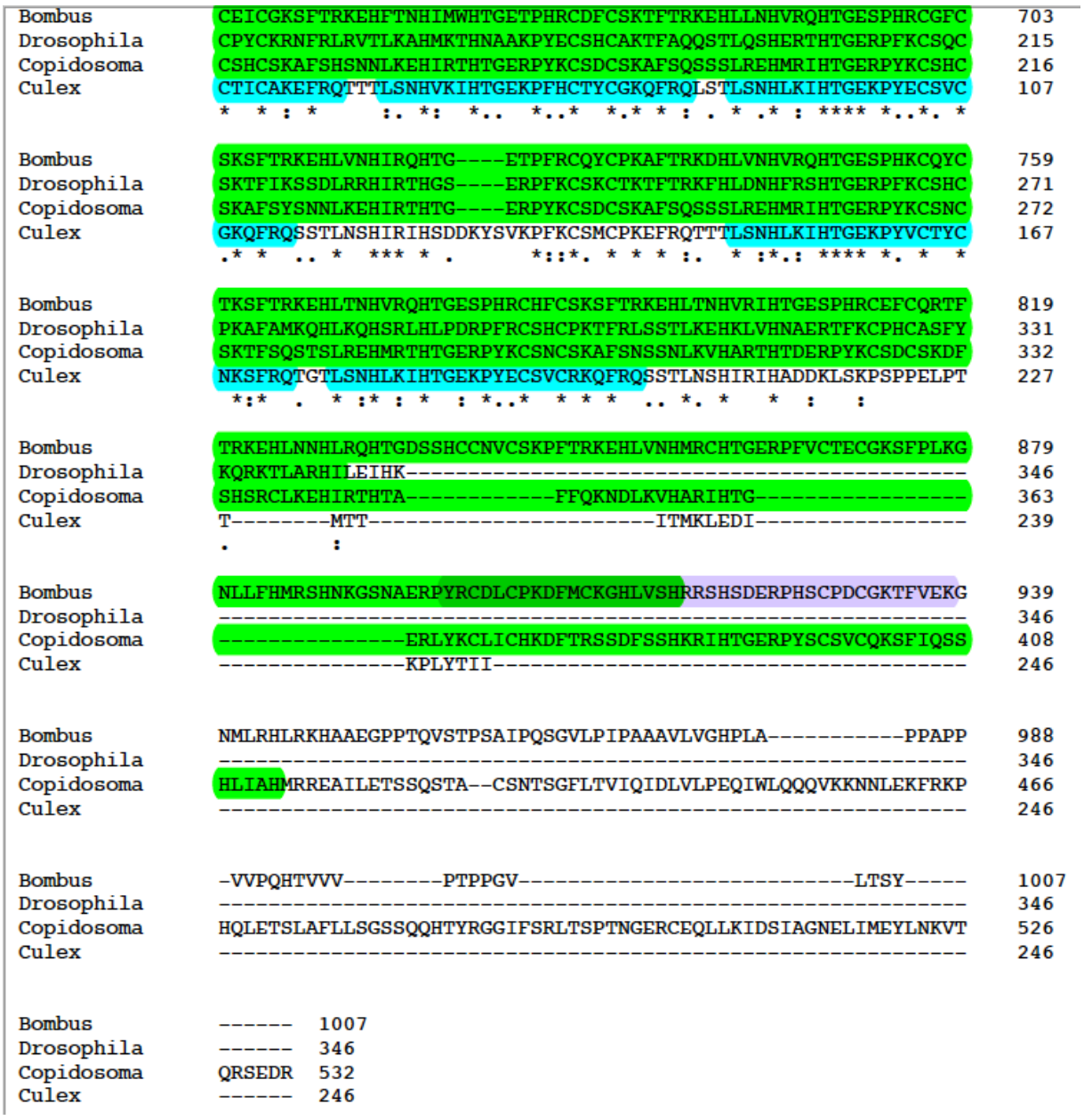
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Copidosoma CSHCSKAFSHSNNLKEHIRTHTGERPYKCSDCSKAFSQSSSLREHMRIHTGERPYKCSHC 216
Culex CTICAKEFRQTTTLSNHVKIHTGEKPFHCTYCGKQFRQLSTLSNHLKIHTGEKPYECSVC 107
Bombus SKSFTRKEHLVNHIRQHTG----ETPFRCQYCPKAFTRKDHLVNHVRQHTGESPHKCQYC 759
Drosophila SKTFIKSSDLRRHIRTHGS----ERPFKCSKCTKTFTRKFHLDNHFRSHTGERPFKCSHC 271
Copidosoma SKAFSYSNNLKEHIRTHTG----ERPYKCSDCSKAFSQSSSLREHMRIHTGERPYKCSNC 272
Culex GKQFRQSSTLNSHIRIHSDDKYSVKPFKCSMCPKEFRQTTTLSNHLKIHTGEKPYVCTYC 167
Bombus TKSFTRKEHLTNHVRQHTGESPHRCHFCSKSFTRKEHLTNHVRIHTGESPHRCEFCQRTF 819
Drosophila PKAFAMKQHLKQHSRLHLPDRPFRCSHCPKTFRLSSTLKEHKLVHNAERTFKCPHCASFY 331
Copidosoma SKTFSQSTSLREHMRTHTGERPYKCSNCSKAFSNSSNLKVHARTHTDERPYKCSDCSKDF 332
Culex NKSFRQTGTLSNHLKIHTGEKPYECSVCRKQFRQSSTLNSHIRIHADDKLSKPSPPELPT 227
Bombus TRKEHLNNHLRQHTGDSSHCCNVCSKPFTRKEHLVNHMRCHTGERPFVCTECGKSFPLKG 879
Drosophila KQRKTLARHILEIHK---------- 346
Copidosoma SHSRCLKEHIRTHTA----------FFQKNDLKVHARIHTG---------- 363
Culex T--------MTT----------ITMKLEDI---------- 239
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Drosophila ---------- 346
Copidosoma ----------ERLYKCLICHKDFTRSSDFSSHKRIHTGERPYSCSVCQKSFIQSS 408
Culex ----------KPLYTII---------- 246
Bombus NMLRHLRKHAAEGPPTQVSTPSAIPQSGVLPIPAAAVLVGHPLA----------PPAPP 988
Drosophila ---------- 346
Copidosoma HLIAHMRREAILETSSQSTA--CSNTSGFLTVIQIDLVLPEQIWLQQQVKKNNLEKFRKP 466
Culex ---------- 246
Bombus -VVPQHTVVV--------PTPPGV----------LTSY----- 1007
Drosophila ---------- 346
Copidosoma HQLETSLAFLLSGSSQQHTYRGGIFSRLTSPTNGERCEQLLKIDSIAGNELIMEYLNKVT 526
Culex ---------- 246
Bombus ------ 1007
Drosophila ------ 346
Copidosoma QRSEDR 532
Culex ------ 246

A

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Mus       ------------------------------------------------------------  0
Danio     ------------------------------------------------------------  0
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Homo      ------------------------------------------------------------  0
Mus       ------------------------------------------------------------  0
Danio     ------------------------------------------------------------  0
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Mus       ------------------------------------------------------------  0
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Mus       ------------------------------------------------------------  0
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Mus       ------------------------------------------------------------  0
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Homo      ------------------------------------------------------------  0
Mus       ------------------------------------------------------------  0
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Gallus    AAHQRAHMDEEACTSLEAGESFRQKSKSSAKGQRSRPSKRANSEKGSGYKYGFVRPNNMV  420

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Mus       ------------------------------------------------------------  0
Danio     ------------------------------------------------------------  0
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Danio     ------------------------------------------------------------  0
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Gallus    EALQHNLESATQTSLFCLKTETKHGGAGARSAERRSRDPEPLGLSPPPSHHRLRRPEVRP  600

Homo      ------------------------------------------------------------  0
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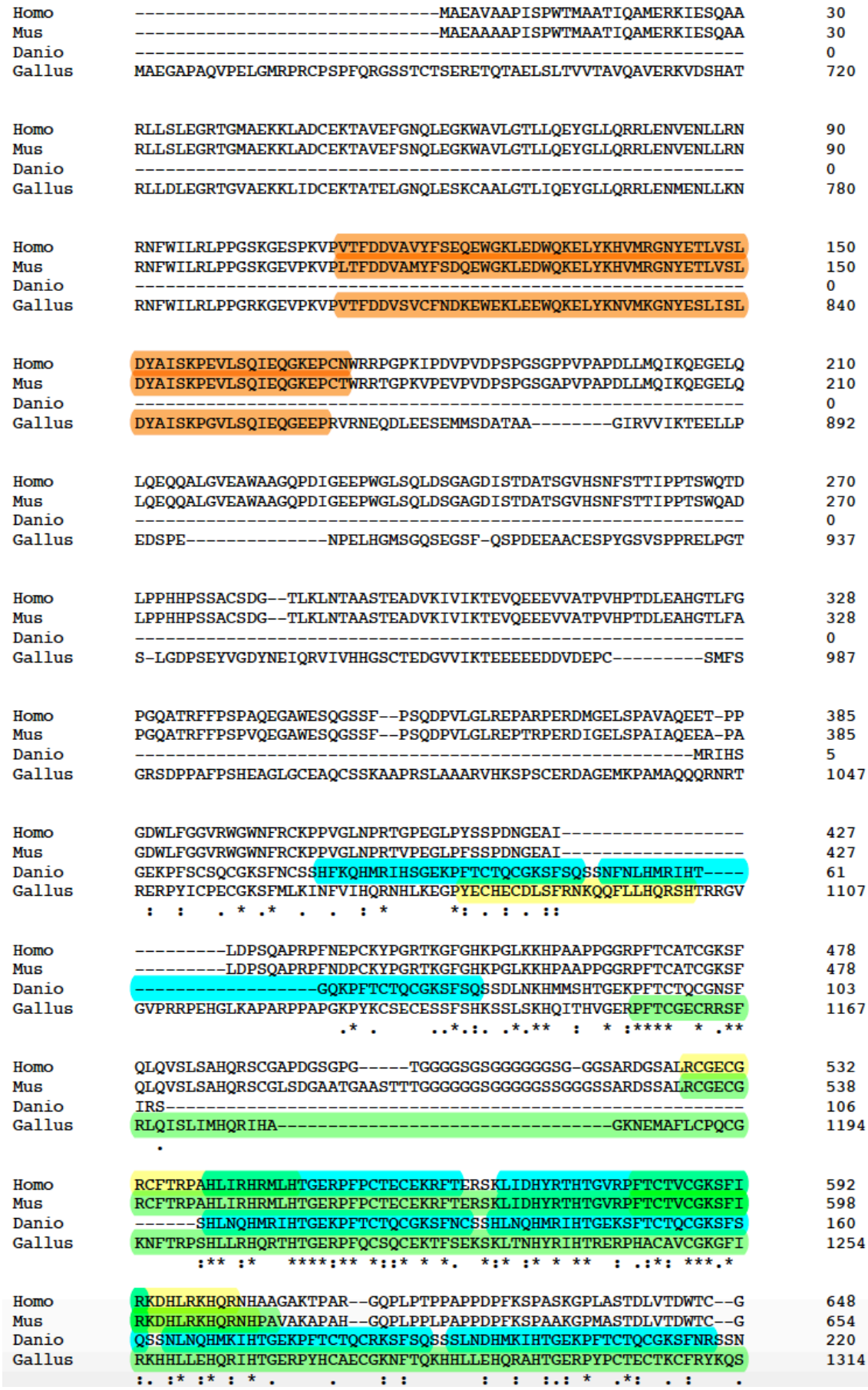
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Danio ---------- 0
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Homo RLLSLEGRTGMAEKKLADCEKTAVEFGNQLEGKWAVLGTLLQEYGLLQRRLENVENLLRN 90
Mus RLLSLEGRTGMAEKKLADCEKTAVEFSNQLEGKWAVLGTLLQEYGLLQRRLENVENLLRN 90
Danio ---------- 0
Gallus RLLDLEGRTGVAEKKLIDCEKTATELGNQLESKCAALGTLIQEYGLLQRRLENMENLLKN 780
Homo RNFWILRLPPGSKGESPKVPVTFDDVAVYFSEQEWGKLEDWQKELYKHVMRGNYETLVSL 150
Mus RNFWILRLPPGSKGEVPKVPLTFDDVAMYFSDQEWGKLEDWQKELYKHVMRGNYETLVSL 150
Danio ---------- 0
Gallus RNFWILRLPPGRKGEVPKVPVTFDDVSVCFNDKEWEKLEEWQKELYKNVMKGNYESLISL 840
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Mus DYAISKPEVLSQIEQGKEPCTWRRTGPKVPEVPVDPSPGSGAPVPAPDLLMQIKQEGELQ 210
Danio ---------- 0
Gallus DYAISKPGVLSQIEQGEEPRVRNEQDLEESEMMSDATAA--------GIRVVIKTEELLP 892
Homo LQEQQALGVEAWAAGQPDIGEEPWGLSQLDSGAGDISTDATSGVHSNFSTTIPPTSWQTD 270
Mus LQEQQALGVEAWAAGQPDIGEEPWGLSQLDSGAGDISTDATSGVHSNFSTTIPPTSWQAD 270
Danio ---------- 0
Gallus EDSPE----------NPELHGMSGQSEGSF-QSPDEEAACESPYGSVSPPRELPGT 937
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Mus LPPHHPSSACSDG--TLKLNTAASTEADVKIVIKTEVQEEEVVATPVHPTDLEAHGTLFA 328
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Mus PGQATRFFPSPVQEGAWESQGSSF--PSQDPVLGLREPTRPERDIGELSPAIAQEEA-PA 385
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Gallus GVPRRPEHGLKAPARPPAPGKPYKCSECESSFSHKSSLSKHQITHVGERPFTCGECRRSF 1167
Homo QLQVSLSAHQRSCGAPDGSGPG-----TGGGGSGSGGGGGGSG-GGSARDGSALRCGECG 532
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Danio IRS---------- 106
