Investigating the role of *Hip1* in Huntington Disease and Parkinson Disease Models in *Drosophila melanogaster*

by

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Abstract

Huntington Disease (HD) is a progressive neurodegenerative disorder characterized by the loss of cognition and motor ability. Beginning in the dysfunction of the GABAergic neurons, HD has been mainly attributed to an increased number of CAG repeats in the *Huntingtin* gene, resulting in an extended polyglutamine tract within the protein. This expansion prevents the normal interaction between the proteins encoded by *Htt* and *Huntingtin interacting protein 1* (*Hip1*). Conserved sequences between human and *Drosophila* Hip1 allow examination of the protein in *D. melanogaster* to be used as a model for human HD. Analysis of the loss of function of *Hip1* in a pan-neuronal fashion resulted in an increase in average lifespan and decrease in motor ability. Overexpression of *Hip1* resulted in a decrease in both lifespan and motor ability, suggesting a delicate balance of *Hip1* in healthy cells. Investigation of the biological effects of altered *Hip1* expression may provide insights into a possible role for *Hip1* in HD pathogenesis.

The progression of Parkinson Disease (PD) is similar to that of HD – generally occurring later in life, and affecting cognitive ability and motor function. Associated with the death of dopamingeric neurons, impairment of a number of genes has been implicated in disease progression. Recently, a polymorphism in *Huntingtin interacting protein 1 Related* (*Hip1R*), was identified as a risk-factor for PD. The *Hip1R* gene is not found in *D. melanogaster*, but is considered an orthologue of *Hip1*, which is conserved across species. Directed manipulation of the single *Drosophila Hip1* in the dopaminergic neurons was carried out to provide an *in vivo* model of disease progression and symptom development. Investigation of motor ability and longevity of *D. melanogaster* upon overexpression and loss of function of *Hip1* was completed. Loss of expression of *Hip1*

in the dopaminergic neurons, through the use of RNAi, decreased the locomotor ability of the flies and increased the average lifespan. Overexpression of *Hip1* in the dopaminergic neurons revealed the opposite effect, improving locomotor ability and slightly decreasing average lifespan. This suggests that a delicate balance of *Hip1* exists in the dopaminergic neurons, and alteration of expression affects the motor ability and life expectancy. Maintaining a healthy balance of *Hip1* in the dopaminergic neurons may offer a new therapeutic option for loss of locomotor ability and pre-mature death. Further investigation of *Hip1R* and its role in human disease progression is needed, and may be crucial to our understanding of PD in order to provide new therapeutic targets.

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ANTH - AP180 N-terminal homology Lipid Binding Domain

D. melanogaster – Drosophila melanogaster

Ddc – Dopa Decarboxylase

dsRNAi – double stranded Ribonucleic Acid Interference

elav – embryonic lethal, abnormal vision

GABA - γ-aminobutyric acid

GFP – Green Fluorescent Protein

HD - Huntington Disease

Hip1 – Huntingtin interacting protein 1

Hip1R - Huntingtin interacting protein 1 Related

Hippi - Huntingtin interacting protein 1 protein interactor (Che-13)

Htt – Huntingtin

I/LWEQ – C-terminal talin-like Cytoskeletal binding domain

lacZ - β-galactosidase

LD50 – Lethal Dose 50

PD – Parkinson Disease

polyQ – polyglutamine

RNAi - Ribonucleic Acid Interference

UAS – Upstream Activating Sequence

w^{1118} – $white^{1118}$

Ubx - *Ultrabithorax*

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Co-Authorship Statement

Chapter 2 is a version of a manuscript in preparation for the journal *Genetics and Molecular Research* (Slade, F.A. and Staveley, B.E.). Modelling Huntington Disease though *Huntingtin interacting protein 1* in *Drosophila melanogaster.* Contributions to this chapter were made by F.A Slade and B.E. Staveley. The project was designed and conceived by B.E. Staveley with input from F.A. Slade. F.A. Slade preformed the longevity, locomotor and eye analysis assays, carried out the statistical analysis and drafted the initial manuscript. B.E. Staveley provided contributions and suggestions throughout the study, and provided feedback and edits on the study and provided edits in order to prepare the final draft of the manuscript for publication.

Chapter 3 is a version of a manuscript in preparation for the journal *Genome* (Slade, F.A. and Staveley, B.E.). A Huntington gene in models of Parkinson Disease: Investigating the effects of altered *Hip1* expression in *Drosophila melanogaster*. Contributions to this chapter were made by F.A Slade and B.E. Staveley. The project was designed and conceived by B.E. Staveley with input from F.A. Slade. F.A. Slade preformed the longevity, locomotor and eye analysis assays, carried out the bioinformatics and statistical analysis and drafted the initial manuscript. B.E. Staveley provided contributions and suggestions throughout the study, and provided feedback and edits on the study and provided edits to prepare the final draft of the manuscript for submission.

Chapter 1 - Introduction and Overview

1.1. Neurodegenerative Disease

A neurodegenerative disease is defined as a chronic condition that is caused by the deterioration or degradation of neuronal tissues over time (1). Advances in medical science have increased both the average lifespan and the quality of life. Unfortunately, this leads to a rise in the incidence of diseases that begin later in life including neurodegenerative diseases such as Huntington Disease (HD), Parkinson Disease (PD) and Alzheimer Disease (AD). The progression of neurodegenerative disease was originally believed to depend upon environmental factors (2). Advances in genomic and proteomic technologies, such as microarrays, RNA-Seq and Next-Gen sequencing have revealed a number of new genes, along with novel functions for genes previously thought to be "junk" or non-functional. The discovery of common genetic risk loci between patients with the same neurodegenerative disorder and heritability patterns suggests instead that genetic factors may be the major contributors to disease development and progression (3). Mutations found in the *TMEM230* gene, for example, have recently been implicated in PD pathogenesis (4). This creates a need to better understand the etiology behind these diseases. The genetic background of Huntington Disease and Parkinson Disease has been partially characterized, but many of the molecular mechanisms and cellular pathways are still largely unknown (5). An investigation into the genes involved in these diseases and the role they play in the cell can increase our understanding of disease pathogenesis and potentially provide novel therapeutic targets and treatment options.

1.1.1. Huntington Disease

Huntington Disease is a progressive neurodegenerative disorder that affects between 4 and 8 people in 100,000, displaying its highest prevalence in Europe and North America (6, 7). Discovered by George Huntington in 1872, HD is inherited in an autosomal dominant fashion, and on-set of disease typically occurs in mid-late life, although juvenile forms of HD do exist. Symptoms of HD include impaired cognition, loss of motor ability, dementia, involuntary convulsive movements, severe weight loss, saccadic eye movements and changes in sleeping patterns, with the end result often being premature death (7, 8). Current treatments and therapeutic agents aid in reducing severity of symptoms but do not address the underlying cause of disease.

Beginning in the spiny GABAergic neurons of the striatum and deep layers of the cortex, neuronal death spreads as the disease progresses (6). Upon reaching the hypothalamus and hippocampus, the sufferer's body begins to shut down major organ function, resulting in death (9). HD occurs in patients which exhibit an expanded number of CAG repeats in exon 1 of the *Huntingtin* (*Htt*) gene, found on the short arm of chromosome 4 (10). This results in an increased number of glutamine residues in the Huntingtin protein. Beginning at the 18^{th} amino acid, the polyQ domain is present in healthy individuals, but contains only 11 to 34 glutamine residues. In general, patients with 37 or more glutamine residues are diagnosed with HD (9). The number of CAG repeats present in disease patients is proportional to disease severity and inversely proportional to age of onset (10). Expansion of the Htt protein influences structure and functionality, rendering the protein unable to interact with a number of its normal binding partners (9). While disease symptoms are generally contributed to the mutation of *Htt*, it

is highly probable that many of the symptoms may be the result of the dysfunctional complexes and downstream cellular pathways.

1.1.2. Hip1 in Huntington Disease

Huntingtin is expressed in a wide variety of tissues and thus the normal functions of the protein have been difficult to characterize. The Huntingtin protein has a number of binding partners, including proteins involved in metabolism, cellular signalling and trafficking, endocytosis and transcription (9). Therefore, disruptions in the proteins' normal binding patterns (such as in HD) can alter cellular pathways, and may contribute to the etiology of Huntington Disease.

One binding partner of Htt, Huntingtin interacting protein 1 (Hip1), binds to the region of the protein that contains amino acids 1-540, and exhibits reduced affinity upon polyQ expansion. Normally involved in trafficking, endocytosis, neurogenesis and apoptosis, Hip1 is left in a 'less-bound' state in the cytoplasm when in the presence of expanded Htt. Not only unable to complete its standard cellular functions, when Htt does not bind Hip1 as it normally does, Hip1 can be sequestered by other proteins to form aggregates, which may provide the potential for disease phenotypes (9, 11-14). *Hip1* is highly conserved throughout evolutionary history (14), and so an overexpression of *Hip1* in *D. melanogaster* is expected to mimic aspects of the effects of Hip1 in HD patients by increasing the amount of unbound Hip1 in the cytoplasm, and providing the opportunity for aggregation. Investigating the effects of altered *Hip1* expression in the fruit fly is expected to provide insights into the pathology of HD in humans.

Huntingtin interacting protein 1 protein interactor (Hippi) (also known as Che-13) is a novel protein that as the name suggests, binds to Hip1 in healthy individuals. A positive

correlation was found between *Hip1* and *Hippi* expression in humans (11). One promising model for HD was proposed based on the Hip1-Hippi interaction, suggesting that when the affinity of Hip1 for Htt is lost due to the polyQ expansion, the free Hip1 binds Hippi instead. This complex not only causes the formation of aggregates, it activates pro-caspase-8, presumably initiating apoptosis (11). Therefore, an investigation of altered *Hippi* expression could contribute to the working knowledge of HD pathogenesis.

1.1.3. Parkinson Disease

Parkinson Disease (PD) is one of the most prevalent progressive neurodegenerative disorders known to date, second only to Alzheimer Disease (15). Affecting 1% of the population above the age of 65 and 4 to 5% of the population above 85 years of age, PD is characterized by the loss of cognitive ability and motor function (16, 17). Initially believed to be the result of environmental factors including brain trauma and chemical exposure, recent advances suggest a significant role of genetic causation (3, 18). The sequencing of the human genome opened up new possibilities, and efforts were focused on finding potential risk factors – mutations that may make a patient more susceptible to disease onset in accordance with environmental factors (19). Beginning in the Dopaminergic neurons of the *substantia nigra*, neuronal death spreads as the disease progresses (17). No single cellular dysfunction has been isolated as the cause of PD, but a number of genes have been implicated as having roles in disease pathogenesis. Mutations in *Pink1*, *parkin*, *PGC-1α*, *PARIS* and *α-synuclein* have all been linked to disease progression (5, 20-23). Understanding the cellular components and molecular pathways involved in PD may provide novel treatment options and therapeutic targets.

1.1.4. Hip1R in Parkinson Disease

Recently, a polymorphism in *Huntingtin interacting protein 1 Related* (*Hip1R*) was identified as a risk factor for PD in a genome wide association study (24, 25). Considered an orthologue of *Hip1*, *Hip1R* shares similar domains and structure but is involved in somewhat different cellular functions (26, 27). Although originally believed to form heterodimers, recent evidence suggests that the Hip1 and Hip1R proteins preferentially form homodimers *in vivo,* interacting through the flexible coiled coil domains found in both proteins. The flexibility observed in the coiled coil domain may contribute to the proteins ability to interact with a number of binding partners including Hippi, Htt and the clathrin light chain (27-31). As the implication of Hip1R in PD is still a novel idea, the role of Hip1R in PD is mainly uncharacterized. Conformational changes resulting in Hip1R interacting with novel binding partners provides a good theory on which to base the investigation of the role of Hip1R in the pathogenesis of PD.

1.1.5. *Drosophila melanogaster* as a model of neurodegenerative disease

The common fruit fly, *Drosophila melanogaster* has been used to model many human diseases. Having a short generation time and numerous progeny, culturing and maintaining *D. melanogaster* in the laboratory is relatively simple and is much less expensive than maintaining other organisms (32). The *D. melanogaster* genome was sequenced in the year 2000, and it revealed that about 75% of disease related genes in humans have Drosophila counterparts (32). Although simpler than the human nervous system, *D. melanogaster* boasts a compartmentalized nervous system that can be genetically manipulated with relative ease (33-35). Forward and reverse genetic screens can be used to identify new genes and their functions, while the *UAS-Gal4* bipartite

ectopic expression system allows different genes to be over expressed or knocked down in a variety of tissues (33). Based on the *Gal4* transcription factor found in yeast (*Saccharomyces cerevisiae*), Gal4 binds to the *Upstream activating sequence (UAS),* driving gene expression. Transgenic organisms formed by insertion of the *UAS* upstream of the target gene and random insertion of *Gal4* in opposite parental lines can then be mated, providing accurate and precise control of gene expression (35) (Figure 1.1). Combined with the use of RNA interference (RNAi), a sequence-specific gene silencing mechanism (36), the *UAS-Gal4* system offers the tools to easily manipulate the Drosophila genome, providing a platform on which to base the study of human disease. Bioinformatic analysis revealed that the closest *D. melanogaster* homologue for both human *Hip1* and *Hip1R* is Drosophila *Hip1* (Figure 1.2). As the two proteins perform different functions but display evolutionary similarity, it is possible that alternative splicing of *Hip1* in *D. melanogaster* could produce alternate versions of the proteins that perform different functions. Alternative first exons produce Hip1 and HipΔANTH, and thus it is possible that other isoforms of the protein exists (14). While no direct evidence for this model exists for *Hip1*, other genes, such as the Drosophila *Hox* gene *Ultrabithorax* (*Ubx*) are known to generate different isoforms that perform different functions through alternative splicing (37-40). Thus, an investigation of altered *Hip1* expression on *D. melanogaster* lifespan and locomotor ability may provide insight into both Huntington Disease and Parkinson Disease.

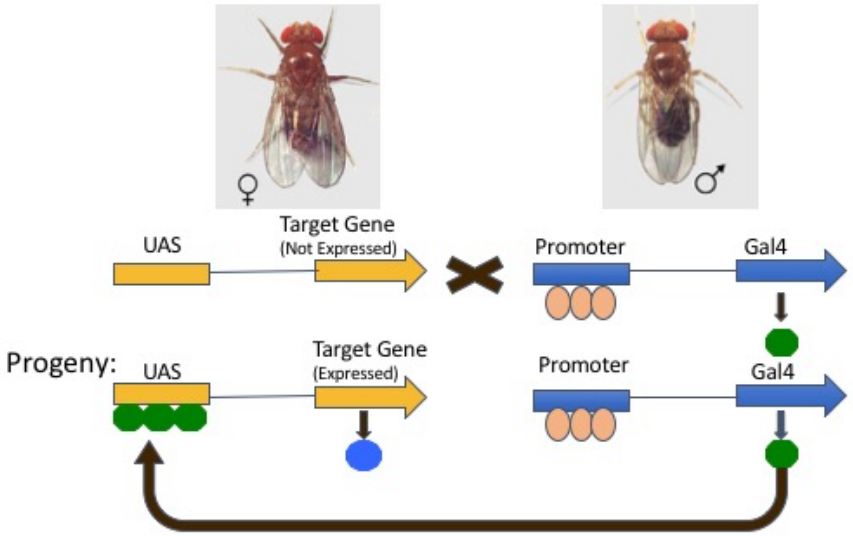


Figure 1.1 - The *UAS-Gal4* expression system in *D. melanogaster*. Maternal *UAS* lines crossed to paternal *Gal4* lines produce progeny in which expression of the target gene occurs through binding of the Gal4 protein to the *UAS* sequence. Parental lines carry only *UAS* or *Gal4*, and, therefore, are viable, fertile and healthy and can be maintained through the standard procedures of Drosophila culture. The use of RNAi transgenes provides the ability to examine loss of function.

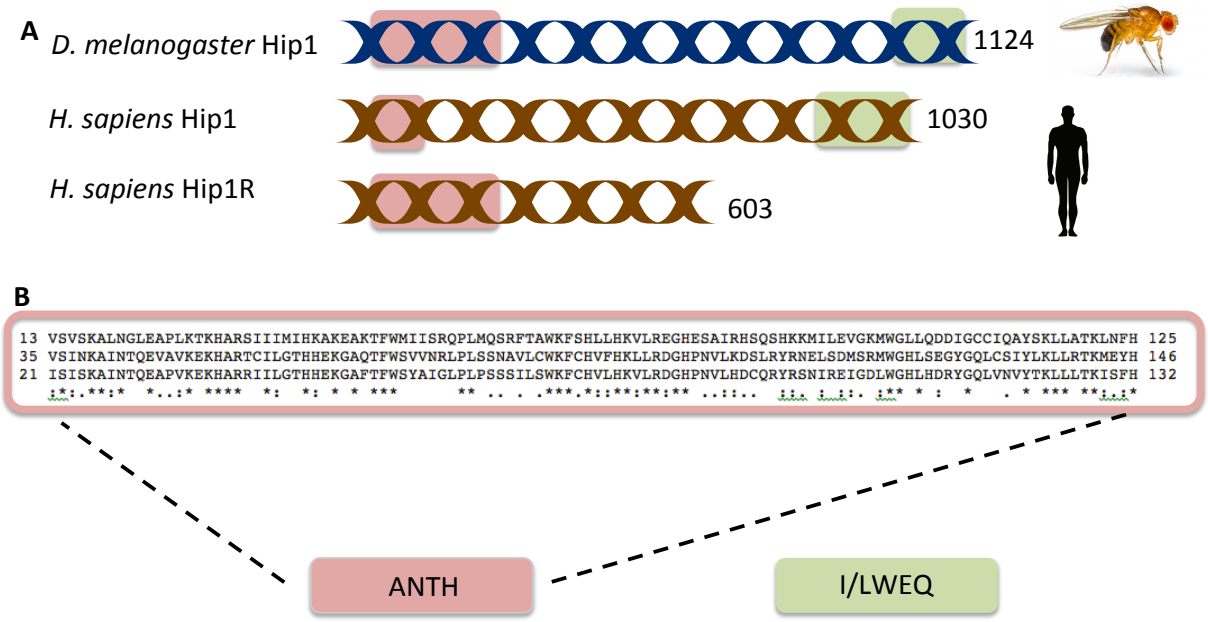


Figure 1.2 - Hip1 and Hip1R share similar domains across species. Conservation of the ANTH and talin-like (I/LWEQ) domains of *D. melanogaster* and human Hip1 suggest similar functions for the protein throughout evolutionary history. The ANTH domain is also conserved in human Hip1R, and the protein itself shows a high degree of conservation, suggesting that *Hip1R* is paralogous to *Hip1*.

A. Visual depiction of proteins.

B. Partial alignment of the ANTH domain from *D. melanogaster* Hip1, Human Hip1 and Human Hip1R in that order. An asterisk (*) indicates amino acids that are fully conserved, A colon (:) indicates conservation between groups of strongly similar properties (>0.5 in the Gonnet PAM 250 matrix), A period (.) indicates conservation between weakly similar properties (<0.5 in the Gonnet PAM 250 matrix).

Refer to appendix A for supplemental data.