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Homo RCFTRPAHLIRHRMLHTGERPFPCTECEKRFTERSKLIDHYRTHTGVRPFTCTVCGKSFI 592
Mus RCFTRPAHLIRHRMLHTGERPFPCTECEKRFTERSKLIDHYRTHTGVRPFTCTVCGKSFI 598
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Gallus KNFTRPSHLLRHQRTHTGERPFQCSQCEKTFSEKSKLTNHYRIHTRERPHACAVCGKGFI 1254
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Mus RKDHLRKHQRNHPAVAKAPAH--GQPLPPLPAPPDPFKSPAAKGPMASTDLVTDWTC--G 654
Danio QSSNLNQHMKIHTGEKPFTCTQCRKSFSQSSSLNDHMKIHTGEKPFTCTQCGKSFNRSSN 220
Gallus RKHHLLEHQRIHTGERPYHCAECGKNFTQKHHLLEHQRAHTGERPYPCTECTKCFRYKQS 1314

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Homo        LSVLGPTDGGDM-       660
Mus         LSVLGPSDGGGDL       667
Danio       LNKHMRIHTG---       230
Gallus      LKYHLRTHVGE--       1325
            *.      . *
```

B

Figure 5: The protein Paris is conserved across vertebrates as well as across invertebrates. A) Clustal Omega multiple alignment of Paris proteins in invertebrates. B) Clustal Omega multiple alignment of Paris proteins in vertebrates. Highlighted are the zinc finger associated domain (dark yellow), C2H2 type domain (pale yellow), the KRAB box (orange), Zinc finger double domain (blue), FOG zinc finger (green), SFP1 super family putative transcriptional repressor (pink) and zinc ribbon domain (purple). "*" indicates amino acids that are identical in all sequences in the alignment. ":" indicates conserved substitutions. "." indicates semi-conserved substitutions. BLAST used to obtain protein sequences and NCBI Conserved Domains Database used to obtain conserved domain areas.

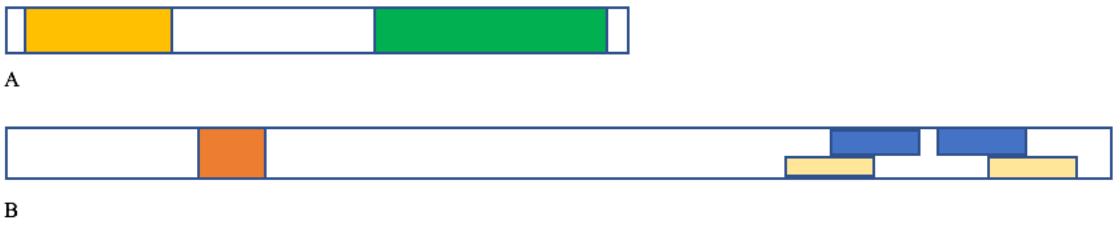


Figure 6: Comparison of *Drosophila melanogaster* CG15436 protein (A) and *Homo sapiens* Paris protein (B) with conserved domains. Highlighted are the zinc finger associated domain (dark yellow), C2H2 type domain (pale yellow), the KRAB box (orange), zinc finger double domain (blue), and FOG zinc finger (green).

Effects of the knockdown of *CG15436* and *spargel*

Knockdown of *CG15436* decreases climbing ability and lifespan

To determine the effects of the knockdown of *CG15436* on climbing ability and lifespan of *D. melanogaster*, the motoneuron specific driver *D42-GAL4*, the dopaminergic neuron specific driver *TH-GAL4*, the neuron specific driver *ddc-GAL4* and the recombinant line *ddc-GAL4;UAS-park*^{RNAi} were used. When using the motoneuron specific driver *D42-GAL4*, there was a significant difference in the climbing ability between *D42-GAL4; UAS-CG15436*^{RNAi} and the control *D42-GAL4; UAS-lacZ* (Figure 7, Table 2). When the dopaminergic neuron specific driver *TH-GAL4* (Figure 9, Table 4), the neuron specific driver *ddc-GAL4* (Figure 11, Table 6) and the recombinant line *ddc-GAL4;UAS-park*^{RNAi} (Figure 13, Table 7), there was no significant difference found in the climbing ability of flies with the knockdown of *CG15436* when compared to the control *UAS-lacZ.*

The knockdown of *spargel* using the neuron-specific driver *ddc-GAL4* resulted in a significant decrease in climbing ability when compared to the control *UAS-lacZ* (Figure 11, Table 6). There was no significant change in climbing ability of flies with the knockdown of *spargel* when compared to the control *UAS-lacZ* when using the driver lines *D42-GAL4* (Figure 7, Table 2), *TH-GAL4* (Figure 9, Table 4) and *ddc-GAL4;UAS-park*^{RNAi} (Figure 13 and Table 8).

The knockdown of *CG15436* using the motorneuron specific driver *D42-GAL4* and the recombinant line *ddc-GAL4;UAS-park*^{RNAi} resulted in a significant decrease in lifespan in comparison to the control *UAS-lacZ* (Figures 8 and 14). The median lifespan for flies with an knockdown of *CG15436* with drivers *D42-GAL4* and *ddc-GAL4;UAS-park*^{RNAi} are 62 and 60, respectively. These median lifespans are shorter than the control *UAS-lacZ* whose median lifespans are 64 and 72, respectively (Tables 3 and 9). The knockdown of *CG15436* using the

dopaminergic neuron specific driver *TH-GAL4* resulted in no significant change in the lifespan of flies when compared to the control *UAS-lacZ* (Figure 12, Table 5). The knockdown of *CG51436* using the neuron specific driver *ddc-GAL4* resulted in a significant increase in lifespan of the flies when compared to the control *UAS-lacZ* (Figure 10). The median lifespan for flies with an knockdown of *CG15436* with driver *ddc-GAL4* is 80 days. This median lifespan is significantly longer than the control *UAS-lacZ* whose median lifespan is 70 days (Table 7).

The knockdown of *spargel* using the motoneuron specific driver *D42-GAL4*, the dopaminergic neuron specific driver *TH-GAL4,* the neuron specific driver *ddc-GAL4* and the recombinant line *ddc-GAL4;UAS-park*^{RNAi} resulted in a significant decrease in lifespan in comparison to the control *UAS-lacZ* (Figures 8, 10, 12 and 14). The median lifespan for flies with an knockdown of *CG15436* with drivers *D42-GAL4, TH-GAL4, ddc-GAL4* and *ddc-GAL4;UAS-park*^{RNAi} are 54, 64, 60 and 58, respectively. These median lifespans are shorter than the control *UAS-lacZ* whose median lifespans are 64, 70, 70 and 72, respectively (Tables 3, 5, 7 and 9).

Knockdown of *CG15436* and *spargel* decreases bristle and ommatidia number

The eye of *Drosophila melanogaster* is a compound eye which has a very specific developmental pattern. Each eye is made up of approximately 800 ommatidia when undergoing normal development. The eye develops as a morphogenetic furrow which then migrates from the posterior imaginal disc to the anterior. The formation and differentiation of these cells then occur behind the furrow (Baker, 2001). If this process is disrupted, characteristic phenotypes are produced. These phenotypes are presented in many ways such as changes in ommatidia number

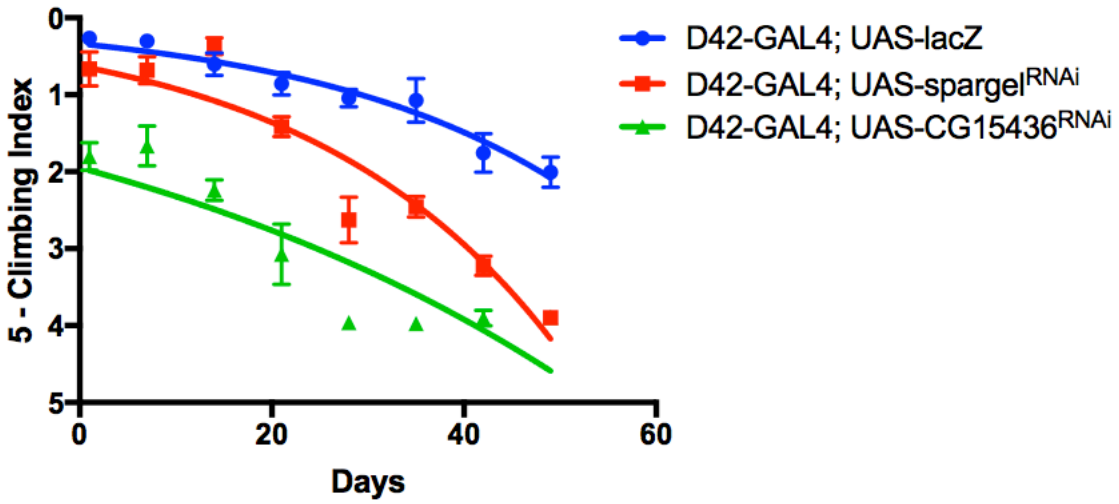


Figure 7: Directed motorneuron specific expression with knockdown of *CG15436* and *spargel* causes a significant decrease in climbing ability over time as flies age. Directed RNA interference of CG15436 shows a significant decrease in climbing ability. Directed RNA interference of s*pargel* shows no significant difference in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 2: Statistical analysis using a non-linear regression curve of locomotor ability with directed motorneuron-specific expression with knockdown of *CG15436* and *spargel*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant compared to D42; UAS-lacZ
D42-GAL4; UAS-lacZ	0.03711 ± 0.00453	0.02869 – 0.04677	N/A
D42-GAL4; UAS-CG15436^{RNAi}	0.01749 ± 0.002233	0.01325 – 0.02185	Yes ↓
D42-GAL4; UAS-spargel^{RNAi}	0.03867 ± 0.003811	0.0316 – 0.04642	No

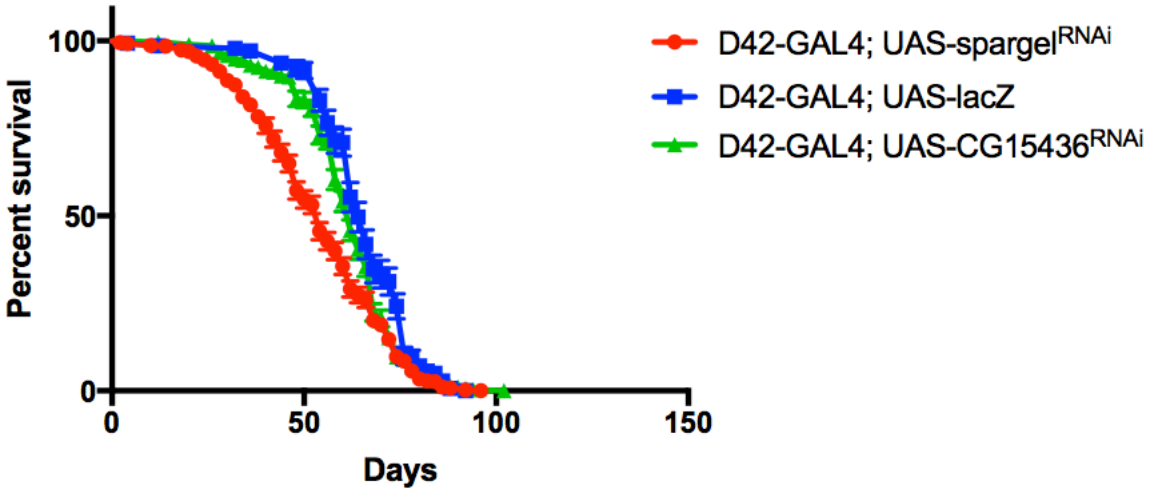


Figure 8: Directed motorneuron specific expression with knockdown of *CG15436* and *spargel* causes a significant decrease in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 3: Log-rank statistical analysis of longevity of flies with directed motorneuron specific expression with knockdown of *CG15436*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi – square value	P – value	Significant
D42-GAL4; UAS-lacZ	141	64	N/A	N/A	N/A
D42-GAL4; UAS-CG15436^{RNAi}	290	62	6.191	0.0128	Yes ↓
D42-GAL4; UAS-spargel^{RNAi}	388	54	27.51	<0.0001	Yes ↓

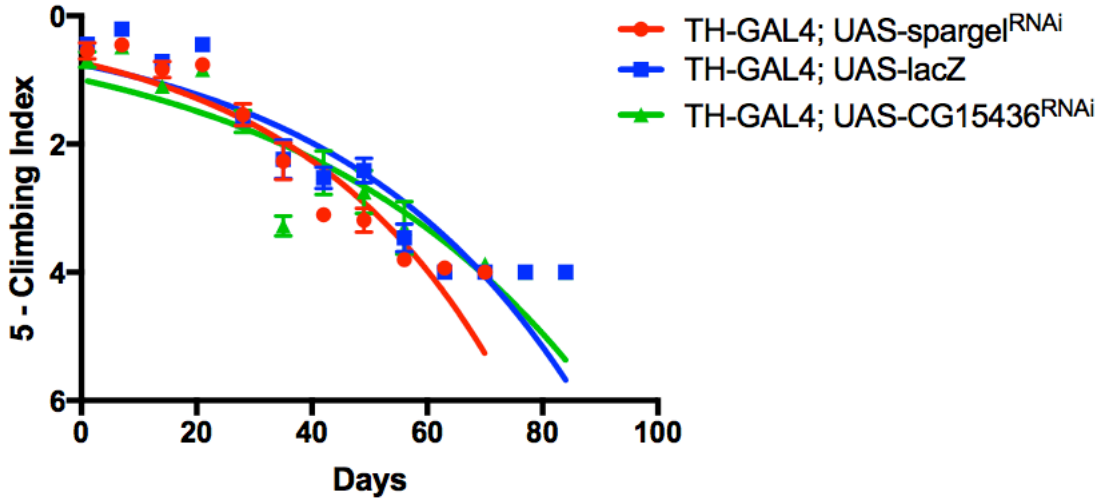


Figure 9: Directed dopaminergic neuron specific expression with knockdown of *CG15436*. Knockdown of *CG15436* does not show a significant decrease in climbing ability over time. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 4: Statistical analysis using a non-linear regression curve of locomotor ability of flies with dopaminergic neuron specific expression with knockdown of *CG15436*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
TH-GAL4; UAS-lacZ	0.02395 ± 0.001932	0.02055 – 0.0275	N/A
TH-GAL4; UAS-CG15436^{RNAi}	0.02006 ± 0.002093	0.01628 – 0.02397	No
TH-GAL4; UAS-spargel^{RNAi}	0.02821 ± 0.00205	0.02454 – 0.03209	No

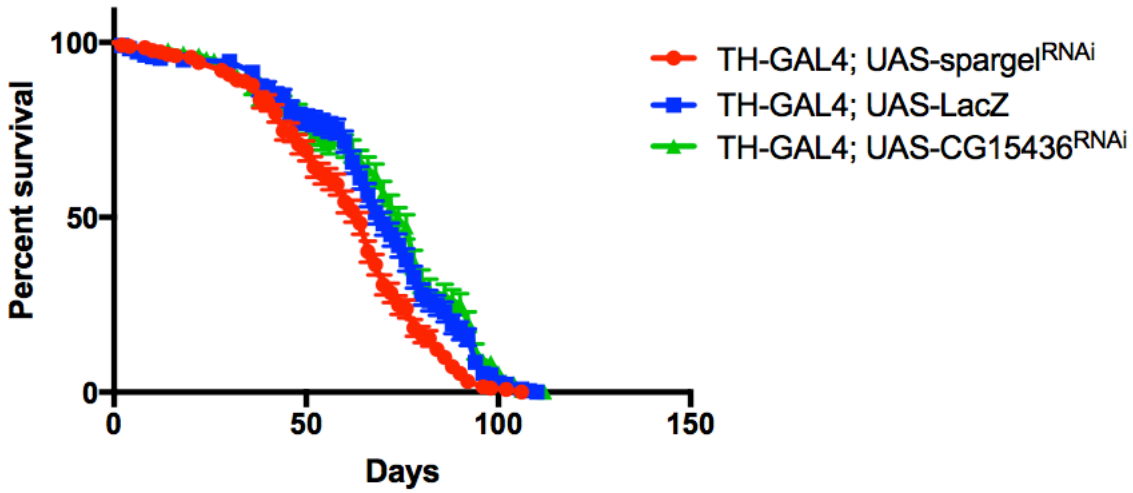


Figure 10: Directed dopaminergic neuron specific expression with knockdown of *CG15436* does not cause a significant increase in longevity and knockdown of *spargel* causes a significant decrease in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 5: Log-rank statistical analysis of longevity of flies with directed dopaminergic neuron specific expression with knockdown of *CG15436* and *spargel*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi – square value	P – value	Significant
TH-GAL4; UAS-lacZ	222	70	N/A	N/A	N/A
TH-GAL4; UAS-CG15436^{RNAi}	199	74	2.198	0.1382	No
TH-GAL4; UAS-spargel^{RNAi}	261	64	21.59	<0.0001	Yes↓

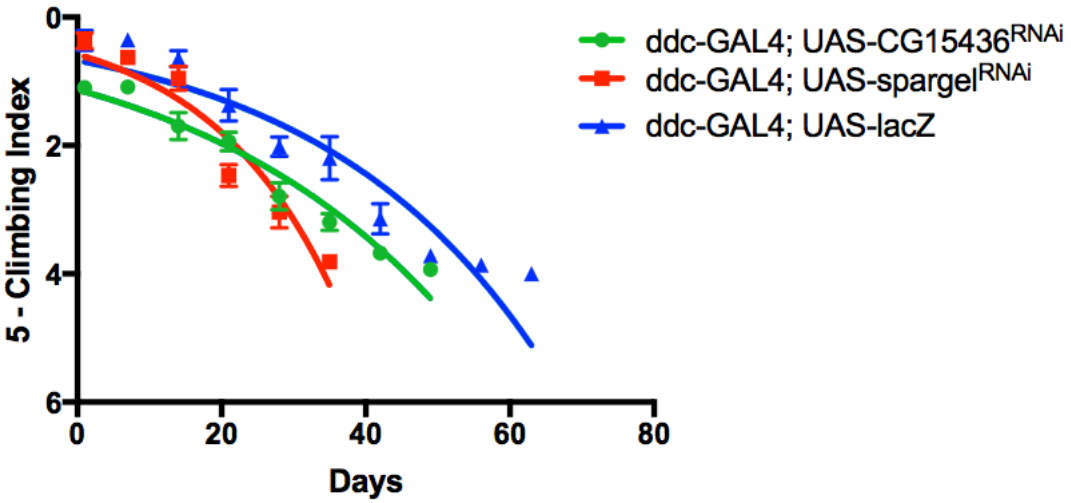


Figure 11: Directed neuron specific expression with knockdown of *CG15436* does not cause a significant decrease in climbing ability and with knockdown of *spargel* does cause a significant decrease in climbing ability over time as flies age. Neuron specific expression of *CG15436* does not show a significant decrease in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 6: Statistical analysis using a non-linear regression curve of locomotor ability of flies with neuron specific expression with knockdown of and *CG15436* and *spargel*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