1.2. Research goals

While *Hip1* and *Hip1R* have been suggested as candidate genes in the development of neurodegenerative disease, their role in disease progression and onset has not been fully characterized. This project aims to examine the effects of overexpression and inhibition of *Hip1* and *Hippi* in a pan-neuronal fashion and specifically in the dopaminergic neurons of *D. melanogaster*. Examining the effects of altered gene expression on *D. melanogaster* lifespan and motor ability may establish a role for *Hip1* and *Hip1R* in the pathogenesis of HD and PD respectively and suggest that *Hip1* and *Hip1R* dysfunction contribute to disease phenotypes. It also aims to expand upon the working knowledge of the Hippi protein, and its role in disease pathogenesis.

1.3 References

1. Merzetti EM, Staveley BE. Mitochondrial dynamics in degenerative disease and disease models. Neuroscience Discovery. 2013;1(1). doi: 10.7243/2052-6946-1-8.
2. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Frontiers in cellular neuroscience. 2015;9:124. Epub 2015/04/29. doi: 10.3389/fncel.2015.00124. PubMed PMID: 25914621; PubMed Central PMCID: PMCPMC4392704.
3. Bereznai B, Molnar MJ. [Genetics and present therapy options in Parkinson's disease: a review]. Ideggyogyaszati szemle. 2009;62(5-6):155-63. Epub 2009/07/08. PubMed PMID: 19579663.
4. Deng H-X, Shi Y, Yang Y, Ahmeti KB, Miller N, Huang C, et al. Identification of TMEM230 mutations in familial Parkinson's disease. Nature genetics. 2016;48(7):733-9. doi: 10.1038/ng.3589 http://www.nature.com/ng/journal/v48/n7/abs/ng.3589.html - supplementary-information.
5. Kitada T, Asakawa S, Matsumine H, Hattori N, Shimura H, Minoshima S, et al. Progress in the clinical and molecular genetics of familial parkinsonism. Neurogenetics. 2000;2(4):207-18. PubMed PMID: 10983716.
6. Petersén Å, Mani K, Brundin P. Recent Advances on the Pathogenesis of Huntington's Disease. Experimental Neurology. 1999;157(1):1-18. doi: http://dx.doi.org/10.1006/exnr.1998.7006.
7. Walling HW, Baldassare JJ, Westfall TC. Molecular aspects of Huntington's disease. Journal of Neuroscience Research. 1998;54(3):301-8. doi: 10.1002/(SICI)1097-4547(19981101)54:3<301::AID-JNR1>3.0.CO;2-W.
8. O'Keeffe GC, Michell AW, Barker RA. Biomarkers in Huntington's and Parkinson's Disease. Annals of the New York Academy of Sciences. 2009;1180(1):97-110. doi: 10.1111/j.1749-6632.2009.04943.x.
9. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics. 2004;20(3):146-54. doi: http://dx.doi.org/10.1016/j.tig.2004.01.008.
10. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971-83. doi: http://dx.doi.org/10.1016/0092-8674(93)90585-E.
11. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst C-A, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4(2):95-105.
12. Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, et al. Huntingtin Interacting Protein 1 Is a Clathrin Coat Binding Protein Required for Differentiation of late Spermatogenic Progenitors. Molecular and Cellular Biology. 2001;21(22):7796-806. doi: 10.1128/MCB.21.22.7796-7806.2001. PubMed PMID: PMC99949.
13. Rao DS, Hyun TS, Kumar PD, Mizukami IF, Rubin MA, Lucas PC, et al. Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is

critical for cellular survival. The Journal of Clinical Investigation. 2002;110(3):351-60. doi: 10.1172/JCI15529. PubMed PMID: PMC151092.
14. Moores JN, Roy S, Nicholson DW, Staveley BE. Huntingtin interacting protein 1 can regulate neurogenesis in Drosophila. The European journal of neuroscience. 2008;28(3):599-609. Epub 2008/08/16. doi: 10.1111/j.1460-9568.2008.06359.x. PubMed PMID: 18702731.
15. Schiesling C, Kieper N, Seidel K, Kruger R. Review: Familial Parkinson's disease--genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol Appl Neurobiol. 2008;34(3):255-71. doi: 10.1111/j.1365-2990.2008.00952.x. PubMed PMID: 18447897.
16. Trinh J, Gustavsson EK, Guella I, Vilarino-Guell C, Evans D, Encarnacion M, et al. The role of SNCA and MAPT in Parkinson disease and LRRK2 parkinsonism in the Tunisian Arab-Berber population. Eur J Neurol. 2014;21(11):e91-2. doi: 10.1111/ene.12489. PubMed PMID: 25303626.
17. de Lau LM, Giesbergen PC, de Rijk MC, Hofman A, Koudstaal PJ, Breteler MM. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. Neurology. 2004;63(7):1240-4. PubMed PMID: 15477545.
18. Vanitallie TB. Parkinson disease: primacy of age as a risk factor for mitochondrial dysfunction. Metabolism: clinical and experimental. 2008;57 Suppl 2:S50-5. Epub 2008/09/23. doi: 10.1016/j.metabol.2008.07.015. PubMed PMID: 18803967.
19. Labbé C, Ross OA. Association Studies of Sporadic Parkinson’s Disease in the Genomic Era. Current Genomics. 2014;15(1):2-10. doi: 10.2174/1389202914666131210212745. PubMed PMID: 24653658; PubMed Central PMCID: PMCPMC3958956.
20. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. Genome / National Research Council Canada = Genome / Conseil national de recherches Canada. 2008;51(12):1040-6. Epub 2008/12/18. doi: 10.1139/g08-085. PubMed PMID: 19088817.
21. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. Science (New York, NY). 1997;276(5321):2045-7. doi: 10.1126/science.276.5321.2045.
22. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998;395(6701):451-2.
23. Castillo-Quan JI. Parkin’ control: regulation of PGC-1α through PARIS in Parkinson’s disease. Disease Models & Mechanisms. 2011;4(4):427-9. doi: 10.1242/dmm.008227. PubMed PMID: PMC3124045.
24. Sharma M, Ioannidis JP, Aasly JO, Annesi G, Brice A, Van Broeckhoven C, et al. Large-scale replication and heterogeneity in Parkinson disease genetic loci. Neurology. 2012;79(7):659-67. Epub 2012/07/13. doi: 10.1212/WNL.0b013e318264e353. PubMed PMID: 22786590; PubMed Central PMCID: PMCPMC3414661.
25. Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS genetics. 2012;8(3):e1002548. Epub 2012/03/23. doi: 10.1371/journal.pgen.1002548. PubMed PMID: 22438815; PubMed Central PMCID: PMCPMC3305333.

26. Bradley SV, Hyun TS, Oravecz-Wilson KI, Li L, Waldorff EI, Ermilov AN, et al. Degenerative phenotypes caused by the combined deficiency of murine HIP1 and HIP1r are rescued by human HIP1. Hum Mol Genet. 2007;16(11):1279-92. doi: 10.1093/hmg/ddm076. PubMed PMID: 17452370.
27. Gottfried I, Ehrlich M, Ashery U. The Sla2p/HIP1/HIP1R family: similar structure, similar function in endocytosis? Biochem Soc Trans. 2010;38(Pt 1):187-91. doi: 10.1042/BST0380187. PubMed PMID: 20074057.
28. Niu Q, Ybe JA. Crystal Structure at 2.8 Å of Huntingtin-Interacting Protein 1 (HIP1) Coiled-Coil Domain Reveals a Charged Surface Suitable for HIP1 Protein Interactor (HIPPI). Journal of Molecular Biology. 2008;375(5):1197-205. doi: https://doi.org/10.1016/j.jmb.2007.11.036.
29. Engqvist-Goldstein ÅEY, Warren RA, Kessels MM, Keen JH, Heuser J, Drubin DG. The actin-binding protein Hip1R associates with clathrin during early stages of endocytosis and promotes clathrin assembly in vitro. The Journal of Cell Biology. 2001;154(6):1209-24. doi: 10.1083/jcb.200106089.
30. Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. ENTH/ANTH proteins and clathrin-mediated membrane budding. Journal of cell science. 2004;117(Pt 1):9-18. Epub 2003/12/06. doi: 10.1242/jcs.00928. PubMed PMID: 14657269.
31. Wilbur JD, Chen C-Y, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM. Actin binding by huntingtin-interacting protein 1 (hip1) and hip1-related protein (Hip1R) is regulated by clathrin light chain. Journal of Biological Chemistry. 2008. doi: 10.1074/jbc.M802863200.
32. Marsh JL, Pallos J, Thompson LM. Fly models of Huntington's disease. Hum Mol Genet. 2003;12 Spec No 2:R187-93. Epub 2003/08/20. doi: 10.1093/hmg/ddg271. PubMed PMID: 12925571.
33. Celotto AM, Palladino MJ. Drosophila: a "model" model system to study neurodegeneration. Molecular interventions. 2005;5(5):292-303. Epub 2005/10/27. doi: 10.1124/mi.5.5.9. PubMed PMID: 16249525.
34. Brand AH, Manoukian AS, Perrimon N. Ectopic expression in Drosophila. Methods Cell Biol. 1994;44:635-54. PubMed PMID: 7707973.
35. Phelps CB, Brand AH. Ectopic gene expression in Drosophila using GAL4 system. Methods (San Diego, Calif). 1998;14(4):367-79. Epub 1998/06/03. doi: 10.1006/meth.1998.0592. PubMed PMID: 9608508.
36. Karlikow M, Goic B, Saleh MC. RNAi and antiviral defense in Drosophila: setting up a systemic immune response. Developmental and comparative immunology. 2014;42(1):85-92. Epub 2013/05/21. doi: 10.1016/j.dci.2013.05.004. PubMed PMID: 23684730.
37. Reed HC, Hoare T, Thomsen S, Weaver TA, White RAH, Akam M, et al. Alternative Splicing Modulates Ubx Protein Function in Drosophila melanogaster. Genetics. 2010;184(3):745-58. doi: 10.1534/genetics.109.112086. PubMed PMID: PMC2845342.
38. Smith CW, Valcarcel J. Alternative pre-mRNA splicing: the logic of combinatorial control. Trends Biochem Sci. 2000;25(8):381-8. Epub 2000/08/01. PubMed PMID: 10916158.

39. Matlin AJ, Clark F, Smith CW. Understanding alternative splicing: towards a cellular code. Nature reviews Molecular cell biology. 2005;6(5):386-98. Epub 2005/06/16. doi: 10.1038/nrm1645. PubMed PMID: 15956978.
40. Wang Z, Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. RNA (New York, NY). 2008;14(5):802-13. Epub 2008/03/29. doi: 10.1261/rna.876308. PubMed PMID: 18369186; PubMed Central PMCID: PMCPMC2327353.

Chapter 2 - Modelling Huntington Disease through *Huntingtin interacting protein 1* in *Drosophila melanogaster*

2.1 Introduction

Huntington Disease (HD) is a progressive neurodegenerative disorder, with symptoms varying from loss of cognition and motor control to weight loss and changes in sleeping patterns, generally ending in premature death (7). HD occurs upon expansion of a CAG repeat in the *Huntingtin* (*Htt*) gene, resulting in an expanded polyglutamine tract of the protein (10). The number of polyglutamine residues present displays a positive correlation with disease severity and a negative correlation with age of onset (9, 10). The increased number of polyglutamine residues has a drastic impact on the cell – effecting protein folding, binding ability and causing aggregate formation, eventually leading to the selective death of neurons in the brain (6, 9). Upon expansion, Htt is unable to bind to a number of its normal binding partners, including Huntingtin interacting protein 1 (Hip1), which is then left unbound in the cytoplasm with the potential to be sequestered by other proteins and then to form aggregates (9). The symptoms of HD were originally contributed to the expansion in the polyglutamine tract of Htt. However, it is probable that several Htt binding partners contribute to disease symptoms and pathogenesis. Involved in trafficking, endocytosis, apoptotic cell signalling and neurogenesis (9, 11-14, 41), a disruption in the interaction between Htt and Hip1 could have detrimental effects within the cell.

The *Hip1* gene has been highly conserved throughout evolutionary history. A comparison of human and *D. melanogaster* Hip1 proteins reveals three highly conserved domains; the N-terminal lipid binding ANTH domain, a coiled coil and a C-terminal

talin-like I/LWEQ domain (14, 28). The degree of evolutionary conservation suggests a highly conserved function and thus an investigation of the effects of altered *Hip1* expression in *D. melanogaster* may provide insights into the pathology of HD human patients. In this study, increased and reduced *Hip1* expression in *D. melanogaster* is used to mimic the same conditions in humans, to examine the effects on lifespan and locomotor ability.

2.2 Materials and Methods

2.2.1 *Drosophila melanogaster* stocks and culture

The *UAS-lacZ* responder line (42), *UAS-GFP* responder line (43), *UAS-Hip1-RNAi*^{32504} responder line (44) along with the *elav-Gal4* (45) and *GawB*$^{l(3)31-1}$ (46) transgenic lines were obtained from the Bloomington *Drosophila* Stock Center (University of Indiana, Bloomington, USA). The *UAS-Hip1*^{L2}, *UAS-Hip*^{L6} and *UAS-Hip1*$^{S11.2}$ (Δ*ANTH*) transgenic lines were generated by our research group by Justin Moores (14). The *UAS-Hip1-RNAi*^{106978} (47, 48) was obtained from the Vienna *Drosophila* Resource Center (Vienna Biocenter, Vienna, USA). The *w*^{1118} stock was generously provided by Dr. Howard Lipshitz, of the University of Toronto (Toronto, Canada). The expression patterns of fly lines used in this analysis and the place of insertion can be found in Table 1.

D. melanogaster were cultured on standard media composed of 65 g/L cornmeal, 10 g/L yeast, 5.5 g/L agar and 50 ml/L fancy grade molasses in water supplemented with 5 ml of 0.1 g/mL methyl paraben in 95% ethanol and 2.5 mL of propionic acid. All experiments were at 25° C.

Crosses were set up by placing three to five *UAS*-bearing virgin females of the corresponding genotype and two to five Gal4-bearing males together on standard media. To increase the number of progeny, parental flies were placed on new media at day 2, 4 and 6 post experimental initiation. Critical class males were selected.

Table 2.1 – Genotypes, location of transgene insertion and expression patterns used in the analysis of *Hip1* overexpression and the loss of function of *Hip1* expressed in a pan-neuronal fashion to determine their role in the pathogenesis of Huntington Disease.

Genotype	Insertion Chromosome	Affected Chromosome	Expression Patterns
w^{1118}	N/A	1	N/A
UAS-lacZ	2	1;2	Expresses *lacZ* under *UAS* control
UAS-GFP	3	3	Expresses *GFP* under *UAS* control
UAS-Hip1-RNAi^{106978}	2	1;2	Expresses *dsRNAi* for *Hip1* under *UAS* control
UAS-Hip1-RNA1^{32504}	3	1;3	Expresses *dsRNAi* for *Hip1* under *UAS* control
UAS-Hip1^{L2}	Unknown	Unknown	Expresses *Hip1* under *UAS* control
UAS-Hip1^{L6}	Unknown	Unknown	Expresses *Hip1* under *UAS* control
UAS-Hip1$^{ST1.2}$	Unknown	Unknown	Expresses *Hip1*ΔANTH under *UAS* control
elav-Gal4	1	1	*Gal4* expressed in all tissues of the embryonic nervous system beginning at stage 12
GawB$^{l(3)31-1}$	3	1;3	*Gal4* expressed in neuroblasts and neurons

2.2.2 Longevity assay

Critical class male progeny were isolated on the day of eclosure and maintained at 25°C on standard media in vials of 23 or less. Flies were scored every two days for the presence of deceased individuals, and provided with fresh media regularly to ensure ideal

conditions (N ≥ 269). Data was analyzed using GraphPad Prism 5.0c or 7.0b (GraphPad Software, Inc., San Diego, California, USA), survival curves were generated and a Mantel-Cox test was performed to determine significance; indicated by a P value of less than 0.05.

2.2.3 Locomotor assay

Critical class male progeny were isolated on the day of eclosure and maintained at 25°C on standard media in vials of 10 or less. Beginning at day 2 post eclosion, and at regular seven day intervals afterward, flies were scored for climbing ability. In glass tubes, flies were given 10 seconds to climb, and then the maximum level that they reached was recorded (20). A climbing index was calculated to determine climbing ability, using the formula described below:

$$\text{Climbing Index} = (nm/N)$$

where n=number of flies at level, m=score for the level and N=total number of flies climbed (49).

A cohort of 50 or less individuals from each genotype were analyzed in groups of 10 or less (N = 50). Each cohort underwent a total of 10 trials, providing a total trial number of 500 flies per genotype per session. This analysis was continued until less than 10 flies remained alive or flies received a minimum score for two consecutive sessions. A climbing curve (non-linear regression curve) was generated using GraphPad Prism 5.0c or 7.0b software (GraphPad Software, Inc., San Diego, California, USA). A comparison of fits of the non-linear regression curves taking the Y-intercept (initial climbing ability) and

slope (rate of decline in climbing ability) into consideration concluded if the curves were significantly different, indicated by a P value less than 0.05.

2.3 Results

Examination of altered *Hip1* expression in a pan-neuronal fashion can provide insights into the role of *Hip1* in neurodegenerative diseases such as Huntington Disease, and determine the effects on disease phenotypes. Investigation with *elav-Gal4* revealed decreased lifespan with *UAS-GFP* (LD50 = 52) compared to *UAS-lacZ* (LD50 = 70) and w^{1118} (LD50 = 70) (Figure 2.1a). Both *UAS-GFP* and w^{1118} displayed a reduction in motor function compared to *UAS-lacZ* (Figure 2.1b). Due to the nature of the control phenotypes, *UAS-lacZ* served as an appropriate control for this analysis.

An investigation of loss of function of *Hip1* revealed an increase in average lifespan with *elav-Gal4; UAS-Hip1-RNAi*^{106978} displaying a median lifespan of 78 days compared to 70 days for the *elav-Gal4; UAS-lacZ* control (Figure 2.1c). The *elav-Gal4; UAS-Hip1-RNAi*^{32504} males had a median lifespan of 70 days, showing no significant difference compared to the *UAS-lacZ* control. Both *elav-Gal4; UAS-Hip1-RNAi*^{106978} and *elav-Gal4; UAS-Hip1-RNAi*^{32504} showed a significant loss in motor ability (Figure 2.1d). Overexpression of *Hip1* by *elav-Gal4* has the opposite effect, decreasing median lifespan to 60 days with *elav-Gal4; UAS-Hip1*^{L2} and 54 days with *elav-Gal4; UAS-Hip1*^{L6}, along with a clear premature loss of climbing ability (Figure 2.1e, f). Overexpression of truncated *Hip1* in *elav-Gal4; UAS-Hip1*Δ*ANTH*$^{S11.2}$ further reduced climbing ability (Figure 2.1f) but had no significant effect on median lifespan (Figure 2.1e), to suggest that the lipid binding domain may play a significant role in motor function.