ddc-GAL4; UAS-lacZ	0.03219 ± 0.002762	0.02721 – 0.03744	N/A
ddc-GAL4; UAS-CG15436^{RNAi}	0.02767 ± 0.001791	0.0242 – 0.03126	No
ddc-GAL4; UAS-spargel^{RNAi}	0.05642 ± 0.005439	0.04653 – 0.06734	Yes ↓

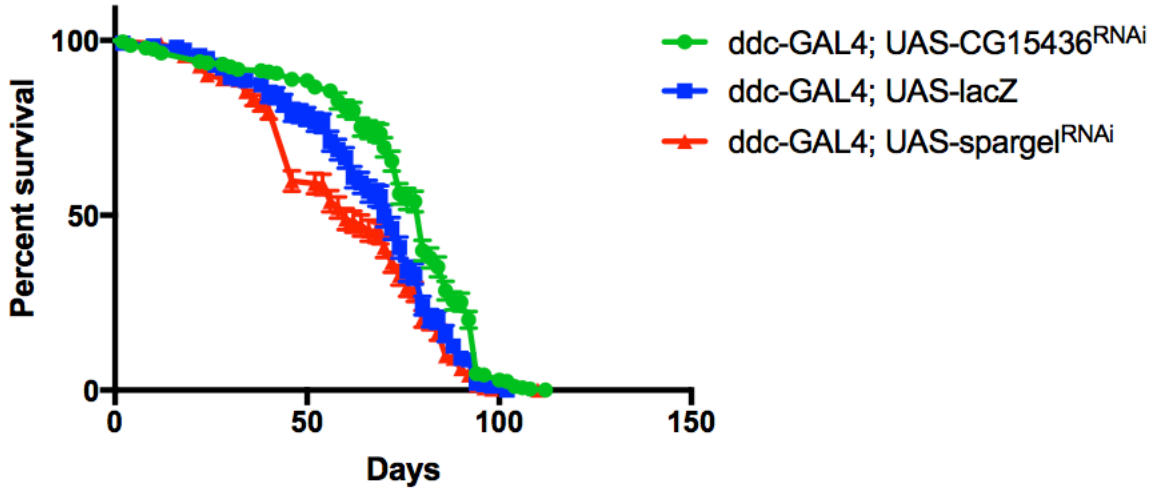


Figure 12: Directed neuron specific expression with knockdown of *CG15436* causes a significant increase in longevity and directed neuron specific expression with knockdown of *spargel* causes a significant decrease in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 7: Log-rank statistical analysis of longevity of flies with directed neuron specific expression with knockdown of *spargel* and *CG15436*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi-square value	P-value	Significant
ddc-GAL4; UAS-lacZ	253	70	N/A	N/A	N/A
ddc-GAL4; UAS-CG15436^{RNAi}	278	80	28.07	<0.0001	Yes ↑
ddc-GAL4; UAS-spargel^{RNAi}	274	60	5.8	0.0160	Yes ↓

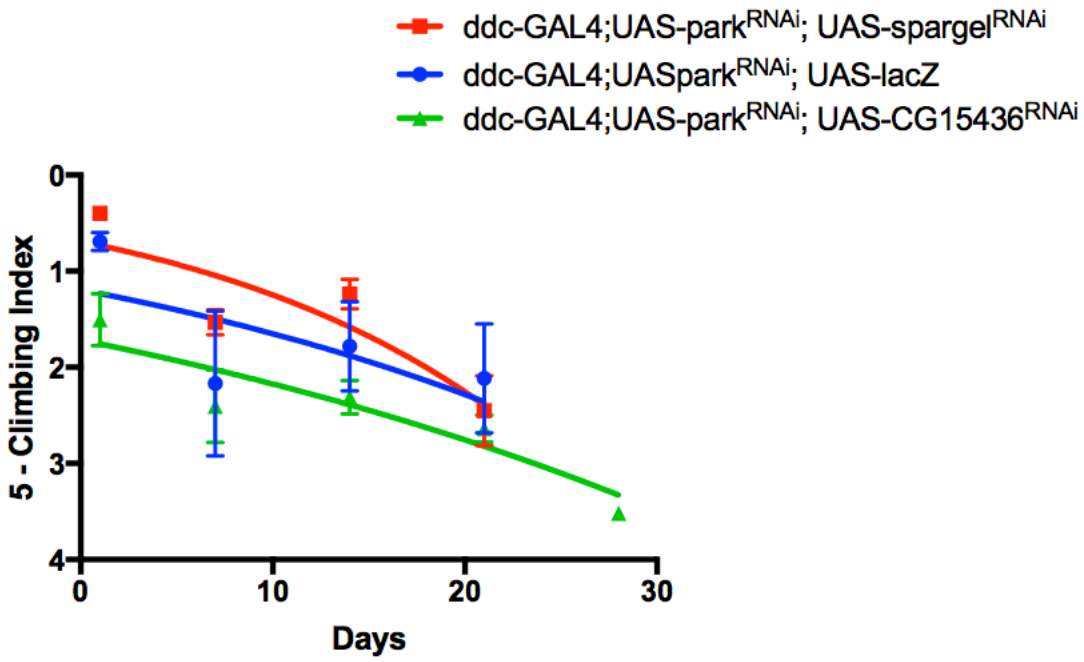


Figure 13: Directed neuron specific expression with knockdown of *parkin* shows that *CG15436* and *spargel* do not cause a significant decrease in climbing ability over time as flies age. Neuron specific expression of *CG15436* does not show a significant decrease in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 8: Statistical analysis using a non-linear regression curve of locomotor ability of flies with neuron specific expression with knockdown of *parkin* and *CG15436* or *spargel*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
ddc-GAL4;UAS-park^{RNAi}; UAS-lacZ	0.03245 ± 0.02488	-0.02106 – 0.08766	N/A
ddc-GAL4;UAS-park^{RNAi}; UAS-CG15436^{RNAi}	0.02368 ± 0.007278	0.008233 – 0.0391	No
ddc-GAL4;UAS-park^{RNAi}; UAS-spargel^{RNAi}	0.05917 ± 0.01319	0.03226 – 0.08941	No

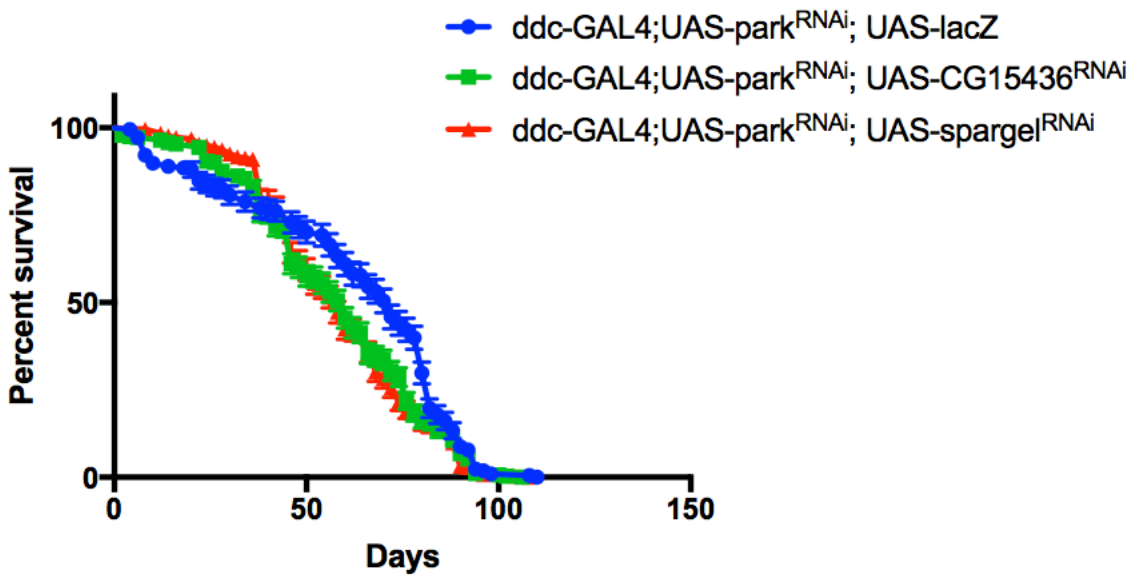


Figure 14: Directed neuron specific expression with knockdown of *parkin* and *CG15436* or *spargel* causes a significant decrease in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 9: Log-rank statistical analysis of longevity of flies with directed neuron specific expression with knockdown of *parkin* and *CG15436* or *spargel*. Chi-square values and p-values were calculated using *LacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi-square value	P-value	Significant
ddc-GAL4;UAS-park^{RNAi}; UAS-lacZ	218	72	N/A	N/A	N/A
ddc-GAL4;UAS-park^{RNAi}; UAS-CG15436^{RNAi}	283	60	9.37	0.0022	Yes ↓
ddc-GAL4;UAS-park^{RNAi}; UAS-spargel^{RNAi}	254	58	11.04	0.0009	Yes ↓

and bristle number. Biometric analysis was conducted to determine the phenotypic changes in the eye to determine the effects of altering gene expression such as the knockdown of *CG15436*. These phenotypic changes include a change in the number of ommatidia or bristles when compared to the control. *GMR-GAL4* is a GAL4 transgene used to determine the effects of the knockdown of *CG15436* and *spargel* in the compound eye. A recombinant driver, *GMR-GAL4; UAS-park*^{RNAi} was also used which consisted of *GMR-GAL4* with the knockdown of *parkin*. Biometric analysis of the scanning electron micrographs shows that there is a significant decrease in ommatidia number and bristle number when *CG15436* and *spargel* are knocked down with the driver *GMR-GAL4* (Figures 15 and 16). When the knockdown of *CG15436* and *spargel* is driven by *GMR-GAL4* the average number of ommatida per eye was shown to be 682.9 ± 10.46 and 619.4 ± 6.058, respectively. This is compared to the control *lacZ* where the average number of ommatidia per eye is 712.1 ± 7.326. The average bristle number for *CG15436* and *spargel* are 491.9 ± 17.17 and 462.2 ± 7.071, respectively. The control *lacZ* had a significantly higher number of bristles with an average of 539.5 ± 11.69 (Table 10). There was no significant difference in ommatidia number or bristle number detected in the knockdown of *CG15436* and *spargel* with the driver line *GMR-GAL4;UAS-park*^{RNAi} (Figure 17 and Table 11).