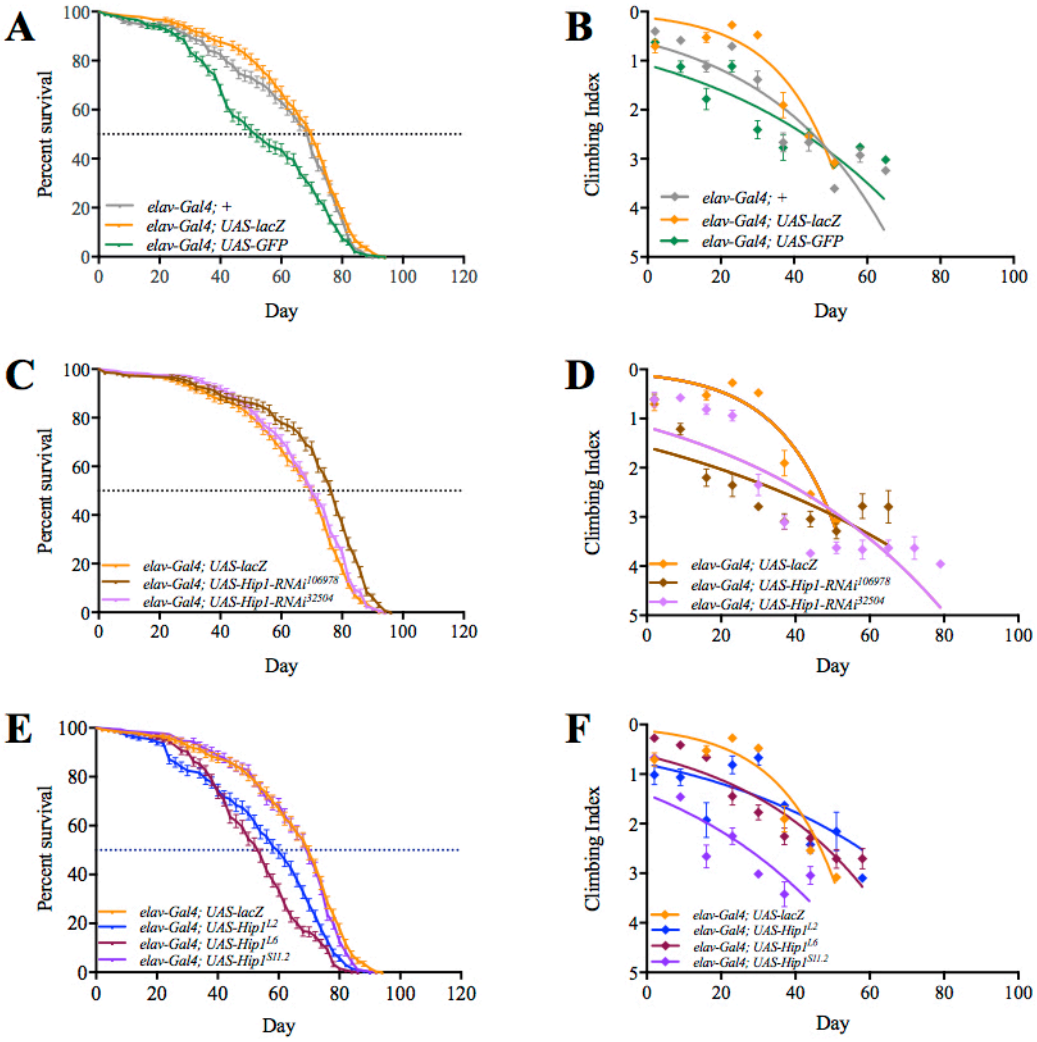


Figure 2.1 - Altered expression of *Hip1* through the *elav-Gal4* transgene affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in a pan-neuronal fashion. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥295). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in a pan-neuronal fashion. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *elav-Gal4; +* (w^{1118}), *elav-Gal4; UAS-lacZ, elav-Gal4; UAS-GFP, elav-Gal4; UAS-Hip1-RNAi*^{106978}, *elav-Gal4; UAS-*

Hip1-RNAi^{32504}*, elav-Gal4; UAS-Hip1*^{L2}*, elav-Gal4; UAS-Hip1*^{L6}*, elav-Gal4; UAS-Hip1ΔANTH*$^{S11.2}$. See Appendix B for supplemental data.

A similar analysis completed using *GawB*$^{l(3)31\text{-}1}$ revealed no significant difference in lifespan between no responder *GawB*$^{l(3)3\text{-}1}$*; +* (LD50 = 52), *GawB*$^{l(3)3\text{-}1}$*; UAS-lacZ* (LD50 = 58) and *UAS-GFP* (LD50 = 52) (Figure 2.2a), although *GawB*$^{l(3)3\text{-}1}$*; +* did display a significant increase in locomotor function (Figure 2.2b). Upon a comparison of *GawB*$^{l(3)3\text{-}1}$*;UAS-lacZ* with *GawB*$^{l(3)31\text{-}1}$*; UAS-Hip1-RNAi*^{106978}*, GawB*$^{l(3)31\text{-}1}$*; UAS-Hip1*^{L2}*, GawB*$^{l(3)31\text{-}1}$*; UAS-Hip1*^{L6}*, GawB*$^{l(3)31\text{-}1}$*; UAS-Hip1*Δ*ANTH*$^{S11.2}$, no significant difference was found in survival or motor function, with median lifespans of 46, 56 and 52 respectively (Figure 2.2c, d, e & f). *GawB*$^{l(3)31\text{-}1}$*; UAS-Hip1-RNAi*^{32504} did display a decrease in motor function, but no change in average lifespan (LD50 = 55). A slight increase in average lifespan (LD50 = 58) and motor ability was observed upon *Hip1*ΔANTH overexpression (Figure 2.2e & f). It is plausible that the *GawB* line may not direct expression as powerfully as other *Gal4* lines, and that this may affect the results shown here.

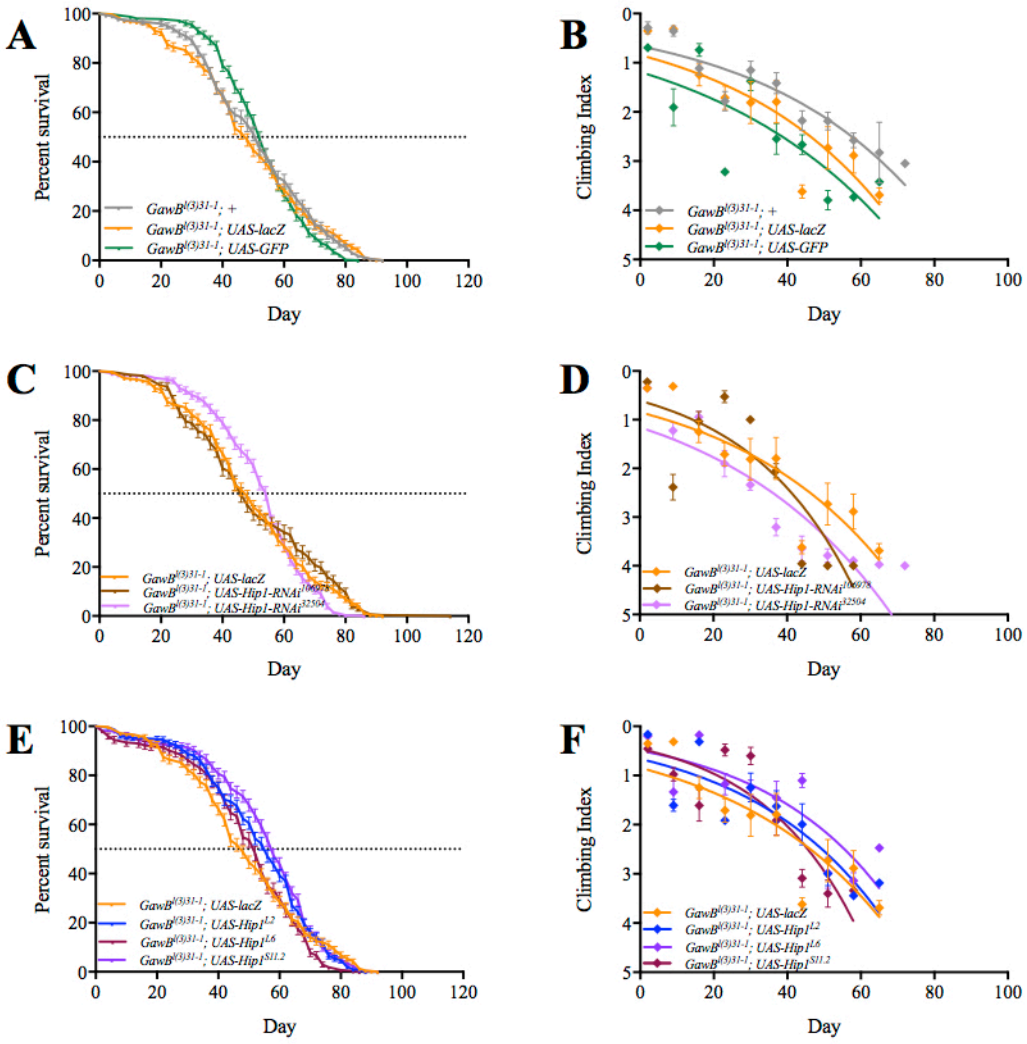


Figure 2.2 - Altered expression of *Hip1* through the *GawB$^{l(3)3\text{-}1}$-Gal4* transgene affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in a pan-neuronal fashion. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥269). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in a pan-neuronal fashion. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *GawB$^{l(3)31\text{-}1}$; +* (*w^{1118}*), *GawB$^{l(3)3\text{-}1}$; UAS-lacZ, GawB$^{l(3)31\text{-}1}$; UAS-GFP, GawB$^{l(3)31\text{-}1}$; UAS-Hip1-RNAi^{106978}, GawB$^{l(3)31\text{-}1}$; UAS-Hip1-RNAi^{32504}, GawB$^{l(3)31\text{-}1}$; UAS-Hip1^{L2}, GawB$^{l(3)31\text{-}1}$; UAS-Hip1^{L6}, GawB$^{l(3)31\text{-}1}$; UAS-Hip1ΔANTH$^{S11.2}$*. See Appendix C for supplemental data.

2.4 Discussion

Alteration of the cellular expression levels of *Hip1* effected whole organism longevity and some aspects of neuronal development – particularly growth. Flies expressing an increased amount of *Hip1* display a reduction in average lifespan and a reduction in motor function over time (Figure 2.1e & f). This reduction in lifespan indicates that Hip1 may be involved in cellular pathways related to cellular and whole organism survival and death. Previously implicated in the induction of apoptosis, Hip1 activates caspase-8 upon the formation of a complex with Hippi. (11). In HD, Hip1 cannot bind the mutated Htt and thus the unbound levels in the cell increase, leaving it free to create other complexes such as the Hip1-Hippi heterodimer (9, 11). The overexpression of *Hip1* experimental line was designed to mimic the suggested HD pathology in this way, and thus the reduction in lifespan and motor ability provides a promising model of neurodegenerative disease.

Overexpression of *Hip1*ΔANTH did not result in the reduction in lifespan observed with the overexpression of full length *Hip1* (Figure 2.1e). The loss of the lipid binding domain occurs naturally upon the expression of alternative first exons, and likely causes conformational changes in the protein, influencing folding and protein binding ability and rendering it less able to interact with its normal binding partners (14). The reduced ability to bind to Hippi and activate Caspase-8, should result in no increase in cell death (11), and thus no reduction in average lifespan. The motor function of flies overexpressing *Hip1*ΔANTH was severely diminished (Figure 2.1f). As the ANTH domain is primarily involved in endocytosis and can affect cytoskeletal interactions (30, 31), the loss of the

ANTH domain likely impairs the proper binding of Hip1 to the cytoskeleton. Cytoskeletal movement is in turn responsible for cellular and muscle movements (50), thus the loss of the ANTH domain of Hip1 may in fact affect motor ability on the tissue level. This suggests a crucial role for the ANTH lipid binding domain in motor function, but not in the regulation of lifespan.

Alternatively, the reduced expression of *Hip1* was examined to investigate the effect of altered *Hip1* levels – which could occur due to a mutation in the *Hip1* gene or during HD, if the cell recognizes the free Hip1 as non-functional and tags the protein for degradation. Flies possessing reduced amounts of normal *Hip1* levels display increased longevity, but a reduction in motor ability through *elav-Gal4* (Figure 2.1c & d). As Hip1 is involved in initiating caspase-8 mediated apoptotic cell pathways, a reduction in protein levels may result in decreased apoptotic cell cycle control (11), thus increasing the lifespan of the cell, and therefore the organism. The decline in motor ability can again be explained by the loss of the interaction of Hip1 with the cytoskeleton (31), causing cytoskeletal dysfunction and affecting locomotor ability of the organism. The effects observed suggest a delicate balance of Hip1 protein concentration levels is necessary in healthy cells, and altered expression levels affect longevity and motor function. The Hip1 RNAi lines used in this analysis have also been shown to increase the microchaetae density of *D. melanogaster*, while a loss of function mutation of Hip1 displayed a reduced longevity and no change in motor function (51). Still a relatively novel gene in the study of Huntington Disease, there is minimal data available on the expression patterns of *Hip1*. As a potential target gene in neurodegenerative disease, an investigation of expression

levels in higher organisms with respect to Huntington Disease will aid in the search for potential treatment options.

2.5 References

6. Petersén Å, Mani K, Brundin P. Recent Advances on the Pathogenesis of Huntington's Disease. Experimental Neurology. 1999;157(1):1-18. doi: http://dx.doi.org/10.1006/exnr.1998.7006.

7. Walling HW, Baldassare JJ, Westfall TC. Molecular aspects of Huntington's disease. Journal of Neuroscience Research. 1998;54(3):301-8. doi: 10.1002/(SICI)1097-4547(19981101)54:3<301::AID-JNR1>3.0.CO;2-W.

9. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics. 2004;20(3):146-54. doi: http://dx.doi.org/10.1016/j.tig.2004.01.008.

10. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971-83. doi: http://dx.doi.org/10.1016/0092-8674(93)90585-E.

11. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst C-A, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4(2):95-105.

12. Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, et al. Huntingtin Interacting Protein 1 Is a Clathrin Coat Binding Protein Required for Differentiation of late Spermatogenic Progenitors. Molecular and Cellular Biology. 2001;21(22):7796-806. doi: 10.1128/MCB.21.22.7796-7806.2001. PubMed PMID: PMC99949.

13. Rao DS, Hyun TS, Kumar PD, Mizukami IF, Rubin MA, Lucas PC, et al. Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival. The Journal of Clinical Investigation. 2002;110(3):351-60. doi: 10.1172/JCI15529. PubMed PMID: PMC151092.

14. Moores JN, Roy S, Nicholson DW, Staveley BE. Huntingtin interacting protein 1 can regulate neurogenesis in Drosophila. The European journal of neuroscience. 2008;28(3):599-609. Epub 2008/08/16. doi: 10.1111/j.1460-9568.2008.06359.x. PubMed PMID: 18702731.

20. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. Genome / National Research Council Canada = Genome / Conseil national de recherches Canada. 2008;51(12):1040-6. Epub 2008/12/18. doi: 10.1139/g08-085. PubMed PMID: 19088817.

28. Niu Q, Ybe JA. Crystal Structure at 2.8 Å of Huntingtin-Interacting Protein 1 (HIP1) Coiled-Coil Domain Reveals a Charged Surface Suitable for HIP1 Protein Interactor (HIPPI). Journal of Molecular Biology. 2008;375(5):1197-205. doi: https://doi.org/10.1016/j.jmb.2007.11.036.

30. Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. ENTH/ANTH proteins and clathrin-mediated membrane budding. Journal of cell science. 2004;117(Pt 1):9-18. Epub 2003/12/06. doi: 10.1242/jcs.00928. PubMed PMID: 14657269.

31. Wilbur JD, Chen C-Y, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM. Actin binding by huntingtin-interacting protein 1 (hip1) and hip1-related protein (Hip1R) is

regulated by clathrin light chain. Journal of Biological Chemistry. 2008. doi: 10.1074/jbc.M802863200.
41. Choi SA, Kim SJ, Chung KC. Huntingtin-interacting protein 1-mediated neuronal cell death occurs through intrinsic apoptotic pathways and mitochondrial alterations. FEBS Letters. 2006;580(22):5275-82. doi: 10.1016/j.febslet.2006.08.076.
42. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118(2):401-15. PubMed PMID: 8223268.
43. Yeh E, Gustafson K, Boulianne GL. Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(15):7036-40. PubMed PMID: PMC41466.
44. Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou D, et al. A genome-scale shRNA resource for transgenic RNAi in Drosophila. 2010.
45. Lin DM, Goodman CS. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron. 1994;13(3):507-23. Epub 1994/09/01. PubMed PMID: 7917288.
46. Ito K, Awano W, Suzuki K, Hiromi Y, Yamamoto D. The Drosophila mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development. 1997;124(4):761-71.
47. Keleman K, Micheler T, members Vp. RNAi-phiC31 construct and insertion data submitted by the Vienna Drosophila RNAi Center. 2009.
48. Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448(7150):151-6.
49. Todd AM, Staveley BE. Novel Assay and Analysis for Measuring Climbing Ability in Drosophila. Drosophila Information Service. 2004;87:101-8. Epub December 2004.
50. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, et al. Structure of the Actin-Myosin Complex and Its Implications for Muscle Contraction. Science (New York, NY). 1993;261(5117):58-65.
51. Slade FA. The effects of altered Hip1 expression in a Huntington disease model in Drosophila melanogaster. St. John's, NL: Memorial University of Newfoundland; 2014.

Chapter 3 - A Huntington gene in models of Parkinson Disease: Investigating the effects of altered *Hip1* expression in *Drosophila melanogaster*

3.1 Introduction

Neurodegenerative diseases are an increasing problem in today's ageing society. Parkinson Disease (PD) is a progressive neurodegenerative disorder, second in prevalence only to Alzheimer Disease (15). The incidence of Parkinson Disease increases with age, affecting over 1% of the human population above 65 and 4 to 5% of the population over the age of 85 (16). Characterized by loss of cognitive ability and motor functions, PD begins with the loss of dopaminergic neurons in the *substantia nigra* of the midbrain, often accompanied by the formation of Lewy bodies (17, 52). PD can occur sporadically or through genetic inheritance, in both autosomal-dominant and autosomal-recessive patterns (5, 52). A number of genes have been implicated in the pathogenesis of Parkinson Disease, including *Pink1*, *Parkin*, *PGC-1α*, *PARIS* and *α-synuclein* just to name a few (5, 20-23), and a number of genes have been associated with different patterns of inheritance (5, 53). An investigation of the expression patterns of the genes involved in PD and the interactions of their respective proteins can provide insights into disease pathogenesis.

Huntingtin interacting protein 1 Related (*Hip1R*) was identified as a risk factor for PD in a genome wide association study in humans (24, 25). A number of functions have been identified for Hip1R, including endocytosis, clathrin assembly, chromosomal segregation and apoptosis (29, 54, 55). Low *Hip1R* expression has been associated with decreased survival in cancer patients, and expression of *Hip1R* in the mouse brain

suggests a role for the protein in development (56, 57). Prominent expression of *Hip1R* is present in the midbrain (56), the area in which the dopaminergic neurons are located (58), suggesting a potential link to PD development.

Hip1R is considered an orthologue of *Hip1* (*Huntingtin interacting protein 1*), which has been implicated in Huntington Disease (14, 59). Although the Hip1 and Hip1R proteins share structural similarity and have some overlapping functions, they play somewhat different roles in the cell (26, 27). Hip1 is involved in trafficking, endocytosis, neurogenesis, cell survival and apoptotic cell signalling (9, 11-14). Bioinformatic analysis of *Hip1* and *Hip1R* revealed that the closest *D. melanogaster* homologue for both genes is Drosophila *Hip1* (Figure 1.2). Thus, an investigation of altered *Hip1* expression on *D. melanogaster* lifespan and locomotor ability may provide insight into both Huntington Disease and Parkinson Disease.

Implications observed upon overexpression and loss of function of *Hip1* in a pan-neuronal fashion suggested a need to investigate differential expression in more detail. To mimic the pathogenesis of Parkinson Disease, in this study the expression of *Hip1* in the dopaminergic neurons was altered, and the effects on longevity and locomotor ability were examined.

3.2 Materials and Methods

3.2.1 *Drosophila melanogaster* stocks and culture

Drosophila were cultured on a standard media containing 65 g/L cornmeal, 10 g/L yeast, 5.5 g/L agar, 50 ml/L fancy grade molasses, 5 ml of 0.1 g/ml methyl 4-hydroxybenzoate in 95% ethanol and 2.5 ml of propionic acid at 25°C.

The *UAS-lacZ* responder line (42), *UAS-GFP* responder line (43), *ple-Gal4* (60) and *UAS-Hip1-RNAi*^{32504} (44) lines were obtained from the Bloomington *Drosophila* Stock Centre (University of Indiana, Bloomington, USA). The *UAS-Hip1*^{L2}, *UAS-Hip*^{L6} and *UAS-Hip1*$^{S11.2}$ (ΔANTH) transgenic lines were generated by Justin Moores (14). The *UAS-Hip1-RNAi*^{106978} (47, 48) was obtained from the Vienna Drosophila Resource Centre (Vienna Biocenter, Vienna, USA). Dr. J. Hirsch (University of Virginia) generously provided the *Ddc-Gal4* $^{HL4.3D}$ and *Ddc-Gal4* $^{HL4.36}$ (61) lines and the combination stock *Ddc-Gal4* $^{HL4.3D}$; *Ddc-Gal4* $^{HL4.36}$ was generated by standard genetic means. Stock of w^{1118} was generously provided by Dr. Howard Lipshitz, of the University of Toronto. Expression patterns of the fly lines used in this analysis can be found in Table 2.

On standard media, crosses were set up by placing three to five virgin females of the corresponding genotype and two to five Gal4 males together. To increase the number of progeny, parental flies were placed on new media at day 2, 4 and 6 post experimental initiation. Critical class males were selected.

3.2.2 Longevity assay

Male progeny of the critical class genotype were isolated on the day of eclosure and maintained on fresh standard media in vials of 25 or less at 25°C. Flies were scored every second day for death ($N \geq 296$). Data was analyzed and survival curves were generated using GraphPad Prism 5.0c or 7.0b (GraphPad Software, Inc., San Diego, California, USA). A Mantel-Cox test indicated significance ($P < 0.05$).

Table 3.1 – Genotypes, location of transgene insertion and expression patterns used in the analysis of *Hip1* overexpression and the loss of function of *Hip1* expressed in the dopaminergic neurons to determine their role in the pathogenesis of Parkinson Disease.

Genotype	Insertion Chromosome	Affected Chromosome	Expression Patterns
w^{1118}	N/A	1	N/A
UAS-lacZ	2	1;2	Expresses *lacZ* under *UAS* control
UAS-GFP	3	3	Expresses *GFP* under *UAS* control
UAS-Hip1-RNAi^{106978}	2	1;2	Expresses *dsRNAi* for *Hip1* under *UAS* control
UAS-Hip1-RNA1^{32504}	3	1;3	Expresses *dsRNAi* for *Hip1* under *UAS* control
UAS-Hip1^{L2}	Unknown	Unknown	Expresses *Hip1* under *UAS* control
UAS-Hip1^{L6}	Unknown	Unknown	Expresses *Hip1* under *UAS* control
UAS-Hip1$^{S11.2}$	Unknown	Unknown	Expresses *Hip1*ΔANTH under *UAS* control
ple-Gal4	3	1;3	*Gal4* expressed in dopaminergic cells
Ddc-Gal4$^{HL4.3D}$	2	1;2	*Gal4* expressed in the dopaminergic and serotonergic neurons
Ddc-Gal4$^{HL4.36}$	3	1;3	*Gal4* expressed in the dopaminergic and serotonergic neurons
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$	2 & 3	1;2;3	*Gal4* expressed in the dopaminergic and serotonergic neurons

3.2.3 Locomotor assay

Male progeny of the critical class were isolated on the day of eclosure and maintained on fresh standard media in vials of 10 or less at 25°C. Flies were scored for climbing ability beginning at day two post eclosion, and at regular seven day intervals afterward. To score for climbing ability, flies were placed in glass tubes and given 10 seconds to climb. The maximum level that they reached was recorded (20). A climbing index was calculated based on these results given the following formula:

Climbing Index = (nm/N)

where n=number of flies at level, m=score for the level and N=total number of flies climbed (49).

A total of 50 individuals (N=50) from each genotype were analyzed in groups of 10 or less. Each group underwent a total of 10 trials, providing a total number of 500 trials per genotype per week. This analysis continued until less than 10 flies remained alive or flies received a minimum score for two consecutive weeks. Data was analyzed and non-linear regression curves with a 95% confidence interval were generated using GraphPad Prism version 5.0c or 7.0b (GraphPad Software, Inc., San Diego, California, USA). A comparison of fits of the non-linear regression curves incorporating the Y-intercept (initial climbing ability) and slope (rate of decline in climbing ability) concluded if the curves were significantly different ($P < 0.05$).

3.3 Results

3.3.1. Hip1 and Hip1R share similar domains across species

Conservation of the ANTH and talin-like structural domains of Hip1 in fruit flies and humans suggests similar functions for the protein throughout evolutionary history

(14). Considered an orthologue of *Hip1*, human *Hip1R* does not have an independent Drosophila homologue. However, The ANTH lipid binding domain is highly conserved in human Hip1R, while the N-terminus of the protein itself shows a high degree of conservation with human and Drosophila Hip1, and they do seem to share similar functions, including endocytosis and apoptosis (11, 27, 29, 54). The Hip1R protein is shorter and missing the talin-like domain at the C-terminus (Figure 1.2), implicating it in cellular functions different than those performed by Hip1 (27, 55). The conservation of *Hip1* across species provides a model with which to study human disease, and suggests that a study of the effects of altered expression of Drosophila *Hip1* may apply to both human *Hip1* and *Hip1R*.

3.3.2. Altered *Hip1* expression in the dopaminergic neurons affects longevity and motor ability

Directed altered expression of *Hip1* in the dopaminergic neurons of *D. melanogaster* can provide insights into the progression of PD. Examination of longevity and locomotor ability will reveal the effects of altered gene expression, and can suggest potential candidates for disease pathogenesis. The use of *ple-Gal4* to drive gene expression in the dopaminergic neurons displayed no significant difference in survival between the benign *UAS-lacZ* and *UAS-GFP* responder lines (Figure 3.1a), both having a median survival of 70 days. Comparing the *UAS-lacZ* benign responder with an average lifespan of 70 days to the 72 day average lifespan of the responder-less (w^{1118}) displayed a significant difference in fly longevity (Figure 3.1a). An examination of motor ability revealed no significant difference in climbing ability between the benign *UAS-lacZ* and *UAS-GFP*

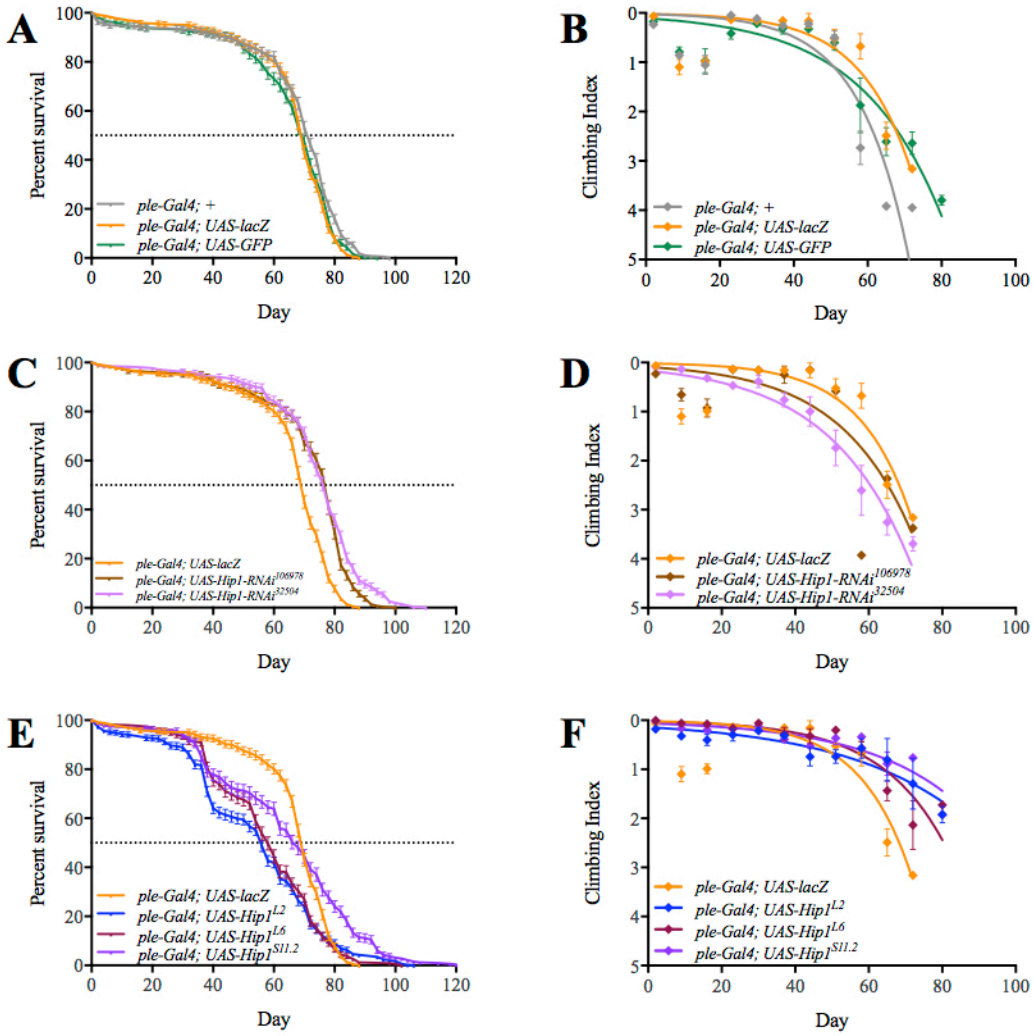


Figure 3.1 – Altered expression of *Hip1* through the *ple-Gal4* transgene affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥296). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *ple-Gal4; +* (w^{1118}), *ple-Gal4; UAS-lacZ, ple-Gal4; UAS-GFP, ple-Gal4; UAS-Hip1-RNAi*^{106978}, *ple-Gal4; UAS-Hip1-RNAi*^{32504}, *ple-Gal4; UAS-Hip1*^{L2}, *ple-Gal4; UAS-Hip1*^{L6}, *ple-Gal4; UAS-Hip1-*ΔANTH$^{S11.2}$. See Appendix D for supplemental data.

controls, and a significant decrease in climbing ability of the responder-less w^{1118} later in life through *ple-Gal4* (Figure 3.1b). As the *UAS-lacZ* control incorporates the activation of the *UAS-Gal4* system while expressing a benign responder, it will be used as a control for the purpose of this analysis.

A loss of function analysis of *Hip1* through RNAi in *ple-Gal4; UAS-Hip1-RNAi*^{106978} and *ple-Gal4; UAS-Hip1-RNAi*^{32504} increased average lifespan to 78 days (Figure 3.1c) compared to the 70-day average lifespan of the *ple-Gal4; UAS-lacZ* control. With *ple-Gal4; UAS-Hip1-RNAi*^{32504}, a marked reduction in motor function was observed. This significant decrease was not seen in *ple-Gal4; UAS-Hip1-RNAi*^{106978} individuals, although there did appear to be a slight decline in motor function (Figure 3.1d).

Analysis of overexpression data of *UAS-Hip1*^{L2} and *UAS-Hip1*^{L6} in the dopaminergic neurons directed by *ple-Gal4* displayed a dramatic reduction in average lifespan (56 days and 58 days respectively) compared to the *UAS-lacZ* control (70 days) (Figure 3.1e). Overexpression of *ple-Gal4; UAS-Hip1*ΔANTH $^{S11.2}$ in the dopaminergic neurons displayed a slight decrease in median lifespan of 68 days (Figure 3.1e). Interestingly, all three *Hip1* overexpression lines seemed to maintain an increased motor ability until later in life, when flies displayed a slight loss of motor function (Figure 3.1f).

Altered expression of *Hip1* in the dopaminergic neurons through *Ddc-Gal4*$^{HL4.3D}$ displayed no significant difference in motor ability between the *UAS-lacZ*, *UAS-GFP* and "no responder" (w^{1118}) controls (Figure 3.2b). Both *Ddc-Gal4*$^{HL4.3D}$*; UAS-GFP* and *Ddc-Gal4*$^{HL4.3D}$*;* + displayed an increased median lifespan of 58 and 74 days compared to the *Ddc-Gal4*$^{HL4.3D}$*; UAS-lacZ* control (LD50 = 54) (Figure 3.2a). For the purpose of this

experiment, the benign *UAS-lacZ* will be used as the control, as it simulates the use of the *UAS-Gal4* system, but the responder is benign. Note that extra care must be taken when considering a control (62).

A loss of function of *Hip1* analysis through *Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1-RNAi*^{106978} and *Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1-RNAi*^{32504} displayed an increase in lifespan, having median lifespans of 62, and 70 days respectively, compared to the *Ddc-Gal4*$^{HL4.3D}$*; UAS-lacZ* control of 54 days (Figure 3.2c). This data trend is similar to the trend observed upon expression with *ple-Gal4* (Figure 3.1c). The locomotor analysis however did not display similar trends, with both *Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1-RNAi*^{106978} and *Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1-RNAi*^{32504} exhibiting increased climbing ability compared to the *Ddc-Gal4*$^{HL4.3D}$*; UAS-lacZ* control (Figure 3.2d).

Overexpression of *Hip1* through *Ddc-Gal4*$^{HL4.3D}$ displayed a slight increase in longevity for *Ddc-Gal4*$^{HL4.3D}$; *UAS-Hip1*^{L2} (LD50=58) and *Ddc-Gal4*$^{HL4.3D}$; *UAS-Hip1*$^{S11.2}$ (LD50=58) compared to the *Ddc-Gal4*$^{HL4.3D}$; *UAS-lacZ* control (LD50=54). *Ddc-Gal4*$^{HL4.3D}$; *UAS-Hip1*^{L6} however, showed a reduction in longevity, with a median survival of 44 days (Figure 3.2e). All three critical class male cohorts that directed versions of *Hip1* overexpression through *Ddc-Gal4*$^{HL4.3D}$*, Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1*^{L2}*, Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1*^{L6}*, Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1*$^{S11.2}$ showed an increase in locomotor ability compared to the *Ddc-Gal4*$^{HL4.3D}$; *UAS-lacZ* control (Figure 3.2f).

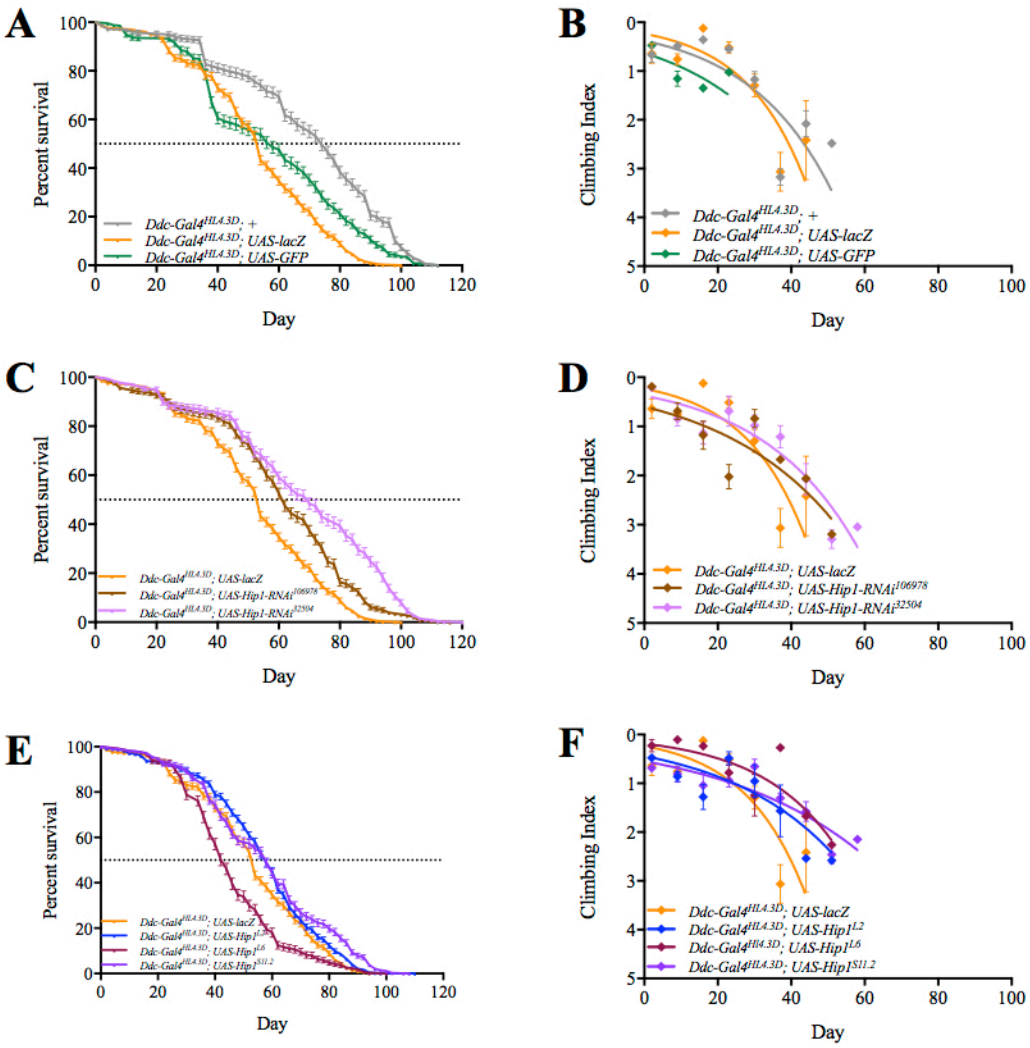


Figure 3.2 - Altered expression of *Hip1* through the *Ddc-Gal4*$^{HL4.3D}$ transgene affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥377). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *Ddc-Gal4*$^{HL4.3D}$; + (*w*^{1118}), *Ddc-Gal4* $^{HL4.3D}$; *UAS-lacZ, Ddc-Gal4* $^{HL4.3D}$; *UAS-GFP, Ddc-Gal4* $^{HL4.3D}$; *UAS-Hip1-RNAi*^{106978}, *Ddc-Gal4* $^{HL4.3D}$; *UAS-Hip1-RNAi*^{32504}, *Ddc-Gal4* $^{HL4.3D}$; *UAS-*

Hip1^{L2}, *Ddc-Gal4*$^{HL4.3D}$; *UAS-Hip1*^{L6}, *Ddc-Gal4*$^{HL4.3D}$; *UAS-Hip1*ΔANTH$^{S11.2}$. See Appendix E for supplemental data.