Effects of the overexpression of *CG15436*

Overexpression of *CG15436* decreases climbing ability and increases longevity

To determine the effects of the overexpression of *CG15436* on climbing ability and lifespan of *Drosophila melanogaster*, the motorneuron specific driver *D42-GAL4*, the neuron specific driver *ddc-GAL4* and the recombinant line *ddc-GAL4;UAS-park*^{RNAi} were used. When using the neuron specific driver *ddc-GAL4*, there was no significant difference in the climbing

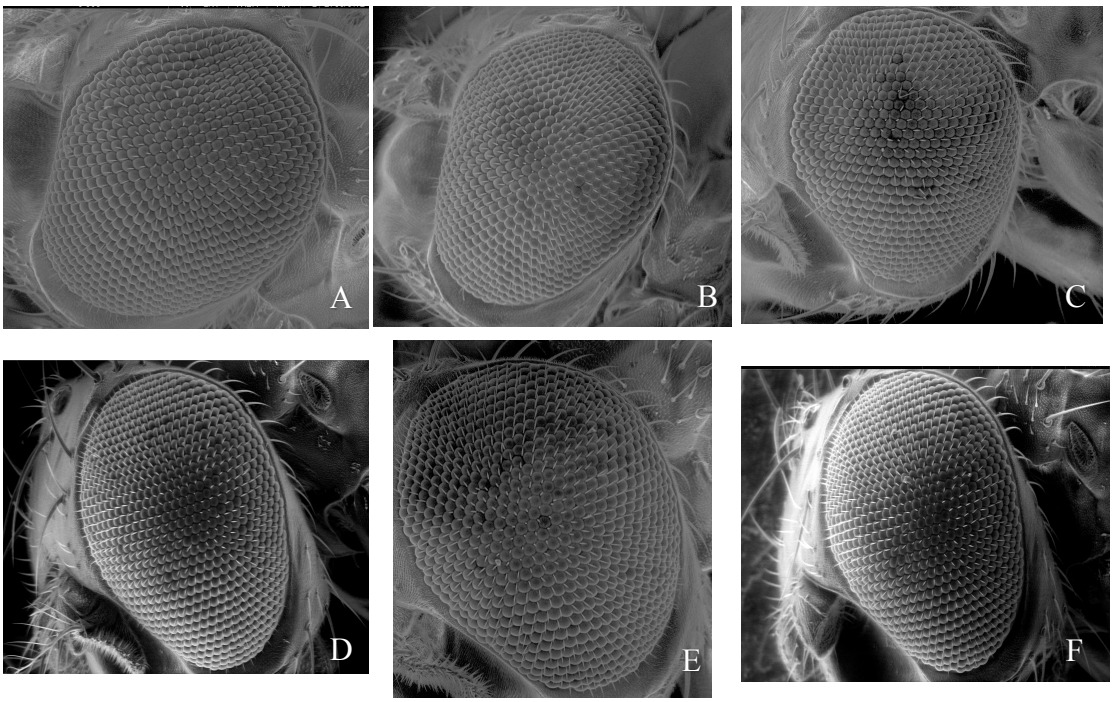
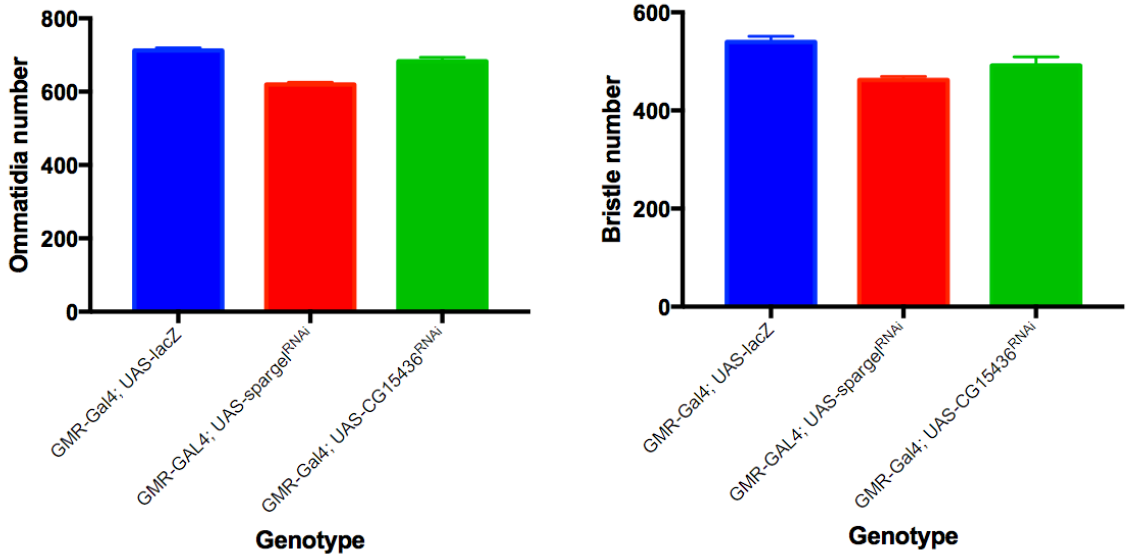
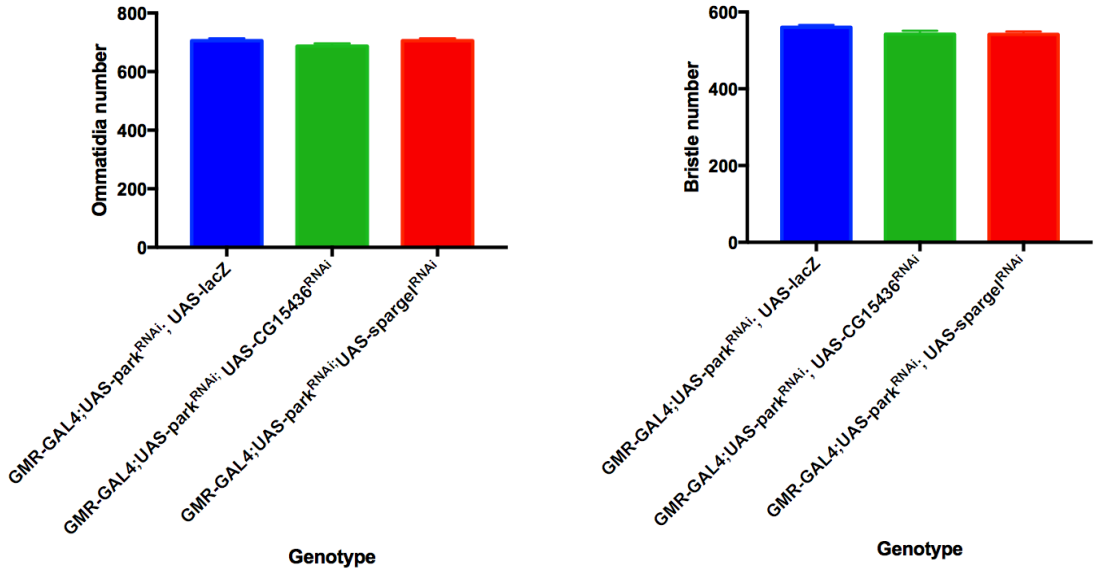


Figure 15: Knockdown of *CG15436* and *spargel* under the control of eye specific drivers influence ommatidia and bristle number. Scanning electron micrographs of A: *GMR-GAL4; UAS-lacZ*, B: *GMR-GAL4; UAS-CG15436^{RNAi}*, C: *GMR-GAL4; UAS-spargel^{RNAi}*, D: *GMR-GAL4;UAS-park^{RNAi}; UAS-lacZ*, E: *GMR-GAL4;UAS-park^{RNAi}; UAS-CG15436^{RNAi}, F: GMR-GAL4;UAS-park^{RNAi}; UAS-spargel^{RNAi}. GMR-GAL4* is an eye specific driver and *GMR-GAL4;UAS-park^{RNAi}* is an eye specific driver with an knockdown of *parkin*.



A B

Figure 16: Biometric analysis of the compound eye under the influence of eye specific expression with the knockdown of *CG15436* and *spargel*. Knockdown of *CG15436* and *spargel* in the eye significantly decreases ommatidia number (A) and bristle number (B). Significance is <0.05. Error bars represent standard error of the mean. *UAS-lacZ* crosses are the comparison controls.



A B

Figure 17: Biometric analysis of the compound eye under the influence of eye specific expression with the knockdown of *CG15436* and *spargel* with a recombinant driver with the knockdown of *parkin*. Knockdown of *CG15436* and *spargel* in the eye does not cause a significant decrease ommatidia number (A) and bristle number (B). Significance is <0.05. Error bars represent standard error of the mean. *UAS-lacZ* crosses are the comparison controls.

Table 10: Summary of ommatidia number and bristle number when *CG15436* and *spargel* are knocked down in the compound eye.

Genotype	Sample Size (n)	Mean ± SEM	P-value compared to control	Significant
Ommatidia Number				
GMR-GAL4; UAS-lacZ	10	712.1 ± 7.326	N/A	N/A
GMR-GAL4; UAS-spargel^{RNAi}	10	619.4 ± 6.058	<0.0001	Yes↓
GMR-GAL4; UAS-CG15436^{RNAi}	10	682.9 ± 10.46	0.0346	Yes↓
Bristle Number				
GMR-GAL4; UAS-lacZ	10	539.5 ± 11.69	N/A	N/A
GMR-GAL4; UAS-spargel^{RNAi}	10	462.2 ± 7.071	<0.0001	Yes↓
GMR-GAL4; UAS-CG15436^{RNAi}	10	491.9 ±17.17	0.0342	Yes↓

Table 11: Summary of ommatidia number and bristle number when *CG15436*, *spargel* and *parkin* are knocked down in the compound eye.

Genotype	Sample Size (n)	Mean ± SEM	P-value compared to control	Significant
Ommatidia Number				
GMR-GAL4; UAS-park^{RNAi}; UAS-lacZ	10	705.4 ± 8.582	N/A	N/A
GMR-GAL4; UAS-park^{RNAi}; UAS-spargel^{RNAi}	10	705.4 ± 8.582	>0.9999	No
GMR-GAL4; UAS-park^{RNAi}; UAS-CG15436^{RNAi}	10	686.9 ± 9.556	0.1669	No
Bristle Number				
GMR-GAL4;UAS-park^{RNAi}; UAS-lacZ	10	560 ± 6.326	N/A	N/A
GMR-GAL4;UAS-park^{RNAi}; UAS-spargel^{RNAi}	10	541.8 ± 7.261	0.0750	No
GMR-GAL4;UAS-park^{RNAi}; UAS-CG15436^{RNAi}	10	542.1 ± 9.197	0.1262	No

ability of flies between *ddc-GAL4; UAS-CG15436*^{ORF} when compared to the control *ddc- GAL4; UAS-lacZ* (Figure 20, Table 14). When the motoneuron specific driver *D42-GAL4* and the recombinant line *ddc-GAL4;UAS-park*^{RNAi}, there was a significant difference found in the climbing ability of flies with the overexpression of *CG15436* when compared to the control *UAS-lacZ* (Figures 18 and 22, Table 12 and 16).

The overexpression of *CG15436* using the motoneuron specific driver *D42-GAL4* and the neuron specific driver *ddc-GAL4* resulted in a significant increase in lifespan in comparison to the control *UAS-lacZ* (Figures 19 and 21). The median lifespan for flies with an overexpression of *CG15436* with driver *D42-GAL4* and *ddc-GAL4* is 74 for both. This median lifespan is shorter than the control *UAS-lacZ* with *D42-GAL4* and *ddc-GAL4* whose median lifespan is 64 and 62, respectively (Table 13 and 15). There was no significant difference in the lifespan of flies using the neuron specific driver *ddc-GAL4;UAS-park*^{RNAi} (Figure 23, Table 17).

Overexpression of *CG15436* decreases bristle and ommatidia number

The eye of *Drosophila melanogaster* is a compound eye which has a very specific developmental pattern. Each eye is made up of approximately 800 ommatidia when undergoing normal development. The eye develops as a morphogenetic furrow which then migrates from the posterior imaginal disc to the anterior. The formation and differentiation of these cells then occur behind the furrow (Baker, 2001). If this process is disrupted, characteristic phenotypes are produced. These phenotypes are presented in many ways such as changes in ommatidia number and bristle number. Biometric analysis was done to determine the phenotypic changes in the eye to determine the effects of altering gene expression such as the overexpression of *CG15436*.

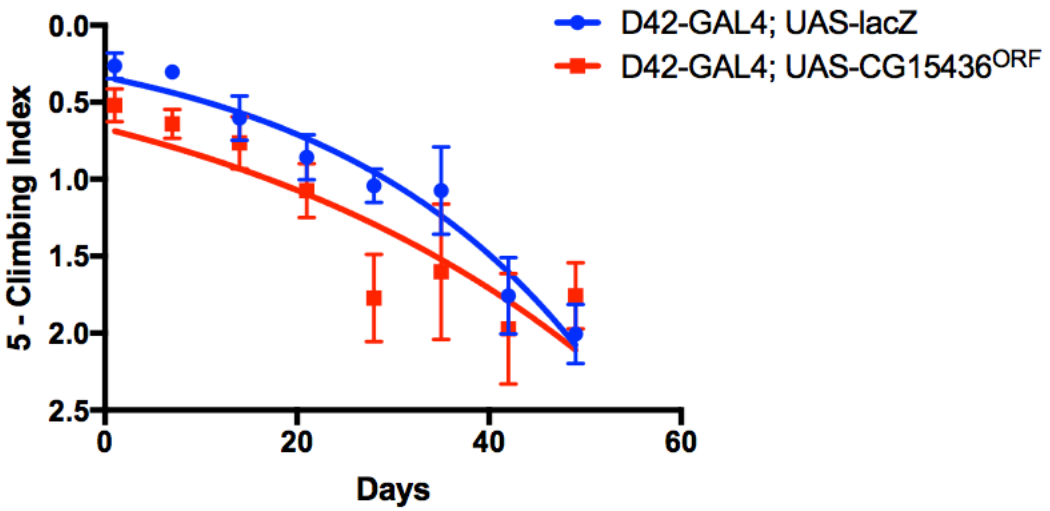


Figure 18: Directed motorneuron specific overexpression of *CG15436* causes a significant decrease in climbing ability over time as flies age. Overexpression of CG15436 shows a significant decrease in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 12: Statistical analysis using a non-linear regression curve of locomotor ability with directed motorneuron-specific overexpression of *CG15436*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
D42; UAS-lacZ	0.03711 ± 0.00453	0.02869 – 0.04677	N/A
D42; UAS-CG15436^{ORF}	0.02336 ± 0.004957	0.01429 – 0.0333	Yes↓

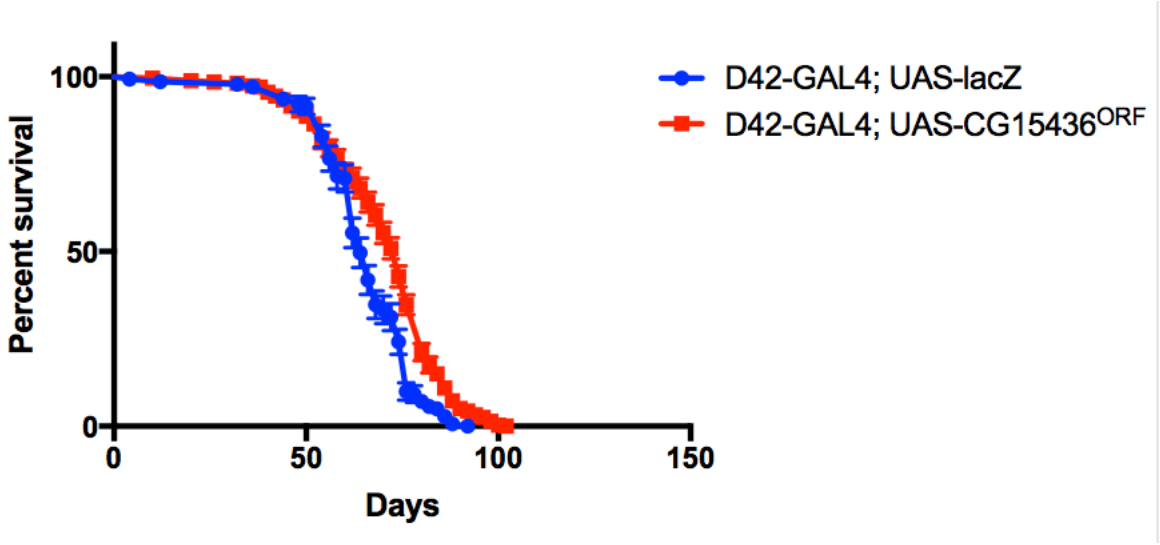


Figure 19: Directed motorneuron specific overexpression of *CG15436* causes a significant increase in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 13: Log-rank statistical analysis of longevity of flies with directed motorneuron specific expression with overexpression of *CG15436*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi – square value	P – value	Significant
D42; UAS-lacZ	141	64	N/A	N/A	N/A
D42; UAS-CG15436^{ORF}	273	74	16.93	<0.0001	Yes ↑

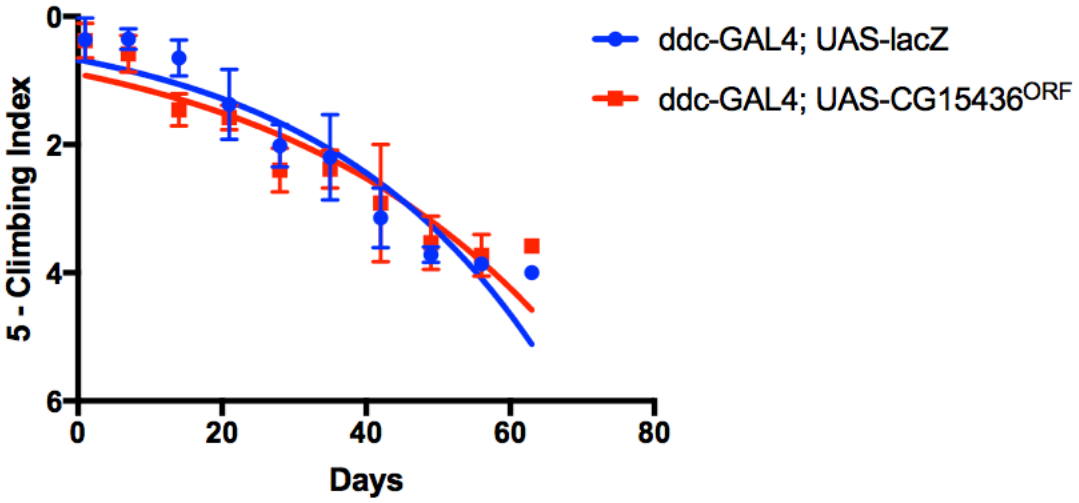


Figure 20: Directed neuron specific expression with overexpression of *CG15436* does not cause a significant decrease in climbing ability over time as flies age. Neuron specific expression of *CG15436* does not show a significant decrease in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 14: Statistical analysis using a non-linear regression curve of locomotor ability of flies with neuron specific expression with overexpression of *CG15436*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
ddc-GAL4; UAS-lacZ	0.03219 ± 0.002762	0.02721 – 0.03744	N/A
ddc-GAL4; UAS-CG15436^{ORF}	0.02589 ± 0.002337	0.02156 – 0.0304	No

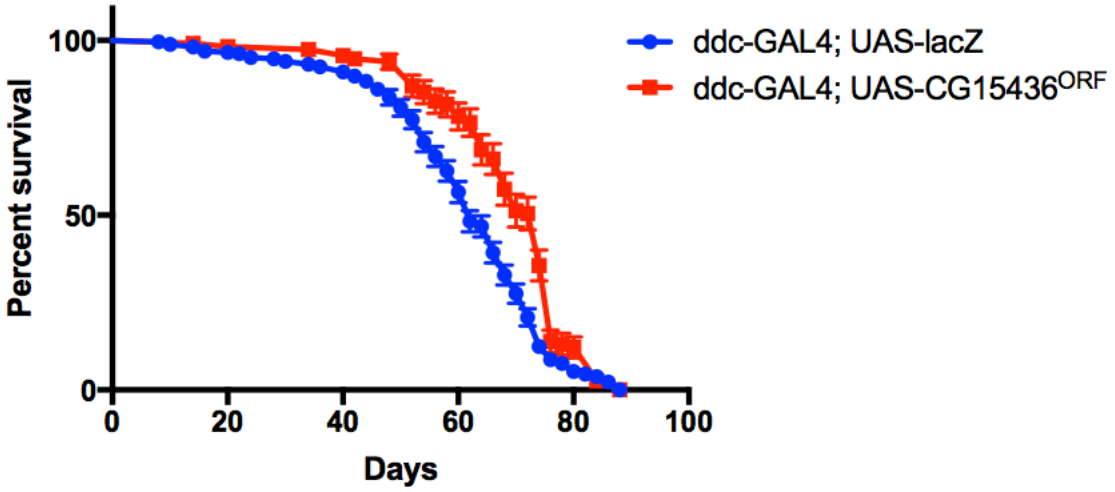


Figure 21: Directed neuron specific expression with overexpression of *CG15436* causes a significant increase in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 15: Log-rank statistical analysis of longevity of flies with neuron specific expression with overexpression of *CG15436*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi – square value	P – value	Significant
ddc-GAL4; UAS-lacZ	265	62	N/A	N/A	N/A
ddc-GAL4; UAS-CG15436^{ORF}	115	74	21.47	<0.0001	Yes↑

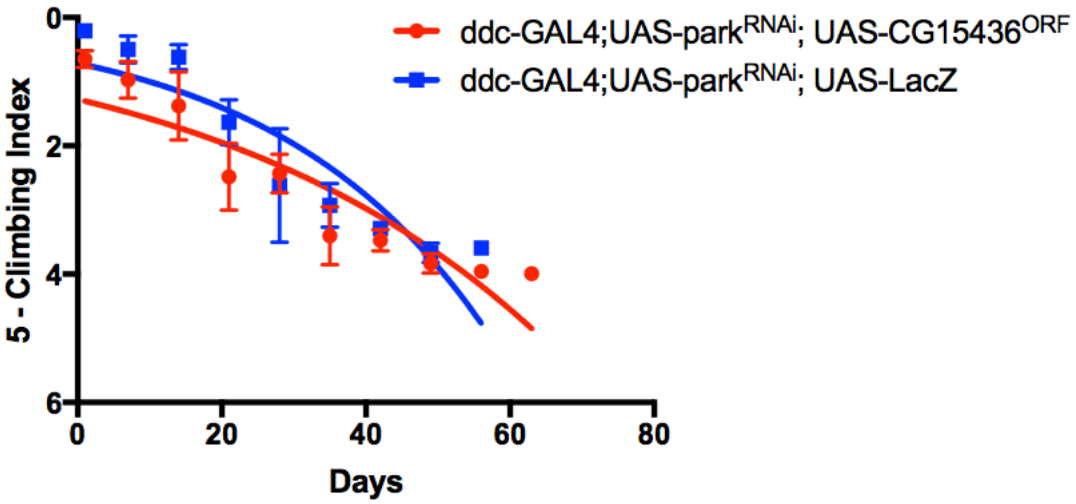


Figure 22: Directed neuron specific expression with knockdown of *parkin* and overexpression of *CG15436* causes a significant decrease in climbing ability over time as flies age. Neuron specific expression of *CG15436* does not show a significant decrease in climbing ability. Data was analyzed by a non-linear curve fit with 95% confidence intervals to determine significance. Error bars represent standard error.

Table 16: Statistical analysis using a non-linear regression curve of locomotor ability of flies with neuron specific expression with knockdown of *parkin* and overexpression of *CG15436*.

Genotype	Slope ± SE	95% Confidence Intervals	Significant
ddc-GAL4;UAS-park^{RNAi}; UAS-lacZ	0.03393 ± 0.003998	0.02683 – 0.04153	N/A
ddc-GAL4;UAS-park^{RNAi}; UAS-CG15436^{ORF}	0.0212 ± 0.002015	0.01749 – 0.02504	Yes↓

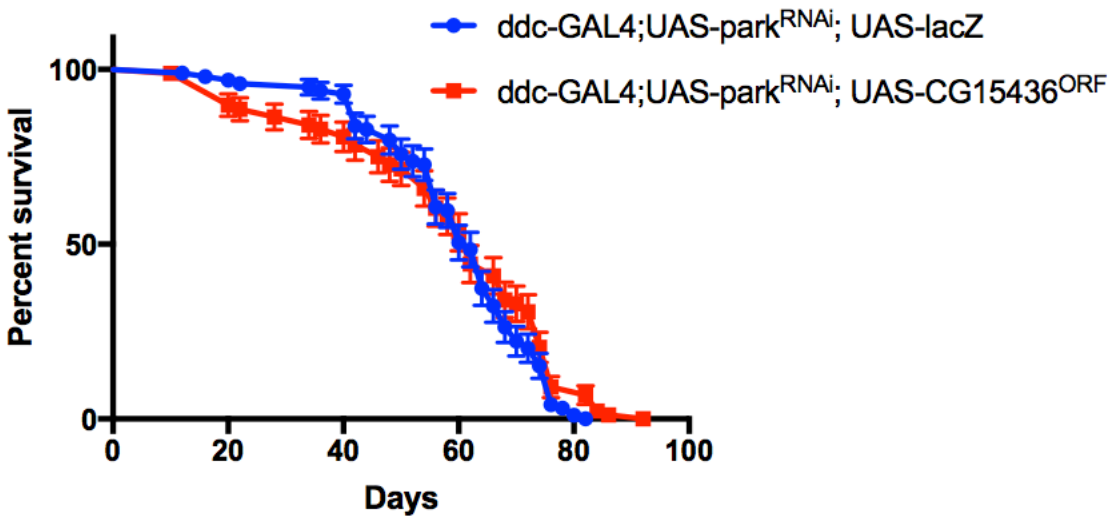


Figure 23: Directed neuron specific expression with knockdown of *parkin* and overexpression of *CG15436* does not cause a significant change in longevity. Longevity is depicted by percent survival. Significance is $p < 0.05$ using the log-rank test. Error bars represent standard error.