Expression through *Ddc-Gal4*$^{HL4.36}$ revealed no significant difference in lifespan between the responder-less, *Ddc-Gal4*$^{HL4.36}$*; +* (LD50 = 58) and those with a benign responder *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* (LD50 = 64), while the *lacZ* flies appeared to have an increased motor function (Figure 3.3a & b). An increase in median lifespan was observed with *Ddc-Gal4*$^{HL4.36}$*; UAS-GFP* (LD50 = 71), while no significant change in motor function was observed (Figure 3.3a & b). As *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* expresses a benign responder, it will serve as a control for the purpose of this analysis. The flies that possessed the *Ddc-Gal4*$^{HL4.36}$ transgene seemed to have a reduced viability compared to the others and a much greater effort to obtain critical class individuals was necessary to obtain the required sample size which might influence the results obtained.

Inhibition of *Hip1* expression in the dopaminergic neurons through *Ddc-Gal4* $^{HL4.36}$*, in Ddc-Gal4* $^{HL4.36}$*; UAS-Hip1-RNAi*^{106978} displays a slight reduction in lifespan (LD50 = 60) and had no effect on motor ability compared to the *Ddc-Gal4* $^{HL4.36}$*; UAS-lacZ* control (LD50 = 64) (Figure 3.3c & d). However, *Ddc-Gal4* $^{HL4.36}$*; UAS-Hip1-RNAi*^{32504} showed increased longevity, having a median lifespan of 76 days, and displayed a reduction in motor control (Figure 3.3c & d). Overexpression of *Hip1*^{L2} in the dopaminergic neurons through *Ddc-Gal4*$^{HL4.36}$*, in Ddc-Gal4* $^{HL4.36}$*; UAS-Hip1*^{L2} males, displayed an increased median lifespan of 72 days, and a reduction in motor function compared to the *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* control (Figure 3.3e & f). *Ddc-Gal4* $^{HL4.36}$*; UAS-Hip1*^{L6} displayed a slight reduction in longevity, having a median lifespan of 60 days, and depreciated locomotor function. *Ddc-Gal4* $^{HL4.36}$*; UAS-Hip1*ΔANTH $^{S11.2}$ (LD50 = 62) displayed a reduced motor function, but survival was not significantly different when compared to the

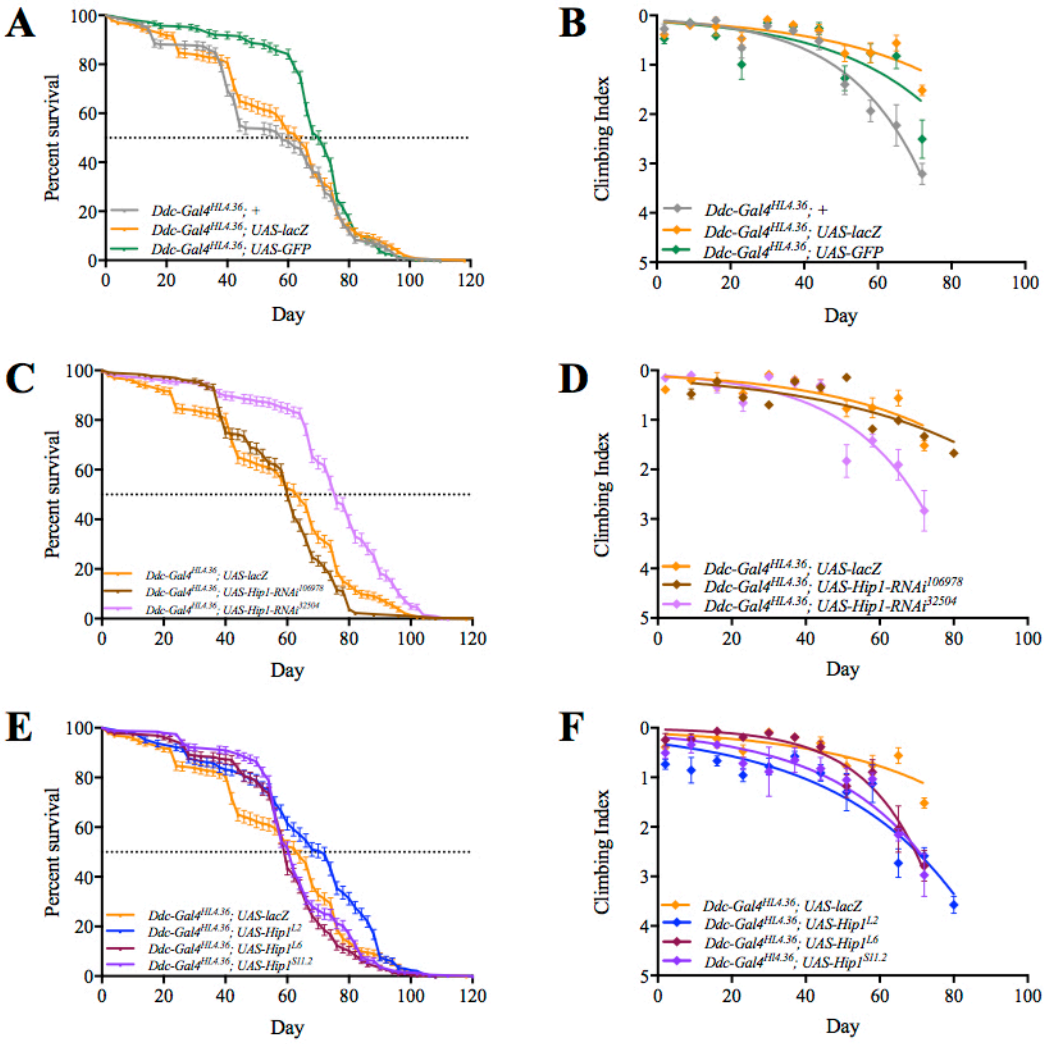


Figure 3.3 - Altered expression of *Hip1* through the *Ddc-Gal4*$^{HL4.36}$ transgene affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥324). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *Ddc-Gal4* $^{HL4.3D}$*; +* (w^{1118}), *Ddc-Gal4* $^{HL4.3D}$*; UAS-lacZ, Ddc-Gal4* $^{HL4.3D}$*; UAS-GFP, Ddc-Gal4* $^{HL4.3D}$*; UAS-Hip1-RNAi*^{106978}, *Ddc-Gal4* $^{HL4.3D}$*; UAS-Hip1-RNAi*^{32504}, *Ddc-Gal4* $^{HL4.3D}$*; UAS-Hip1*^{L2},

Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*^{L6}*, Ddc-Gal4*$^{HL4.3D}$*; UAS-Hip1*ΔANTH$^{S11.2}$. See Appendix F for supplemental data.

Ddc-Gal4$^{HL4.36}$*; UAS-lacZ* control (Figure 3.3e & f). It is important to note that the entire curve, not just the median survival is considered when determining significance.

A final analysis, this one utilizing a composite line, *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$, that was originally constructed to increase the expression of *Ddc-Gal4* was completed to determine the effects of altered *Hip1* expression in the dopaminergic neurons. This combined transgenic stock seems to be less active than anticipated based on the results observed. No significant difference was observed in a survival analysis comparing *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; +* (LD50 = 70), *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* (LD50 = 63) and *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-GFP* (LD50 = 68) (Figure 3.4a). *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; +* flies demonstrated a slight increase in climbing ability (Figure 3.4b). For the purpose of this analysis, the flies expressing the benign responder (*UAS-lacZ*), as in *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ,* will function as the control.

Reduced dopaminergic expression through RNAi in *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1-RNAi*^{32504}*,* males revealed a decrease in motor ability and an increase in lifespan (LD50 = 76) compared to *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* (LD50 = 63) upon expression through *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$ (Figure 3.4b & c). These changes in lifespan and motor ability were not observed upon loss of function of Hip1 in *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1-RNAi*^{106978} (LD50 = 60) (Figure 3.4b & c).

Overexpression of full length *Hip1* in the dopaminergic neurons through the double *Ddc-Gal4* transgenes, in *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1*^{L2} and *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1*^{L6} flies and of truncated *Hip1, in Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1*ΔANTH$^{S11.2}$ flies displayed reduced average lifespans of 52,

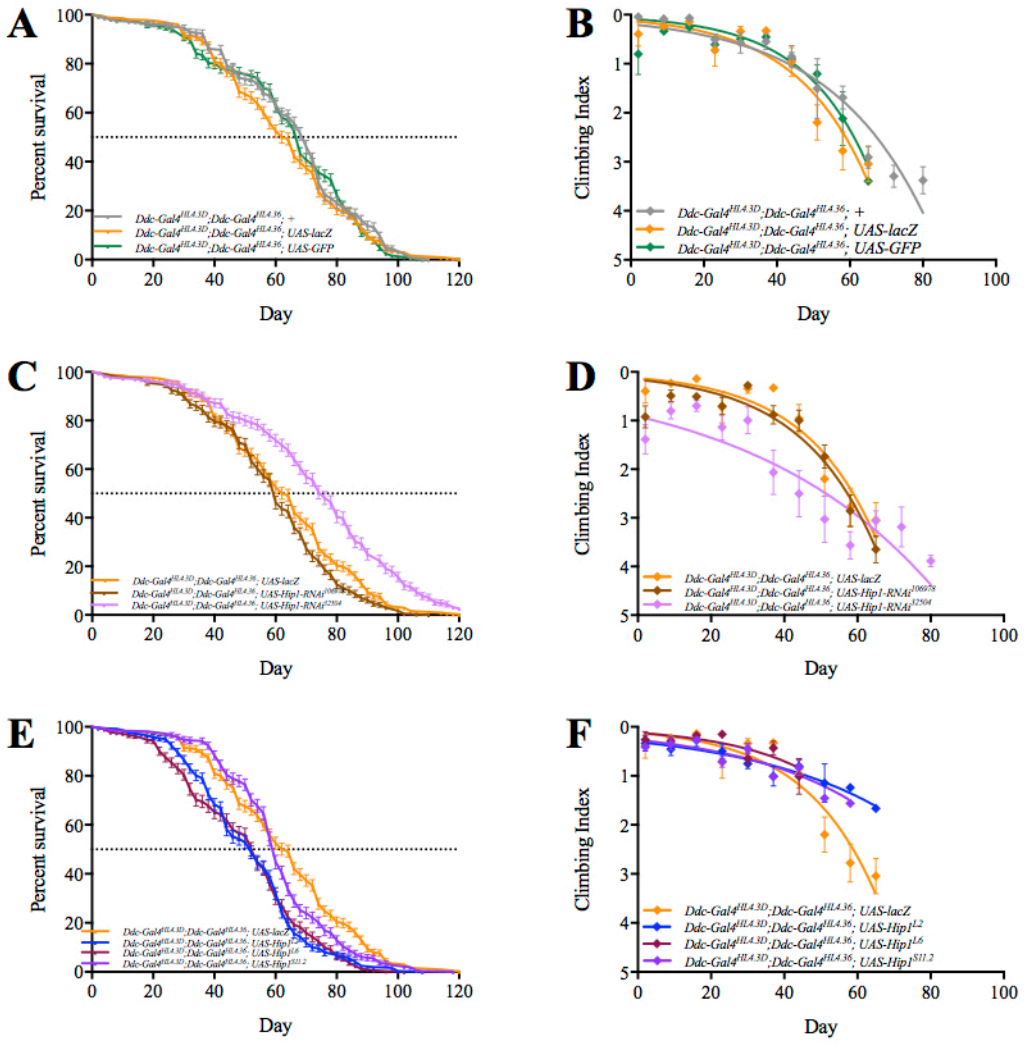


Figure 3.4 - Altered expression of *Hip1* through the combined *Ddc-Gal4*$^{HL4.3D}$and *Ddc-Gal4*$^{HL4.36}$ transgenes affects longevity and motor function. A, C and E: Longevity assay of *Drosophila melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥322). B, D and F: Locomotor assay of *D. melanogaster* males displaying altered *Hip1* expression in the dopaminergic neurons. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; + (w^{1118}), *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-lacZ, Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-GFP, Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{106978}, *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{32504}, *Ddc-Gal4*$^{HL4.3D}$;

Ddc-Gal4$^{HL4.36}$*; UAS-Hip1*^{L2}*, Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$ *; UAS-Hip1*^{L6}*, Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hip1*ΔANTH$^{S11.2}$. See Appendix G for supplemental data.

53 and 60 days respectively, compared to the 63 days displayed by *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* (Figure 3.4d). Upon examination of locomotor ability, an increase in locomotor ability was observed with expression of *UAS-Hip1*^{L2}, *UAS-Hip1*^{L6} and *UAS-Hip1*$^{S11.2}$ via the double *Ddc-Gal4* transgenes when compared to the *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* control (Figure 3.4e).

3.4 Discussion

The progression and pathogenesis of neurodegenerative diseases has long been a focus of scientific research. New technology has allowed the discovery of new genes, many of which still have unknown functions and implications. Characterized by loss of cognitive ability and motor function, the degradation of the dopaminergic neurons and the formation of Lewy bodies is the most commonly recognized pathogenic marker of Parkinson Disease. Similar symptoms are exhibited by HD patients, although the primary cause is suspected to be an expansion of a CAG repeat in the *Htt* gene, with degeneration beginning in the GABAergic neurons (9, 10). While a number of genes have been implicated, the molecular mechanisms and cellular pathways behind the progression of HD and PD remain largely unknown (7, 8, 15, 17). After being identified as a risk factor in a genome wide association study, the precise role of *Hip1R* in the pathogenesis of Parkinson Disease is under investigation (24). Involved in endocytosis, clathrin assembly, chromosomal segregation and apoptosis, Hip1R is a great candidate for a role in disease progression (29, 54, 55). *Hip1R* is considered an orthologue of *Hip1*, which has been implicated in the pathogenesis of HD (14, 59). Involved in trafficking, endocytosis, neurogenesis and apoptosis, the two proteins share similar but distinctive cellular

functions (9, 11-14, 26, 27). An understanding of the functions of *Hip1* and *Hip1R* in the pathogenesis of HD and PD will aid in understanding the disease itself.

D. melanogaster was used as a model to examine the role of *Hip1R* in PD pathogenesis, as the Hip1 protein is conserved across species, sharing domains with human Hip1 and Hip1R (Figure 1.2)(14). This protein conservation across species suggests a functionally important role, and thus alterations or mutations of this gene may have a clinical impact. To investigate the implications of *Hip1R* in Parkinson disease, targeted expression of *Hip1* was altered in the dopaminergic neurons of *D. melanogaster*. Loss of function of *Hip1* through *ple-Gal4* and *Ddc-Gal4* generally increased the average lifespan of the fly, but depressed locomotor skills (Figure 3.1c, d; 3.2c, d; 3.3c, d & 3.4c, d). Cohorts of individuals expressing the inhibitory transgene *UAS-Hip1-RNAi*^{32504}, generally displayed a stronger phenotype than those expressing the RNAi via *UAS-Hip1-RNAi*^{106978} and this may be related to the efficiency of the *RNAi* transgene or it's inhibitory transcript. In contrast, overexpression of full length *Hip1* generally decreased the average life span of *D. melanogaster*, but improved locomotor skills over time (Figure 3.1e, f; 3.2e, f; & 3.4e, f). The exception to this was observed with the *Ddc-Gal4*$^{HL4.36}$ (Figure 3.3) that displayed an increased lifespan when directing the expression of *Hip1*^{L2}, and a reduction in motor ability through expression of *Hip1*^{L2}, *Hip1*^{L6} and *Hip1*$^{S11.2}$.

In general, groups of males that overexpress the truncated *UAS-Hip1*ΔANTH displayed results similar to those of overexpression of full length *Hip1*. This suggests that the I/LWEQ domain may be the cause of any changes observed upon expression in the

dopaminergic neurons, as loss of the ANTH domain had little effect. As human *Hip1R* lacks the I/LWEQ domain, the role for *Hip1R* in the pathogenesis of PD should be further investigated.

It should be noted that throughout the experiment *Ddc-Gal4*$^{HL4.36}$ flies had a reduced viability compared to those bearing other *Gal4* transgenes, and multiple crosses and repetitions were necessary to obtain the required sample size, possibly affecting the lifespan and locomotor ability of the flies, and skewing the results (63). Otherwise, the inverse relationship between lifespan and motor ability suggests a delicate balance of *Hip1* is required, and suggests a role for Hip1 (and potentially Hip1R) in the development of the dopaminergic neurons. A change in *Hip1* expression during development may therefore contribute to the pathogenesis of HD and PD, affecting both length and quality of life.

An investigation of the implications of altered *Hip1* and *Hip1R* expression, and how they affect disease progression is needed. Involved in neurogenesis, endocytosis and apoptosis, altered expression may produce dramatic changes in cellular function (9, 11, 13, 14). The investigation of the role of *Hip1* in Huntington Disease and *Hip1R* in Parkinson Disease is just beginning, and the impact it may have on the understanding of disease pathogenesis and potential treatments is notable. A thorough and precise understanding of all genes involved in disease progression, their functions, interactions and their implications will greatly advance treatment and potentially provide a cure for Parkinson Disease.

3.5 References

5. Kitada T, Asakawa S, Matsumine H, Hattori N, Shimura H, Minoshima S, et al. Progress in the clinical and molecular genetics of familial parkinsonism. Neurogenetics. 2000;2(4):207-18. PubMed PMID: 10983716.

7. Walling HW, Baldassare JJ, Westfall TC. Molecular aspects of Huntington's disease. Journal of Neuroscience Research. 1998;54(3):301-8. doi: 10.1002/(SICI)1097-4547(19981101)54:3<301::AID-JNR1>3.0.CO;2-W.

8. O'Keeffe GC, Michell AW, Barker RA. Biomarkers in Huntington's and Parkinson's Disease. Annals of the New York Academy of Sciences. 2009;1180(1):97-110. doi: 10.1111/j.1749-6632.2009.04943.x.

9. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics. 2004;20(3):146-54. doi: http://dx.doi.org/10.1016/j.tig.2004.01.008.

10. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971-83. doi: http://dx.doi.org/10.1016/0092-8674(93)90585-E.

11. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst C-A, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4(2):95-105.

12. Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, et al. Huntingtin Interacting Protein 1 Is a Clathrin Coat Binding Protein Required for Differentiation of late Spermatogenic Progenitors. Molecular and Cellular Biology. 2001;21(22):7796-806. doi: 10.1128/MCB.21.22.7796-7806.2001. PubMed PMID: PMC99949.

13. Rao DS, Hyun TS, Kumar PD, Mizukami IF, Rubin MA, Lucas PC, et al. Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival. The Journal of Clinical Investigation. 2002;110(3):351-60. doi: 10.1172/JCI15529. PubMed PMID: PMC151092.

14. Moores JN, Roy S, Nicholson DW, Staveley BE. Huntingtin interacting protein 1 can regulate neurogenesis in Drosophila. The European journal of neuroscience. 2008;28(3):599-609. Epub 2008/08/16. doi: 10.1111/j.1460-9568.2008.06359.x. PubMed PMID: 18702731.

15. Schiesling C, Kieper N, Seidel K, Kruger R. Review: Familial Parkinson's disease--genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol Appl Neurobiol. 2008;34(3):255-71. doi: 10.1111/j.1365-2990.2008.00952.x. PubMed PMID: 18447897.

16. Trinh J, Gustavsson EK, Guella I, Vilarino-Guell C, Evans D, Encarnacion M, et al. The role of SNCA and MAPT in Parkinson disease and LRRK2 parkinsonism in the Tunisian Arab-Berber population. Eur J Neurol. 2014;21(11):e91-2. doi: 10.1111/ene.12489. PubMed PMID: 25303626.

17. de Lau LM, Giesbergen PC, de Rijk MC, Hofman A, Koudstaal PJ, Breteler MM. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. Neurology. 2004;63(7):1240-4. PubMed PMID: 15477545.

20. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. Genome / National Research Council Canada = Genome / Conseil national de recherches Canada. 2008;51(12):1040-6. Epub 2008/12/18. doi: 10.1139/g08-085. PubMed PMID: 19088817.
21. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. Science (New York, NY). 1997;276(5321):2045-7. doi: 10.1126/science.276.5321.2045.
22. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998;395(6701):451-2.
23. Castillo-Quan JI. Parkin' control: regulation of PGC-1α through PARIS in Parkinson's disease. Disease Models & Mechanisms. 2011;4(4):427-9. doi: 10.1242/dmm.008227. PubMed PMID: PMC3124045.
24. Sharma M, Ioannidis JP, Aasly JO, Annesi G, Brice A, Van Broeckhoven C, et al. Large-scale replication and heterogeneity in Parkinson disease genetic loci. Neurology. 2012;79(7):659-67. Epub 2012/07/13. doi: 10.1212/WNL.0b013e318264e353. PubMed PMID: 22786590; PubMed Central PMCID: PMCPMC3414661.
25. Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS genetics. 2012;8(3):e1002548. Epub 2012/03/23. doi: 10.1371/journal.pgen.1002548. PubMed PMID: 22438815; PubMed Central PMCID: PMCPMC3305333.
26. Bradley SV, Hyun TS, Oravecz-Wilson KI, Li L, Waldorff EI, Ermilov AN, et al. Degenerative phenotypes caused by the combined deficiency of murine HIP1 and HIP1r are rescued by human HIP1. Hum Mol Genet. 2007;16(11):1279-92. doi: 10.1093/hmg/ddm076. PubMed PMID: 17452370.
27. Gottfried I, Ehrlich M, Ashery U. The Sla2p/HIP1/HIP1R family: similar structure, similar function in endocytosis? Biochem Soc Trans. 2010;38(Pt 1):187-91. doi: 10.1042/BST0380187. PubMed PMID: 20074057.
29. Engqvist-Goldstein ÅEY, Warren RA, Kessels MM, Keen JH, Heuser J, Drubin DG. The actin-binding protein Hip1R associates with clathrin during early stages of endocytosis and promotes clathrin assembly in vitro. The Journal of Cell Biology. 2001;154(6):1209-24. doi: 10.1083/jcb.200106089.
42. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118(2):401-15. PubMed PMID: 8223268.
43. Yeh E, Gustafson K, Boulianne GL. Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(15):7036-40. PubMed PMID: PMC41466.
44. Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou D, et al. A genome-scale shRNA resource for transgenic RNAi in Drosophila. 2010.
47. Keleman K, Micheler T, members Vp. RNAi-phiC31 construct and insertion data submitted by the Vienna Drosophila RNAi Center. 2009.

48. Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448(7150):151-6.
49. Todd AM, Staveley BE. Novel Assay and Analysis for Measuring Climbing Ability in Drosophila. Drosophila Information Service. 2004;87:101-8. Epub December 2004.
52. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. The Lancet Neurology. 2006;5(6):525-35. Epub 2006/05/23. doi: 10.1016/s1474-4422(06)70471-9. PubMed PMID: 16713924.
53. Shin J-H, Ko HS, Kang H, Lee Y, Lee Y-I, Pletinkova O, et al. PARIS (ZNF746) Repression of PGC-1α Contributes to Neurodegeneration in Parkinson's Disease. Cell. 2011;144(5):689-702. doi: 10.1016/j.cell.2011.02.010. PubMed PMID: PMC3063894.
54. Hayashi T, Ishimori C, Takahashi-Niki K, Taira T, Kim YC, Maita H, et al. DJ-1 binds to mitochondrial complex I and maintains its activity. Biochem Biophys Res Commun. 2009;390(3):667-72. doi: 10.1016/j.bbrc.2009.10.025. PubMed PMID: 19822128.
55. Park SJ. Huntingtin-interacting protein 1-related is required for accurate congression and segregation of chromosomes. BMB reports. 2010;43(12):795-800. Epub 2010/12/30. doi: 10.5483/BMBRep.2010.43.12.795. PubMed PMID: 21189155.
56. Masuda T, Sakuma C, Ueno T, Yamada Y, Ohmomo H, Ueda S, et al. Spatiotemporal patterns of the Huntingtin-interacting protein 1-related gene in the mouse head. Congenit Anom (Kyoto). 2013;53(4):141-8. doi: 10.1111/cga.12023. PubMed PMID: 24712472.
57. Wong KK, Ch'ng ES, Loo SK, Husin A, Muruzabal MA, Moller MB, et al. Low HIP1R mRNA and protein expression are associated with worse survival in diffuse large B-cell lymphoma patients treated with R-CHOP. Experimental and molecular pathology. 2015;99(3):537-45. Epub 2015/09/06. doi: 10.1016/j.yexmp.2015.08.019. PubMed PMID: 26341140.
58. Nelson EL, Liang CL, Sinton CM, German DC. Midbrain dopaminergic neurons in the mouse: computer-assisted mapping. The Journal of comparative neurology. 1996;369(3):361-71. Epub 1996/06/03. doi: 10.1002/(SICI)1096-9861(19960603)369:3<361::AID-CNE3>3.0.CO;2-3. PubMed PMID: 8743418.
59. Seki N, Muramatsu M, Sugano S, Suzuki Y, Nakagawara A, Ohhira M, et al. Cloning, expression analysis, and chromosomal localization of HIP1R, an isolog of huntingtin interacting protein (HIP1). J Hum Genet. 1998;43(4):268-71. doi: 10.1007/s100380050087. PubMed PMID: 9852681.
60. Friggi-Grelin F, Coulom H, Meller M, Gomez D, Hirsh J, Birman S. Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. J Neurobiol. 2003;54(4):618-27. Epub 2003/01/30. doi: 10.1002/neu.10185. PubMed PMID: 12555273.
61. Li H, Chaney S, Roberts IJ, Forte M, Hirsh J. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Current biology : CB. 2000;10(4):211-4. Epub 2000/03/08. PubMed PMID: 10704417.
62. Slade FA, Staveley BE. Arm-Gal4 inheritance influences development and lifespan in Drosophila melanogaster. Genetics and molecular research : GMR.

2015;14(4):12788-96. Epub 2015/10/28. doi: 10.4238/2015.October.19.22. PubMed PMID: 26505429.
63. Haywood AFM, Saunders LD, Staveley BE. Dopa decarboxylase(Ddc)-GAL4 dramatically reduces life span. Drosophila Information Service. 2002;85:42-5.

Chapter 4 – Investigating the role of *Huntingtin interacting protein 1 protein interactor* in Huntington Disease and Parkinson Disease in a *Drosophila melanogaster* model

4.1 Introduction

Expansion of the polyglutamine tract of Htt in HD patients renders a number of the Htt protein's normal binding partners unable to bind. These binding partners, now free in the cell, have the potential to form aggregates and have downstream cellular effects (9, 10). One of the normal binding partners of Htt, Huntingtin interacting Protein 1, is unable to bind to the expanded polyglutamine tract (9). This increases the amount of free Hip1 in the cell, which is then able to form aggregates, specifically a complex with Huntingtin interacting protein 1 protein interactor (Hippi). Interacting through their pDED (coiled coil) domains, this Hip1-Hippi complex activates pro-caspase-8, and has the ability to initiate apoptosis (9, 11). Hippi has been implicated in other apoptotic pathways, including activation of caspase-1 and caspase-3 (64, 65). On the other hand, interaction of Hippi with Apoptin, a chicken anemia virus-encoded protein, seems to function in the suppression of apoptosis (66). The normal function of Hippi has been difficult to characterize, but a role has been established for the protein in regulation of transcription, Sonic hedgehog signalling, cilia assembly and neuronal development (14, 67-69). The implication of Hippi in the activation of apoptotic cell pathways and potential involvement in neurodegenerative disorders suggests a need to further investigate the role of the protein in disease progression, and may provide valuable information in the search for treatments and cures.

4.2 Materials and Methods

4.2.1 *Drosophila melanogaster* stocks and culture

The *UAS-lacZ* responder line (42), *elav-Gal4* (45), *GawB*$^{l(3)31\text{-}1}$ (46) and *ple-Gal4* (60) transgenic lines were obtained from the Bloomington *Drosophila* Stock Centre (University of Indiana, Bloomington, USA). The *UAS-Hippi-RNAi* (*Che-13*) line (47) was obtained from the Vienna Drosophila Resource Centre (Vienna Biocenter, Vienna, USA). The *Ddc-Gal4* $^{HL4.3D}$ and *Ddc-Gal4* $^{HL4.36}$ lines (61) were provided by Dr. J. Hirsch at the University of Virginia, and the *Ddc-Gal4* $^{HL4.3D}$; *Ddc-Gal4* $^{HL4.36}$ combination line was generated by standard genetic means. The expression patterns of fly lines used in this analysis and the place of insertion can be found in Table 3.

D. melanogaster stocks were cultured and maintained on media consisting of 65 g/L cornmeal, 10 g/L yeast, 5.5 g/L agar and 50 ml/L fancy grade molasses, 5 ml of 0.1 g/ml methyl 4-hydroxybenzoate in 95% ethanol and 2.5 ml of propionic acid at 25°C. All experiments were at 25° C.

Crosses were set up by placing three to five *UAS* virgin females of the corresponding genotype and two to five Gal4 males together on standard media. Parental flies were placed on new media at day 2, 4 and 6 after experimental set up, to increase the number of progeny. Critical class males were selected.

4.2.2 Longevity assay

Critical class male progeny were separated on the day of eclosure and maintained at 25°C on standard media in vials of 25 or less. Flies were scored every second day for the presence of deceased individuals, and media was regularly changed to ensure ideal conditions ($N \geq 208$). Data was analyzed using GraphPad Prism 5.0c or 7.0b (GraphPad

Software, Inc., San Diego, California, USA), survival curves were generated and a Mantel-Cox test was performed to determine significance ($P < 0.05$).

Table 4.1 - Genotypes, location of transgene insertion and expression patterns used in the analysis of the loss of function of *Hippi* expressed in a pan-neuronal fashion and in the dopaminergic neurons to determine their role in the pathogenesis of neurodegenerative disease.

Genotype	Insertion Chromosome	Affected Chromosome	Expression Patterns
UAS-lacZ	2	1;2	Expresses *lacZ* under *UAS* control
UAS-Hippi-RNAi	2	1;2	Expressed *dsRNAi* for *Hippi* under *UAS* control
elav-Gal4	1	1	*Gal4* expressed in all tissues of the embryonic nervous system beginning at stage 12
$GawB^{l(3)31\text{-}1}$	3	1;3	*Gal4* expressed in neuroblasts and neurons
ple-Gal4	3	1;3	*Gal4* expressed in dopaminergic cells
$Ddc\text{-}Gal4^{HL4.3D}$	2	1;2	*Gal4* expressed in the dopaminergic and serotonergic neurons
$Ddc\text{-}Gal4^{HL4.36}$	3	1;3	*Gal4* expressed in the dopaminergic and serotonergic neurons
$Ddc\text{-}Gal4^{HL4.3D}$; $Ddc\text{-}Gal4^{HL4.36}$	2 & 3	1;2;3	*Gal4* expressed in the dopaminergic and serotonergic neurons

4.2.3 Locomotor assay

Critical class male progeny were separated on the day of eclosure and maintained at 25°C on fresh standard media in vials of 10 or less. Starting at day 2 post eclosion, and at regular seven day intervals afterward, flies were scored for climbing ability by placing them in glass tubes, and recording the maximum level reached within 10 seconds (20). A climbing index was calculated using the formula described below:

Climbing Index = (nm/N)

where n=number of flies at level, m=score for the level and N=total number of flies climbed (49).

50 individuals from each genotype were analyzed in groups of 10 or less (N = 50). Each group underwent a total of 10 trials, providing a total trial number of 500 flies per genotype per week. This analysis was continued until less than 10 flies remained alive or flies received a minimum score for two consecutive sessions. A climbing curve (non-linear regression curve) was generated using GraphPad Prism 5.0c or 7.0b software (GraphPad Software, Inc., San Diego, California, USA). A comparison of fits of the non-linear regression curves taking the Y-intercept (initial climbing ability) and slope (rate of decline in climbing ability) into consideration concluded if the curves were significantly different ($P < 0.05$).

4.3 Results

Loss of function of *Hippi* (*Che-13*), in *elav-Gal4; UAS-Hippi-RNAi* revealed a reduction in both lifespan (median lifespan = 64 days) and locomotor function compared to the *elav-Gal4; UAS-lacZ* control (median lifespan = 70 days) (Figure 4.1a & b), establishing a role for *Hippi* in HD pathogenesis. Loss of function of *Hippi* (*Che-13*), in *GawB*$^{l(3)31\text{-}1}$*; UAS-Hippi-RNAi* revealed an increase in average lifespan (LD50 = 64) and an increase in motor function compared to the *UAS-lacZ* control, providing further evidence that *Hippi* may play a role in neurogenesis and disease pathogenesis, but its expression patterns may affect the outcome. A loss of function analysis of *Hippi* (*Che-13*), in *ple-Gal4; UAS-Hippi-RNAi* males resulted in a lifespan of 70 days, identical to the

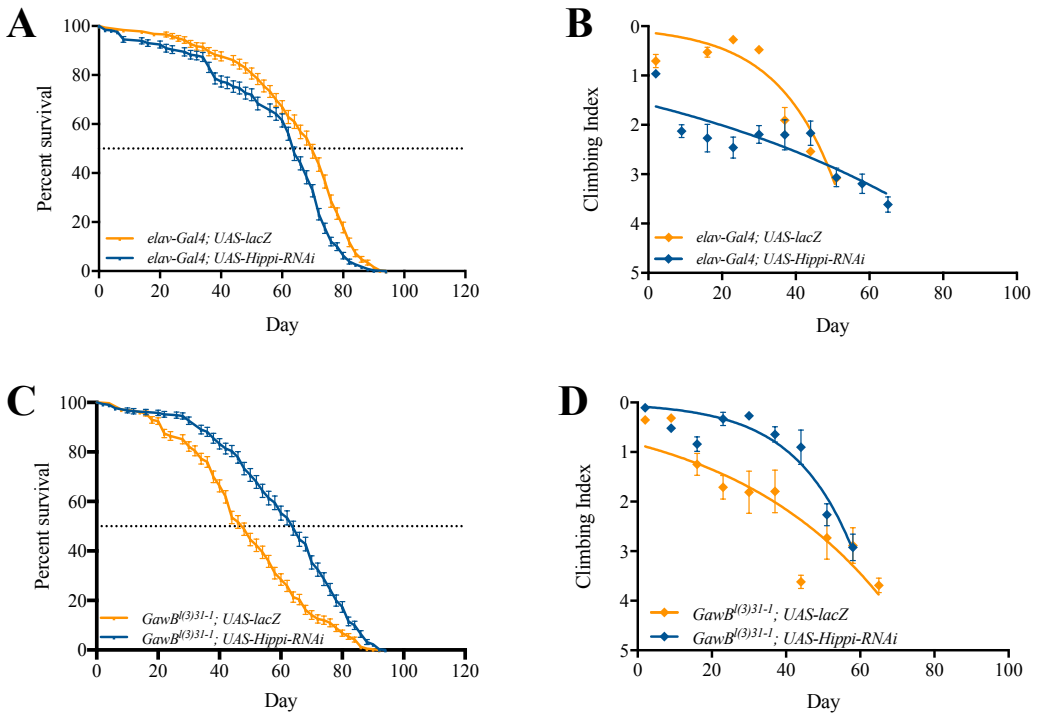


Figure 4.1 – Altered expression of *Hippi* through the *elav-Gal4* and the *GawB*$^{l(3)3-1}$ *-Gal4* transgene affects longevity and motor function. A and C: Longevity assay of *Drosophila melanogaster* males displaying reduced *Hippi* expression in a pan-neuronal fashion. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of flies. Error bars represent standard error of the mean (N≥311). B and D: Locomotor assay of *D. melanogaster* males displaying reduced *Hippi* expression in a pan-neuronal fashion. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *elav-Gal4; UAS-lacZ, elav-Gal4; UAS-Hippi-RNAi*, *GawB*$^{l(3)3-1}$*; UAS-lacZ* and *GawB*$^{l(3)31-1}$*; UAS-Hippi-RNAi*. See Appendix H for supplemental data.