Table 17: Log-rank statistical analysis of longevity of flies with neuron specific expression with knockdown of *parkin* and overexpression of *CG15436*. Chi-square values and p-values were calculated using *lacZ*-expressing controls.

Genotype	Number of flies	Median Survival (days)	Chi – square value	P – value	Significant
ddc-GAL4; UAS-park^{RNAi}; UAS-lacZ	99	62	N/A	N/A	N/A
ddc-GAL4; UAS-park^{RNAi}; UAS-CG15436^{ORF}	88	62	1.837	0.1753	No

GMR-GAL4 is a GAL4 transgene used to determine the effects of the overexpression of *CG15436* in the compound eye. A recombinant driver, *GMR-GAL4; UAS-park*^{RNAi} was used which consisted of *GMR-GAL4* with the knockdown of *parkin*. Biometric analysis of the scanning electron micrographs show that there is a significant decrease in ommatidia number and bristle number when *CG15436* is overexpressed with the driver *GMR-GAL4; UAS-park*^{RNAi} (Figure 24 and 25). When the overexpression of *CG15436* is driven by *GMR-GAL4; UAS-park*^{RNAi} the average number of ommatidia per eye was shown to be 669.7 ± 6.549. This is compared to the control *lacZ* where the average number of ommatidia per eye is 734.2 ± 15.44. The

overexpression of *CG15436* with the driver *GMR-GAL4; UAS-park*^{RNAi} results in a significant decrease in bristle number. The average bristle number for *CG15436* is 518.6 ± 9.597. The control *lacZ* had a significantly higher number of bristles with an average of 573.3 ± 10.99 (Table 18).

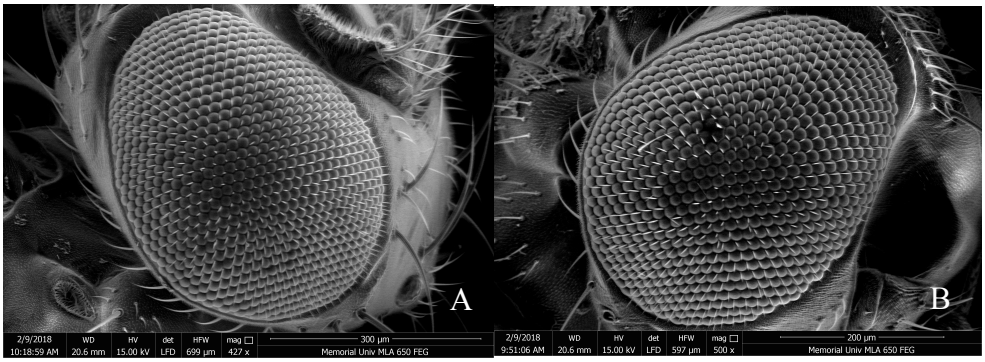
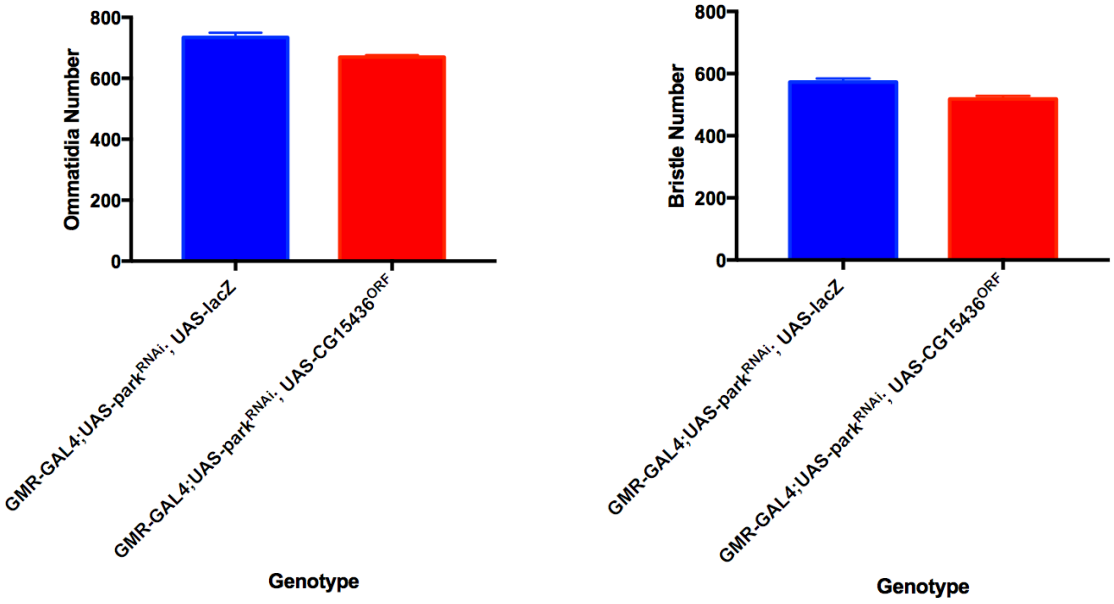


Figure 24: Overexpression of *CG15436* under the control of eye specific drivers with knockdown of *parkin* influence ommatidia and bristle number. Scanning electron micrographs of A: *GMR-GAL4;UAS-park^{RNAi}; UAS-lacZ, B: GMR-GAL4;UAS-park^{RNAi}; UAS-CG15436^{ORF}. GMR-GAL4;UAS-park^{RNAi}* is an eye specific driver with an knockdown of *parkin*.



A B

Figure 25: Biometric analysis of the compound eye under the influence of eye specific expression with the overexpression of *CG15436* with a recombinant driver with the knockdown of *parkin*. Overexpression of *CG15436* with knockdown of *parkin* in a driver line in the eye causes a significant decrease ommatidia number (A) and bristle number (B). Significance is <0.05. Error bars represent standard error of the mean. *UAS-lacZ* crosses are the comparison controls.

Table 18: Summary of ommatidia number and bristle number when *CG15436* is overexpressed and *parkin* is knocked down in the compound eye.

Genotype	Sample Size (n)	Mean ± SEM	P-value compared to control	Significant
Ommatidia Number				
GMR-GAL4;UAS-park^{RNAi}; UAS-lacZ	10	734.2 ± 15.44	N/A	N/A
GMR-GAL4;UAS-park^{RNAi}; UAS-CG15436^{ORF}	10	669.7 ± 6.549	0.0012	Yes ↓
Bristle Number				
GMR-GAL4;UAS-park^{RNAi}; UAS-lacZ	10	573.3 ± 10.99	N/A	N/A
GMR-GAL4;UAS-park^{RNAi}; UAS-CG15436^{ORF}	10	518.6 ± 9.597	0.0015	Yes ↓

Discussion

Parkinson disease (PD) is a neurodegenerative movement disorder that affects 1 to 2% of the human population over the age of 65. This makes PD one of the most prevalent diseases in our world today (Weintraub et al., 2008). Characteristics of this disease include resting tremor, rigidity, bradykinesia and postural instability (Trinh et al., 2014). Mitochondrial autophagy (mitophagy) is important in metabolism as it is involved in adjusting mitochondrial mass and removing mitochondria during differentiation processes. The failure of mitochondrial surveillance supported by autophagy is linked to PD (Franz et al., 2015). *Paris* is a gene of interest in PD research due to its involvement in a pathway with *parkin* and *PCG-1α,* inactivation of both *parkin* and *PCG-1α* have been associated with PD. *Paris* is required for the loss of dopaminergic neurons in adult conditional parkin knockout mice (Stevens et al., 2015). A potential *Paris* homologue in *Drosophila* has not been widely studied in research. Potential *Paris* homologues have been investigated (Merzetti and Staveley, 2016) and *CG15436*, my gene of interest, was identified as the most likely *Paris* homologue in *D. melanogaster.* We must further study the role of *Paris* and its interactions in these pathways in order to open up new opportunities for causes and treatments of this disease. This study explored various aspects of the potential *D. melanogaster* homologue of *Paris*. *CG15436* was ectopically expressed as well as knocked down in *D. melanogaster* to determine its effects on cell death, cell growth, longevity and locomotor ability.

Drosophila CG15436* is conserved across mammalian *Paris

Bioinformatic analysis was conducted to determine the similarity of *Paris* homologues across vertebrates and invertebrates. Through this bioinformatics analysis, it is suggested that

the *Drosophila melanogaster CG15436* and *Homo sapiens Paris* are functional homologues. CG15436 in *Drosophila* and the zinc finger protein 746 (Paris) in humans share structural features which include the zinc finger associated domain and the C2H2 type domain. However, the placement of these domains in the proteins are different from one another as is the size of the proteins as a whole. CG15436 in *Drosophila* is a much smaller protein in comparison to the zinc finger protein 746 in humans. The KRAB box that was identified in *H. sapiens* and *M. musculus* was not identified in *Drosophila.* This KRAB box is a highly conserved motif that is found in over one third of all mammalian zinc-finger transcription factors. CG15436 has previously been characterized as encoding zinc-finger-containing proteins (Merzetti and Staveley, 2017). With the presence of conserved domains in *H. sapiens* Paris and *D. melanogaster* CG15436, there is evidence of homology and, as a result, further evidence of similar function in the two species. The phylogenetic tree constructed with Clustal Omega (Figure 4) gives some insight into the homology of the gene across species. The distance values are the smallest between *H. sapiens* and *M. musculus*. Comparisons with *D. melanogaster* show the largest numbers, which indicates a larger amount of genetic change across more distantly related species. The numbers are produced as the output of the multiple sequence alignment and represent the "length" of the branches. This is an indication of the evolutionary distance between the sequences.

Different families of zinc finger proteins are expanded in different eukaryotic lineages. These expansions include the KRAB family in mammals and the ZAD family in dipterian insects. There is clustering at specific chromosome locations and lineage specific enrichment in both these families of zinc finger proteins (Krystel and Ayyanathan, 2012). Zinc-finger proteins containing the KRAB are the largest single family of transcriptional regulators in mammals. These proteins contain a DNA-binding domain and a KRAB domain (Urrutia, 2003). Zinc finger

proteins are more expanded in higher eukaryotic species. The evolutionary expansion that occurred in humans included zinc-finger proteins that contain evolutionarily conserved SCAN or KRAB domains. These domains are restricted to vertebrate species (Chung et al., 2002). Due to this divergence of proteins which led to different domains in vertebrates compared to invertebrates, one part of the alignment shows a conservation among proteins in *Drosophila melanogaster* and *Homo sapiens*. The bioinformatics analysis suggests that the *Drosophila melanogaster CG15436* and *Homo sapiens Paris* are functional homologues.

Effect of *CG15436* knockdown in *Drosophila*

The *D. melanogaster* eye is made up of a repetitive pattern of ommatidia. The differentiation of the specialized cells that become the photoreceptors starts in the eye imaginal disc with clusters of differentiating neurons. Usually, the fully formed adult *D. melanogaster* eye has in the range of 750 to 800 ommatidia. There are 8 photoreceptors which are photosensitive neurons inside each ommatidia. This amounts to a total of over 6000 neurons in each *D. melanogaster* eye (Frankfort and Mardon, 2002). Neurodegeneration can be measured using the eye structure due to its close association with neurons (Marsh et al., 2003). This highly regulated pattern of the eye allows any defect, big or small, to be detected during the process of neural development in ommatidia and bristle number.