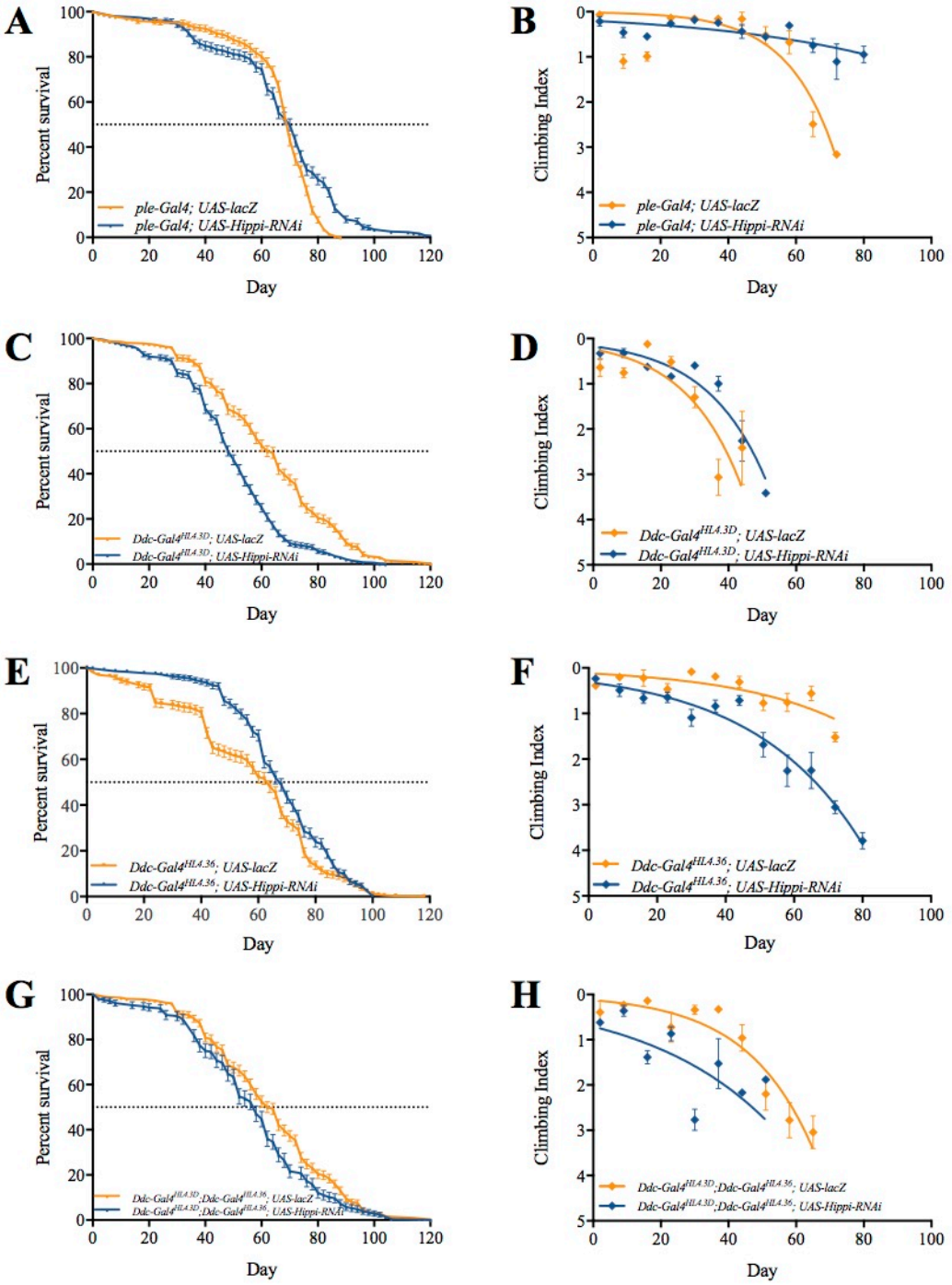


Figure 4.2 – Altered expression of *Hippi* in the dopaminergic neurons affects longevity and motor function. A, C, E and G: Longevity assay of *D. melanogaster* males displaying reduced *Hippi* expression in the dopaminergic neurons. Longevity is shown as percent survival ($P < 0.05$, determined by log rank). The dotted line represents median survival of

flies. Error bars represent standard error of the mean (N≥208). B, D, F and G: Locomotor assay of *D. melanogaster* males displaying reduced *Hippi* expression in the dopaminergic neurons. Climbing ability was determined via nonlinear curve fit (CI=95%). Error bars indicate standard error of the mean, N = 50. Genotypes are as follows: *ple-Gal4; UAS-lacZ, ple-Gal4; UAS-Hippi-RNAi, Ddc-Gal4$^{HL4.3D}$; UAS-lacZ, Ddc-Gal4$^{HL4.3D}$; UAS-Hippi-RNAi, Ddc-Gal4$^{HL4.3D}$; UAS-lacZ, Ddc-Gal4$^{HL4.3D}$; UAS-Hippi-RNAi, Ddc-Gal4$^{HL4.3D}$*; *Ddc-Gal4$^{HL4.36}$; UAS-lacZ* and *Ddc-Gal4$^{HL4.3D}$*; *Ddc-Gal4$^{HL4.36}$; UAS-Hippi-RNAi.* See Appendix I for supplemental data.

UAS-lacZ control (Figure 4.2a). Locomotor ability of *UAS-Hippi-RNAi* flies remained relatively steady until death, displaying an increase in motor function compared to *UAS-lacZ* (Figure 4.2b). Loss of function of *Hippi* (*Che-13*) directed to dopaminergic (and serotonergic) neurons in *Ddc-Gal4*$^{HL4.3D}$*; UAS-Hippi-RNAi* critical class cohorts displayed a reduction in median lifespan (LD50 = 50) and an increase in locomotor ability (Figure 4.2c & d). A comparison of the inhibition of *Hippi* in *Ddc-Gal4*$^{HL4.36}$*; UAS-Hippi-RNAi flies* revealed an increased survival with a median lifespan of 68 days and a decline in motor function compared to *UAS-lacZ* (LD50 = 64) (Figure 4.2e & f).

Finally, a reduction in *Hippi* expression in *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hippi-RNAi* displayed a reduction in lifespan (LD50 = 58) and motor ability compared to *Ddc-Gal4*$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ* (LD50 = 63) (Figure 4.2g & h).

4.4 Discussion

The loss of function of *Hippi* expressed in a pan-neuronal fashion and in the dopaminergic neurons displayed effects on longevity and motor control, but the results are inconclusive, varying based on the transgene used to drive expression. This suggests that *Hippi* may play a role in neurodegenerative disease, but further investigation into the role of *Hippi* in HD pathogenesis is needed. Investigations into the effects of *Hip1* and *Hippi* expression levels in higher organisms may be beneficial in the search for a cure and treatments for HD. The loss of function of *Hippi* through the pan-neuronal *elav-Gal4* transgene displayed a reduction in longevity and motor ability (Figure 4.1a, b), while loss of function with the *GawB*$^{l(3)31-1}$ transgene displayed an increase in longevity and motor ability (Figure 4.1c & d). Loss of function in the dopaminergic neurons displayed inconclusive results, with increases and decreases in lifespan and motor ability varying

based on the transgene (Figure 4.2). Although no conclusive results can be drawn from this data, it is plausible that the regulatory role of *Hippi* and its implication in the activation of apoptosis may contribute to the results observed (11, 14, 68). More data is needed to determine the role of *Hippi* in the pathogenesis of neurodegenerative disease. It would be beneficial to investigate the combined effects of altered *Hip1* and *Hippi* expression, to determine the effects on a cellular and tissue level.

4.5 References

9. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics. 2004;20(3):146-54. doi: http://dx.doi.org/10.1016/j.tig.2004.01.008.

10. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971-83. doi: http://dx.doi.org/10.1016/0092-8674(93)90585-E.

11. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst C-A, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4(2):95-105.

14. Moores JN, Roy S, Nicholson DW, Staveley BE. Huntingtin interacting protein 1 can regulate neurogenesis in Drosophila. The European journal of neuroscience. 2008;28(3):599-609. Epub 2008/08/16. doi: 10.1111/j.1460-9568.2008.06359.x. PubMed PMID: 18702731.

20. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. Genome / National Research Council Canada = Genome / Conseil national de recherches Canada. 2008;51(12):1040-6. Epub 2008/12/18. doi: 10.1139/g08-085. PubMed PMID: 19088817.

42. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118(2):401-15. PubMed PMID: 8223268.

45. Lin DM, Goodman CS. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron. 1994;13(3):507-23. Epub 1994/09/01. PubMed PMID: 7917288.

46. Ito K, Awano W, Suzuki K, Hiromi Y, Yamamoto D. The Drosophila mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development. 1997;124(4):761-71.

47. Keleman K, Micheler T, members Vp. RNAi-phiC31 construct and insertion data submitted by the Vienna Drosophila RNAi Center. 2009.

49. Todd AM, Staveley BE. Novel Assay and Analysis for Measuring Climbing Ability in Drosophila. Drosophila Information Service. 2004;87:101-8. Epub December 2004.

60. Friggi-Grelin F, Coulom H, Meller M, Gomez D, Hirsh J, Birman S. Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. J Neurobiol. 2003;54(4):618-27. Epub 2003/01/30. doi: 10.1002/neu.10185. PubMed PMID: 12555273.

61. Li H, Chaney S, Roberts IJ, Forte M, Hirsh J. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Current biology : CB. 2000;10(4):211-4. Epub 2000/03/08. PubMed PMID: 10704417.

64. Majumder P, Chattopadhyay B, Mazumder A, Das P, Bhattacharyya NP. Induction of apoptosis in cells expressing exogenous Hippi, a molecular partner of

huntingtin-interacting protein Hip1. Neurobiology of Disease. 2006;22(2):242-56. doi: http://dx.doi.org/10.1016/j.nbd.2005.11.003.
65. Wanker EE. Hip1 and Hippi Participate in a Novel Cell Death-Signaling Pathway. Developmental Cell. 2002;2(2):126-8. doi: http://dx.doi.org/10.1016/S1534-5807(02)00121-1.
66. Cheng C-M, Huang S-p, Chang Y-F, Chung W-Y, Yuo C-Y. The viral death protein Apoptin interacts with Hippi, the protein interactor of Huntingtin-interacting protein 1. Biochemical and Biophysical Research Communications. 2003;305(2):359-64. doi: http://dx.doi.org/10.1016/S0006-291X(03)00764-2.
67. Stanton SE, Blanck JK, Locker J, Schreiber-Agus N. Rybp interacts with Hippi and enhances Hippi-mediated apoptosis. Apoptosis. 2007;12(12):2197-206. doi: 10.1007/s10495-007-0131-3.
68. Houde C, Dickinson RJ, Houtzager VM, Cullum R, Montpetit R, Metzler M, et al. Hippi is essential for node cilia assembly and Sonic hedgehog signaling. Developmental Biology. 2006;300(2):523-33. doi: https://doi.org/10.1016/j.ydbio.2006.09.001.
69. Datta M, Choudhury A, Lahiri A, Bhattacharyya NP. Genome wide gene expression regulation by HIP1 Protein Interactor, HIPPI: Prediction and validation. BMC Genomics. 2011;12(1):463. doi: 10.1186/1471-2164-12-463.

Chapter 5 - Summary

The search for treatments and cures for neurodegenerative diseases is ongoing, and the use of model organisms provides a simple way to investigate the consequences of the disease pathways. The reduction in *D. melanogaster* lifespan and motor ability observed upon pan-neuronal overexpression of *Hip1* levels is an attempt to mimic the effects seen in HD patients, as the expansion of the polyglutamine tract results in increased cytoplasmic Hip1 (9). Flies subjected to loss of function of *Hip1* displayed an increased average lifespan and reduced motor ability, providing a model of neurodegenerative disease. This provides a clue into the role that Hip1 plays in the cell and how it may contribute to a number of symptoms associated with HD and neurodegenerative disease. Further investigation into the role of Hip1 in healthy individuals and the mechanism by which its expression is altered in diseased individuals may lead to novel treatment options for HD patients.

The conservation of *Hip1* and *Hip1R* across species makes investigating the effects of altered *Hip1* expression specifically in the dopaminergic neurons possible (14). This can provide a model of Parkinson Disease, and the results observed are similar to those seen in pan-neuronal expression; an increase in lifespan and reduction in motor ability upon loss of function of *Hip1*, and a reduction in lifespan upon overexpression. Interestingly, overexpression of *Hip1* in the dopaminergic neurons increases average motor ability, suggesting that an increased level of *Hip1* in the dopaminergic neurons may actually be beneficial in terms of motor skills, but still reduces average lifespan. The current understanding of the role of *Hip1R* in Parkinson Disease is limited, and

expanding on this knowledge may prove beneficial in the search for novel treatments and cures for PD.

Investigating the loss of *Hippi* expression was inconclusive, providing inconsistent results dependent upon the Gal4 transgene utilized. As the Gal4 transgenes possess different expression patterns, the possibility remains that loss of function of Hippi in other neuronal patterns, such as through *arm-Gal4* or *Ple-Gal4* affects longevity and motor ability, and that interactions of Hippi with other proteins is vital for neuronal development (51, 62). The observation that reduced *Hippi* expression altered the lifespan and motor ability of flies suggests a need for further investigation into its effects on neurodegenerative disease. As a binding partner of Hip1, and with the Hip1-Hippi complex potentially involved in the initiation of apoptosis, the altered effects of *Hippi* expression could contribute to disease phenotypes (11). A more in depth understanding of the role of *Hippi* and the effects of its altered expression on the cell is needed to broaden our knowledge of HD pathogenesis.

Neurodegenerative diseases are an increasing problem in today's ageing population, and the molecular mechanisms and cellular pathways of many are still under investigation. The implication of *Hip1* in Huntington Disease and *Hip1R* in Parkinson Disease may be of clinical significance, as its expression affects the lifespan and motor ability of *Drosophila melanogaster*. Investigation into the role of *Hip1* and *Hip1R* in the pathogenesis of neurodegenerative disease may provide novel treatment targets and therapeutic options.

Chapter 6 - References

1. Merzetti EM, Staveley BE. Mitochondrial dynamics in degenerative disease and disease models. Neuroscience Discovery. 2013;1(1). doi: 10.7243/2052-6946-1-8.
2. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Frontiers in cellular neuroscience. 2015;9:124. Epub 2015/04/29. doi: 10.3389/fncel.2015.00124. PubMed PMID: 25914621; PubMed Central PMCID: PMCPMC4392704.
3. Bereznai B, Molnar MJ. [Genetics and present therapy options in Parkinson's disease: a review]. Ideggyogyaszati szemle. 2009;62(5-6):155-63. Epub 2009/07/08. PubMed PMID: 19579663.
4. Deng H-X, Shi Y, Yang Y, Ahmeti KB, Miller N, Huang C, et al. Identification of TMEM230 mutations in familial Parkinson's disease. Nature genetics. 2016;48(7):733-9. doi: 10.1038/ng.3589 http://www.nature.com/ng/journal/v48/n7/abs/ng.3589.html - supplementary-information.
5. Kitada T, Asakawa S, Matsumine H, Hattori N, Shimura H, Minoshima S, et al. Progress in the clinical and molecular genetics of familial parkinsonism. Neurogenetics. 2000;2(4):207-18. PubMed PMID: 10983716.
6. Petersén Å, Mani K, Brundin P. Recent Advances on the Pathogenesis of Huntington's Disease. Experimental Neurology. 1999;157(1):1-18. doi: http://dx.doi.org/10.1006/exnr.1998.7006.
7. Walling HW, Baldassare JJ, Westfall TC. Molecular aspects of Huntington's disease. Journal of Neuroscience Research. 1998;54(3):301-8. doi: 10.1002/(SICI)1097-4547(19981101)54:3<301::AID-JNR1>3.0.CO;2-W.
8. O'Keeffe GC, Michell AW, Barker RA. Biomarkers in Huntington's and Parkinson's Disease. Annals of the New York Academy of Sciences. 2009;1180(1):97-110. doi: 10.1111/j.1749-6632.2009.04943.x.
9. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics. 2004;20(3):146-54. doi: http://dx.doi.org/10.1016/j.tig.2004.01.008.
10. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971-83. doi: http://dx.doi.org/10.1016/0092-8674(93)90585-E.
11. Gervais FG, Singaraja R, Xanthoudakis S, Gutekunst C-A, Leavitt BR, Metzler M, et al. Recruitment and activation of caspase-8 by the Huntingtin-interacting protein Hip-1 and a novel partner Hippi. Nat Cell Biol. 2002;4(2):95-105.
12. Rao DS, Chang JC, Kumar PD, Mizukami I, Smithson GM, Bradley SV, et al. Huntingtin Interacting Protein 1 Is a Clathrin Coat Binding Protein Required for Differentiation of late Spermatogenic Progenitors. Molecular and Cellular Biology. 2001;21(22):7796-806. doi: 10.1128/MCB.21.22.7796-7806.2001. PubMed PMID: PMC99949.

13. Rao DS, Hyun TS, Kumar PD, Mizukami IF, Rubin MA, Lucas PC, et al. Huntingtin-interacting protein 1 is overexpressed in prostate and colon cancer and is critical for cellular survival. The Journal of Clinical Investigation. 2002;110(3):351-60. doi: 10.1172/JCI15529. PubMed PMID: PMC151092.
14. Moores JN, Roy S, Nicholson DW, Staveley BE. Huntingtin interacting protein 1 can regulate neurogenesis in Drosophila. The European journal of neuroscience. 2008;28(3):599-609. Epub 2008/08/16. doi: 10.1111/j.1460-9568.2008.06359.x. PubMed PMID: 18702731.
15. Schiesling C, Kieper N, Seidel K, Kruger R. Review: Familial Parkinson's disease--genetics, clinical phenotype and neuropathology in relation to the common sporadic form of the disease. Neuropathol Appl Neurobiol. 2008;34(3):255-71. doi: 10.1111/j.1365-2990.2008.00952.x. PubMed PMID: 18447897.
16. Trinh J, Gustavsson EK, Guella I, Vilarino-Guell C, Evans D, Encarnacion M, et al. The role of SNCA and MAPT in Parkinson disease and LRRK2 parkinsonism in the Tunisian Arab-Berber population. Eur J Neurol. 2014;21(11):e91-2. doi: 10.1111/ene.12489. PubMed PMID: 25303626.
17. de Lau LM, Giesbergen PC, de Rijk MC, Hofman A, Koudstaal PJ, Breteler MM. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. Neurology. 2004;63(7):1240-4. PubMed PMID: 15477545.
18. Vanitallie TB. Parkinson disease: primacy of age as a risk factor for mitochondrial dysfunction. Metabolism: clinical and experimental. 2008;57 Suppl 2:S50-5. Epub 2008/09/23. doi: 10.1016/j.metabol.2008.07.015. PubMed PMID: 18803967.
19. Labbé C, Ross OA. Association Studies of Sporadic Parkinson's Disease in the Genomic Era. Current Genomics. 2014;15(1):2-10. doi: 10.2174/1389202914666131210212745. PubMed PMID: 24653658; PubMed Central PMCID: PMCPMC3958956.
20. Todd AM, Staveley BE. Pink1 suppresses alpha-synuclein-induced phenotypes in a Drosophila model of Parkinson's disease. Genome / National Research Council Canada = Genome / Conseil national de recherches Canada. 2008;51(12):1040-6. Epub 2008/12/18. doi: 10.1139/g08-085. PubMed PMID: 19088817.
21. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. Science (New York, NY). 1997;276(5321):2045-7. doi: 10.1126/science.276.5321.2045.
22. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998;395(6701):451-2.
23. Castillo-Quan JI. Parkin' control: regulation of PGC-1α through PARIS in Parkinson's disease. Disease Models & Mechanisms. 2011;4(4):427-9. doi: 10.1242/dmm.008227. PubMed PMID: PMC3124045.
24. Sharma M, Ioannidis JP, Aasly JO, Annesi G, Brice A, Van Broeckhoven C, et al. Large-scale replication and heterogeneity in Parkinson disease genetic loci. Neurology. 2012;79(7):659-67. Epub 2012/07/13. doi: 10.1212/WNL.0b013e318264e353. PubMed PMID: 22786590; PubMed Central PMCID: PMCPMC3414661.
25. Lill CM, Roehr JT, McQueen MB, Kavvoura FK, Bagade S, Schjeide BM, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS genetics. 2012;8(3):e1002548. Epub 2012/03/23.