My experiments demonstrate that in *Drosophila melanogaster*, knockdown of *CG15436* and *spargel* directly in the eye through eye-specific expression as well as in concert with the knockdown of *parkin* results in a decrease in both ommatidia number and bristle number. The decrease in ommatidia number and bristle number is slight but significant, as it is demonstrated through biometric analysis. This decrease can be due to an increase in apoptosis or a decrease in

cell growth and survival signalling that is required for normal and successful eye development. In previous studies, *Paris* has been shown to be required for the loss of dopaminergic neurons in *parkin* knockout mice (Stevens et al., 2015). Therefore, when both *parkin* and *Paris* are knocked down, there may be loss of regulation of the survival of these neurons and neurodegeneration would occur. This would be present in the phenotype of the eye through these experiments, in terms of bristle and ommatidia number. *Paris* is a regulator of *PGC-1α* and therefore with the knockdown of *Paris*, *PGC-1α* (*spargel* in *D. melanogaster*) will not be regulated in its pathway. *PGC-1α* is responsible for mitochondrial function and defects in mitochondria is a characteristic of Parkinson disease (Stevens et al., 2015). With the knockdown of *CG15436, spargel* may not be regulated and mitochondrial defects could occur and with the knockdown of *spargel* itself. Therefore, a potential increase in apoptosis may be the reason for the decrease in ommatidia and bristle number when *CG15436* and *spargel* are knocked down.

Longevity assays were conducted to determine the effects of the knockdown of *CG15436* and *spargel*. Varied results were obtained in the experiments. However, the pathways and functions of these genes are indicative of the results found in this study.

When there is a directed motorneuron specific expression (*D42-GAL4*) and directed neuron specific expression with the knockdown of *parkin* in the driver line, there is a significant decrease in the longevity of flies that are knocked down by *CG15436* or *spargel*. When there is directed dopaminergic neuron specific expression (*TH-GAL4*), there is no significant difference in the longevity of flies with the knockdown of *CG15436* although there is a decrease in the longevity of flies with an knockdown of *spargel*. For the directed neuron specific expression (*ddc-GAL4*) there is an increase in the longevity of flies that have knockdown of *CG15436* and a decrease in the longevity of flies that have an knockdown of *spargel*.

Similar to our results, previous research conducted by Merzetti and Staveley (2016) has shown similar results for the increase in lifespan of *CG15436*^{RNAi} when crossed to the directed neuron specific driver *ddc-GAL4*. The knockdown *of CG15436* should result in an increase in mitochondrial biogenesis in its pathway with *spargel.*

I have significantly extended the investigation into *CG15436* through use of 2 other drivers *TH-GAL4* and *D42-GAL4* and furthermore, though use of a complex line, *ddc-GAL4;UAS-park*^{RNAi}*,* a model of Parkinson Disease. Using the *D42-GAL4* driver, there is motorneuron expression. The decrease in lifespan is not expected as this would mean there would be a decrease in mitochondrial biogenesis when *CG15436* is knocked down in the pathway. The complex line which showed a decrease in the lifespan of flies when *CG15436* was knocked down was *ddc-GAL4;UAS-park*^{RNAi}. This line has neuron specific expression with an knockdown of *parkin*. It has been shown in the pathway that a decrease in *parkin* leads to an increase in *Paris* and then a decrease in *PGC-1α* (*spargel* in *D. melanogaster*). With an knockdown of *parkin* as well as *CG15436* (the potential *Paris* homologue), there would be no regulation of *spargel* in the pathway. This may have detrimental effects and therefore cause the decrease in lifespan in the flies with the knockdown of both of these genes. The inactivation of *parkin* has been shown to contribute to the pathogenesis of Parkinson disease. *PGC-1α* is a transcriptional repressor that is involved in mitochondrial function (Stevens et al., 2015). It controls the transcription of many genes that are involved in cellular metabolism, mitochondrial biogenesis and mitochondrial respiration (Shin et al., 2011). When *PGC-1α* is repressed by the accumulation of *Paris* it is likely a hindrance of the production of mitochondrial proteins (Stevens et al., 2015).

The decrease in lifespan of the flies when *spargel* was knocked down was a constant result across all drivers used in the longevity experiments. This can be explained by the role of *spargel* in the function of mitochondria and cellular mechanisms. The knockdown of *spargel* results in the lack of functional mitochondria. Mitochondrial defects are a characteristic of PD and therefore explain the shorter lifespan of these flies when compared to the lacZ-expressing controls (Stevens et al., 2015). The pathways and functions of these genes are therefore indicative of the results found in this study.

Due to the characteristics of PD that include resting tremor and rigidity, climbing analyses were conducted to determine the effects genes have on locomotor ability of *Drosophila*. When using a motorneuron specific driver (*D42-GAL4*), there was a very significant decrease in the climbing ability of flies with an knockdown of *CG15436*, compared to the *lacZ*-expressing control. In contrast to my results, a study using a c-Abl inhibitor which reduces c-Abl activation and therefore reduces the levels of Paris and represses the expression of *PGC-1α* show that these cause an improvement in motor and cognitive functions in PD patients. C-Abl is an Abelson non-receptor tyrosine kinase and is involved in neurodegerative diseases such as PD. The activation of c-Abl is increased in PD (Zhou et al., 2017). Directed dopaminergic neuron specific expression (*TH-GAL4*), directed neuron specific expression (*ddc-GAL4*) and directed neuron specific expression with an knockdown of *parkin* in the complex line (*ddc-GAL4;UAS-park*^{RNAi}) showed no significant decrease in climbing ability in flies with an knockdown of *CG15436*. For the flies with an knockdown of *spargel*, there was a significant decrease in the climbing ability of the flies with directed neuron specific expression (*ddc-GAL4).* For motorneuron specific expression, dopaminergic neuron expression and directed neuron specific expression with an knockdown of *parki*n, there was no significant change in the climbing ability of the flies. When

there is an accumulation of *Paris*, it becomes a pathogenic substrate. This accumulation occurs in patients with PD. When this accumulation occurs, *PGC-1α* is repressed (Shin et al., 2011). This should produce similar effects to having an knockdown of *spargel* in flies. *PGC-1α* is a transcriptional coactivator and is involved in the transcription of genes involved in metabolic processes such as mitochondrial biogenesis (Shin et al., 2011). If this coactivator is repressed, then these processes may not be carried out properly. This can be detrimental to cells and therefore affect many other developmental processes such as cognitive and motor ability.

Effect of *CG15436* overexpression in *Drosophila*

Overexpression of *CG15436* under the control of the eye specific driver, *GMR-GAL4*, with an knockdown of *parkin* in the driver causes a significant decrease in the number of ommatidia and bristles in the eye of *Drosophila melanogaster.* The slight decrease in the numbers of ommatidia and bristles has been determined through biometric analysis. This decrease may be due to influence of the mechanism that includes *parkin* and *PGC-1α.* Parkin is an ubiquitin E3 ligase that is associated with autosomal recessive PD as well as sporadic PD. When there is a loss of parkin's ubiquitin E3 ligase activity, there is a loss of dopamine neurons which is linked to PD. Paris is shown to be involved in the loss of dopamine neurons. When there is a deletion in parkin, Paris accumulates and a progressive loss of dopamine neurons occurs. Paris overexpression which occurs in adult conditional parkin knockout mice results in defects in PGC-1α. The maintenance of mitochondrial biogenesis is very critical for dopamine neuron survival (Stevens et al., 2015). This knockout of *parkin* and overexpression of *Paris* is replicated with the flies used in this experiment. Flies used have an knockdown of *parkin* and an overexpression of *CG15436*, the potential *Drosophila* homologue of *Paris.* These results suggest

that the loss of *parkin* and the overexpression of *CG15436* impairs mitochondrial biogenesis, which leads to a decrease in mitochondrial function and an increase in cell death. This is characterized by the eye phenotype with decreased number of ommatidia and bristles when compared to the *lacZ* control.

Standard longevity assays were also conducted to determine the effects of overexpression of *CG15436* on the lifespan of *Drosophila.* Overexpression of *CG15436* with motorneuron-specific and neuron-specific overexpression causes a significant increase in lifespan when compared to the *lacZ*-expressing control. This was not an expected result in this experiment. An accumulation of *Paris* makes it an attractive pathogenic substrate. However, it is not certain whether Paris is the only contributing mechanism to the degeneration of dopamine neurons (Shin et al., 2011). Therefore, if there are other contributing factors to this neurodegeneration, solely overexpressing Paris may not cause the expected decrease in lifespan. There may be a sort of counterbalancing effect occurring with the other parts of the pathway including *parkin* and *spargel* (Shin et al., 2011). More research into the potential of this counterbalancing effect must be done to fully understand the expression of this gene.

Due to the characteristics of PD such as rigidity and resting tremor, locomotor analysis was conducted to determine the climbing ability of *Drosophila* over time. Consistent with these characteristics of PD and the study of *Paris*' involvement in PD genetic pathways, there is a significant decrease in climbing ability of flies with an overexpression of *CG15436* when crossed with the driver line *D42-GAL4* and the complex line *ddc-GAL4;UAS-park*^{RNAi}. There is a progression of neuronal degradation and Lewy bodies in the cerebral cortex and limbic structures of PD patients (de Lau and Breteler, 2006). As a consequence, PD leads to the loss of the cognitive and locomotor function of those affected. The identification of the new parkin-

interacting substrate provides insights into the molecular mechanisms involved in the neurodegeneration due to its relation to the inactivation of *parkin* in PD patients (Shin et al., 2011). One of the major drivers in the degeneration of dopaminergic neurons and the defects in mitochondrial biogenesis is the *Paris*-mediated downregulation of *PGC-1α* which is due to the absence of *parkin*. This is then a hinderance of mitochondrial protein production. An increase in the levels of *Paris* in PD patients are likely a contributing factor to the pathogenesis of this neurodegenerative disease (Stevens et al., 2015). *CG15436* overexpression in flies producing a decreased climbing ability in motorneuron and neuron specific driver lines makes it a potential homologue for *Paris. CG15436* knockdown produces similar effects in *Drosophila* as what would be expected to happen in PD patients with an overexpression of *Paris*. The climbing ability of the flies is comparable to the motor functions of humans. As well, the neuron specific driver *ddc-GAL4* which was combined with an knockdown line of *parkin* and crossed to *CG15436* further demonstrates the degeneration and defects in mitochondrial biogenesis that occurs when there is an absence of *parkin* and a subsequent accumulation of Paris in PD patients. The decreased climbing ability of the flies shown to be significant in this experiment is relatable to the symptoms of PD in these patients. Flies have phenotypes that are consistent with modelling PD in *Drosophila* through alteration of *Paris*. One of the drivers, *ddc-GAL4*, which has neuron specific expression did not show a significant decrease in the climbing ability of the flies as was shown with the other two drivers. As explained previously, there may be other contributing factors to this neurodegeneration in the *Paris*-associated pathway. This may include a counterbalancing effect with other parts of the pathway including *parkin* and *spargel* (Shin et al., 2011). In addition, *CG15436* may not be overexpressed as strongly with certain drivers such as the neuron specific driver, *ddc-GAL4*, used in this part of the experiment.

Conclusion

With the present study, the foundation has been done for the characterization and identification of the *Drosophila melanogaster* homologue of *Paris.* Although this protein is not highly conserved at the amino acid level due to the evolutionary divergence between vertebrates and invertebrates of these groups of genes, as seen in bioinformatics analyses, there are many factors showing its conservation functionally. This has been shown through the overexpression and knockdown of the gene in biometric, longevity and locomotor analyses. Further analyses should be carried out at the cellular and molecular levels such as microarray and PCR analyses especially due to the involvement of *Paris* in mitochondrial biogenesis and surveillance. As well, further research into the interaction of *Paris* with other genes such as *spargel, parkin* and *PINK1*. This current study has laid the foundation for more studies of the gene *Paris.*

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