doi: 10.1371/journal.pgen.1002548. PubMed PMID: 22438815; PubMed Central PMCID: PMCPMC3305333.
26. Bradley SV, Hyun TS, Oravecz-Wilson KI, Li L, Waldorff EI, Ermilov AN, et al. Degenerative phenotypes caused by the combined deficiency of murine HIP1 and HIP1r are rescued by human HIP1. Hum Mol Genet. 2007;16(11):1279-92. doi: 10.1093/hmg/ddm076. PubMed PMID: 17452370.
27. Gottfried I, Ehrlich M, Ashery U. The Sla2p/HIP1/HIP1R family: similar structure, similar function in endocytosis? Biochem Soc Trans. 2010;38(Pt 1):187-91. doi: 10.1042/BST0380187. PubMed PMID: 20074057.
28. Niu Q, Ybe JA. Crystal Structure at 2.8 Å of Huntingtin-Interacting Protein 1 (HIP1) Coiled-Coil Domain Reveals a Charged Surface Suitable for HIP1 Protein Interactor (HIPPI). Journal of Molecular Biology. 2008;375(5):1197-205. doi: https://doi.org/10.1016/j.jmb.2007.11.036.
29. Engqvist-Goldstein ÅEY, Warren RA, Kessels MM, Keen JH, Heuser J, Drubin DG. The actin-binding protein Hip1R associates with clathrin during early stages of endocytosis and promotes clathrin assembly in vitro. The Journal of Cell Biology. 2001;154(6):1209-24. doi: 10.1083/jcb.200106089.
30. Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. ENTH/ANTH proteins and clathrin-mediated membrane budding. Journal of cell science. 2004;117(Pt 1):9-18. Epub 2003/12/06. doi: 10.1242/jcs.00928. PubMed PMID: 14657269.
31. Wilbur JD, Chen C-Y, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM. Actin binding by huntingtin-interacting protein 1 (hip1) and hip1-related protein (Hip1R) is regulated by clathrin light chain. Journal of Biological Chemistry. 2008. doi: 10.1074/jbc.M802863200.
32. Marsh JL, Pallos J, Thompson LM. Fly models of Huntington's disease. Hum Mol Genet. 2003;12 Spec No 2:R187-93. Epub 2003/08/20. doi: 10.1093/hmg/ddg271. PubMed PMID: 12925571.
33. Celotto AM, Palladino MJ. Drosophila: a "model" model system to study neurodegeneration. Molecular interventions. 2005;5(5):292-303. Epub 2005/10/27. doi: 10.1124/mi.5.5.9. PubMed PMID: 16249525.
34. Brand AH, Manoukian AS, Perrimon N. Ectopic expression in Drosophila. Methods Cell Biol. 1994;44:635-54. PubMed PMID: 7707973.
35. Phelps CB, Brand AH. Ectopic gene expression in Drosophila using GAL4 system. Methods (San Diego, Calif). 1998;14(4):367-79. Epub 1998/06/03. doi: 10.1006/meth.1998.0592. PubMed PMID: 9608508.
36. Karlikow M, Goic B, Saleh MC. RNAi and antiviral defense in Drosophila: setting up a systemic immune response. Developmental and comparative immunology. 2014;42(1):85-92. Epub 2013/05/21. doi: 10.1016/j.dci.2013.05.004. PubMed PMID: 23684730.
37. Reed HC, Hoare T, Thomsen S, Weaver TA, White RAH, Akam M, et al. Alternative Splicing Modulates Ubx Protein Function in Drosophila melanogaster. Genetics. 2010;184(3):745-58. doi: 10.1534/genetics.109.112086. PubMed PMID: PMC2845342.

38. Smith CW, Valcarcel J. Alternative pre-mRNA splicing: the logic of combinatorial control. Trends Biochem Sci. 2000;25(8):381-8. Epub 2000/08/01. PubMed PMID: 10916158.
39. Matlin AJ, Clark F, Smith CW. Understanding alternative splicing: towards a cellular code. Nature reviews Molecular cell biology. 2005;6(5):386-98. Epub 2005/06/16. doi: 10.1038/nrm1645. PubMed PMID: 15956978.
40. Wang Z, Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. RNA (New York, NY). 2008;14(5):802-13. Epub 2008/03/29. doi: 10.1261/rna.876308. PubMed PMID: 18369186; PubMed Central PMCID: PMCPMC2327353.
41. Choi SA, Kim SJ, Chung KC. Huntingtin-interacting protein 1-mediated neuronal cell death occurs through intrinsic apoptotic pathways and mitochondrial alterations. FEBS Letters. 2006;580(22):5275-82. doi: 10.1016/j.febslet.2006.08.076.
42. Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993;118(2):401-15. PubMed PMID: 8223268.
43. Yeh E, Gustafson K, Boulianne GL. Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(15):7036-40. PubMed PMID: PMC41466.
44. Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou D, et al. A genome-scale shRNA resource for transgenic RNAi in Drosophila. 2010.
45. Lin DM, Goodman CS. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. Neuron. 1994;13(3):507-23. Epub 1994/09/01. PubMed PMID: 7917288.
46. Ito K, Awano W, Suzuki K, Hiromi Y, Yamamoto D. The Drosophila mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development. 1997;124(4):761-71.
47. Keleman K, Micheler T, members Vp. RNAi-phiC31 construct and insertion data submitted by the Vienna Drosophila RNAi Center. 2009.
48. Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448(7150):151-6.
49. Todd AM, Staveley BE. Novel Assay and Analysis for Measuring Climbing Ability in Drosophila. Drosophila Information Service. 2004;87:101-8. Epub December 2004.
50. Rayment I, Holden HM, Whittaker M, Yohn CB, Lorenz M, Holmes KC, et al. Structure of the Actin-Myosin Complex and Its Implications for Muscle Contraction. Science (New York, NY). 1993;261(5117):58-65.
51. Slade FA. The effects of altered Hip1 expression in a Huntington disease model in Drosophila melanogaster. St. John's, NL: Memorial University of Newfoundland; 2014.
52. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. The Lancet Neurology. 2006;5(6):525-35. Epub 2006/05/23. doi: 10.1016/s1474-4422(06)70471-9. PubMed PMID: 16713924.

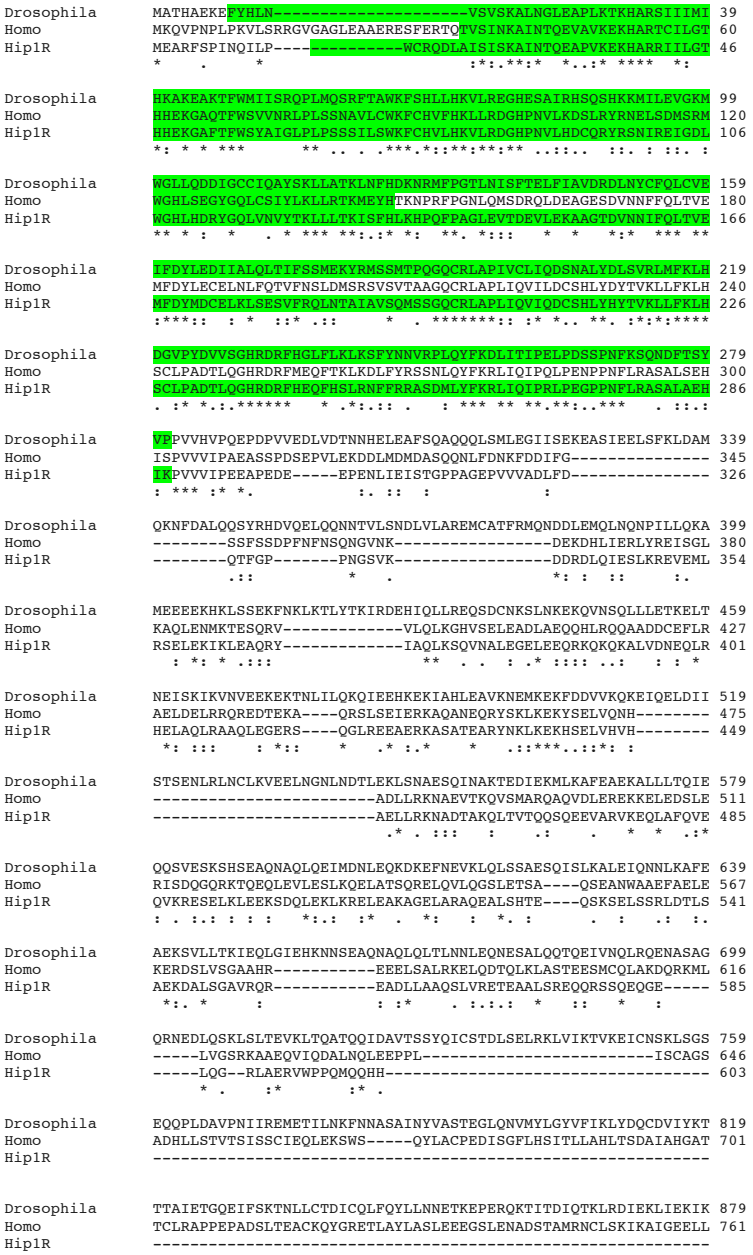
53. Shin J-H, Ko HS, Kang H, Lee Y, Lee Y-I, Pletinkova O, et al. PARIS (ZNF746) Repression of PGC-1α Contributes to Neurodegeneration in Parkinson's Disease. Cell. 2011;144(5):689-702. doi: 10.1016/j.cell.2011.02.010. PubMed PMID: PMC3063894.
54. Hayashi T, Ishimori C, Takahashi-Niki K, Taira T, Kim YC, Maita H, et al. DJ-1 binds to mitochondrial complex I and maintains its activity. Biochem Biophys Res Commun. 2009;390(3):667-72. doi: 10.1016/j.bbrc.2009.10.025. PubMed PMID: 19822128.
55. Park SJ. Huntingtin-interacting protein 1-related is required for accurate congression and segregation of chromosomes. BMB reports. 2010;43(12):795-800. Epub 2010/12/30. doi: 10.5483/BMBRep.2010.43.12.795. PubMed PMID: 21189155.
56. Masuda T, Sakuma C, Ueno T, Yamada Y, Ohmomo H, Ueda S, et al. Spatiotemporal patterns of the Huntingtin-interacting protein 1-related gene in the mouse head. Congenit Anom (Kyoto). 2013;53(4):141-8. doi: 10.1111/cga.12023. PubMed PMID: 24712472.
57. Wong KK, Ch'ng ES, Loo SK, Husin A, Muruzabal MA, Moller MB, et al. Low HIP1R mRNA and protein expression are associated with worse survival in diffuse large B-cell lymphoma patients treated with R-CHOP. Experimental and molecular pathology. 2015;99(3):537-45. Epub 2015/09/06. doi: 10.1016/j.yexmp.2015.08.019. PubMed PMID: 26341140.
58. Nelson EL, Liang CL, Sinton CM, German DC. Midbrain dopaminergic neurons in the mouse: computer-assisted mapping. The Journal of comparative neurology. 1996;369(3):361-71. Epub 1996/06/03. doi: 10.1002/(SICI)1096-9861(19960603)369:3<361::AID-CNE3>3.0.CO;2-3. PubMed PMID: 8743418.
59. Seki N, Muramatsu M, Sugano S, Suzuki Y, Nakagawara A, Ohhira M, et al. Cloning, expression analysis, and chromosomal localization of HIP1R, an isolog of huntingtin interacting protein (HIP1). J Hum Genet. 1998;43(4):268-71. doi: 10.1007/s100380050087. PubMed PMID: 9852681.
60. Friggi-Grelin F, Coulom H, Meller M, Gomez D, Hirsh J, Birman S. Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. J Neurobiol. 2003;54(4):618-27. Epub 2003/01/30. doi: 10.1002/neu.10185. PubMed PMID: 12555273.
61. Li H, Chaney S, Roberts IJ, Forte M, Hirsh J. Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Current biology : CB. 2000;10(4):211-4. Epub 2000/03/08. PubMed PMID: 10704417.
62. Slade FA, Staveley BE. Arm-Gal4 inheritance influences development and lifespan in Drosophila melanogaster. Genetics and molecular research : GMR. 2015;14(4):12788-96. Epub 2015/10/28. doi: 10.4238/2015.October.19.22. PubMed PMID: 26505429.
63. Haywood AFM, Saunders LD, Staveley BE. Dopa decarboxylase(Ddc)-GAL4 dramatically reduces life span. Drosophila Information Service. 2002;85:42-5.
64. Majumder P, Chattopadhyay B, Mazumder A, Das P, Bhattacharyya NP. Induction of apoptosis in cells expressing exogenous Hippi, a molecular partner of huntingtin-interacting protein Hip1. Neurobiology of Disease. 2006;22(2):242-56. doi: http://dx.doi.org/10.1016/j.nbd.2005.11.003.

65. Wanker EE. Hip1 and Hippi Participate in a Novel Cell Death-Signaling Pathway. Developmental Cell. 2002;2(2):126-8. doi: http://dx.doi.org/10.1016/S1534-5807(02)00121-1.
66. Cheng C-M, Huang S-p, Chang Y-F, Chung W-Y, Yuo C-Y. The viral death protein Apoptin interacts with Hippi, the protein interactor of Huntingtin-interacting protein 1. Biochemical and Biophysical Research Communications. 2003;305(2):359-64. doi: http://dx.doi.org/10.1016/S0006-291X(03)00764-2.
67. Stanton SE, Blanck JK, Locker J, Schreiber-Agus N. Rybp interacts with Hippi and enhances Hippi-mediated apoptosis. Apoptosis. 2007;12(12):2197-206. doi: 10.1007/s10495-007-0131-3.
68. Houde C, Dickinson RJ, Houtzager VM, Cullum R, Montpetit R, Metzler M, et al. Hippi is essential for node cilia assembly and Sonic hedgehog signaling. Developmental Biology. 2006;300(2):523-33. doi: https://doi.org/10.1016/j.ydbio.2006.09.001.
69. Datta M, Choudhury A, Lahiri A, Bhattacharyya NP. Genome wide gene expression regulation by HIP1 Protein Interactor, HIPPI: Prediction and validation. BMC Genomics. 2011;12(1):463. doi: 10.1186/1471-2164-12-

Appendix A- Supplemental Data for Figure 1.2

A1. Protein sequence alignment of *Drosophila melanogaster* Hip1 and *Homo sapiens* Hip1 and Hip1R. Alignment performed in ClustalW2.

% Similarity *Drosophila* Hip1 and Human Hip1: 96%
% Similarity *Drosophila Hip1* and Human Hip1R: 31%



Appendix B - Supplemental data for Figure 2.1

Table B1. Statistical analysis and comparison of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *elav-Gal4.*

B1.1. Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
elav-Gal4; +	366	70	0.0368	Yes
elav-Gal4; UAS-lacZ	315	70	N/A	N/A
elav-Gal4; UAS-GFP	401	52	<0.0001	Yes
elav -Gal4; UAS-Hip1-RNAi^{106978}	295	78	<0.0001	Yes
elav-Gal4; UAS-Hip1-RNAi^{32504}	371	70	0.1199	No
elav-Gal4; UAS-Hip1^{L2}	326	60	<0.0001	Yes
elav-Gal4; UAS-Hip1^{L6}	333	54	<0.0001	Yes
elav-Gal4; UAS-Hip1$^{S11.2}$	301	70	0.1383	No

Table B2. Statistical analysis and comparison of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *elav-Gal4*.

B2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
elav-Gal4; +	0.02975	0.002844	0.02466 to 0.03509	0.7539	<0.0001	Yes
elav-Gal4; UAS-lacZ	0.06359	0.008349	0.04609 to 0.08364	0.6669	N/A	N/A
elav-Gal4; UAS-GFP	0.01946	0.00275	0.01429 to 0.02462	0.574	<0.0001	Yes
elav-Gal4; UAS-Hip1-RNAi^{106978}	0.01256	0.001983	0.00897 to 0.01626	0.4912	<0.0001	Yes
elav-Gal4; UAS-Hip1-RNAi^{32504}	0.01793	0.0018	0.01478 to 0.02124	0.6814	<0.0001	Yes
elav-Gal4; UAS-Hip1^{L2}	0.01975	0.004955	0.008783 to 0.03136	0.2736	0.0004	Yes
elav-Gal4; UAS-Hip1^{L6}	0.02831	0.002668	0.02356 to 0.03338	0.7721	<0.0001	Yes
elav-Gal4; UAS-Hip1$^{S11.2}$	0.02117	0.003288	0.01509 to 0.02757	0.6051	<0.0001	Yes

Appendix C – Supplemental data for Figure 2.2

Table C1. Statistical analysis and comparison of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through $GawB^{l(3)31\text{-}1}$.

C1.1. Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
$GawB^{l(3)31\text{-}1}$; +	322	52	0.7466	No
$GawB^{l(3)31\text{-}1}$; *UAS-lacZ*	383	48	N/A	N/A
$GawB^{l(3)31\text{-}1}$; *UAS-GFP*	313	52	0.4768	No
$GawB^{l(3)31\text{-}1}$; $UAS\text{-}Hip1\text{-}RNAi^{106978}$	269	46	0.4018	No
$GawB^{l(3)31\text{-}1}$; $UAS\text{-}Hip1\text{-}RNAi^{32504}$	326	55	0.5759	No
$GawB^{l(3)31\text{-}1}$; $UAS\text{-}Hip1^{L2}$	385	56	0.1423	No
$GawB^{l(3)31\text{-}1}$; $UAS\text{-}Hip1^{L6}$	270	52	0.2574	No
$GawB^{l(3)31\text{-}1}$; $UAS\text{-}Hip1^{S11.2}$	336	58	0.0061	Yes

Table C2. Statistical analysis and comparison of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through $GawB^{l(3)31\text{-}1}$.

C2.1. Longevity at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
$GawB^{l(3)31\text{-}1}$; +	0.02299	0.00258	0.01780 to 0.02817	0.6277	0.0024	Yes
$GawB^{l(3)31\text{-}1}$; *UAS-lacZ*	0.02342	0.002958	0.01751 to 0.02934	0.5906	N/A	N/A
$GawB^{l(3)31\text{-}1}$; *UAS-GFP*	0.01929	0.003806	0.01161 to 0.02698	0.3826	0.089	No
$GawB^{l(3)31\text{-}1}$; *UAS-Hip1-RNAi*^{106978}	0.03422	0.005982	0.02206 to 0.04638	0.4458	0.2025	No
$GawB^{l(3)31\text{-}1}$; *UAS-Hip1-RNAi*^{32504}	0.0214	0.001862	0.01767 to 0.02513	0.7536	0.0008	Yes
$GawB^{l(3)31\text{-}1}$; *UAS-Hip1*^{L2}	0.02681	0.003852	0.01904 to 0.03458	0.5407	0.4355	No
$GawB^{l(3)31\text{-}1}$; *UAS-Hip1*^{L6}	0.0372	0.005401	0.02625 to 0.04815	0.5525	0.0987	No
$GawB^{l(3)31\text{-}1}$; *UAS-Hip1*$^{S11.2}$	0.02916	0.00463	0.01978 to 0.03854	0.5203	0.0089	Yes

Appendix D – Supplemental data for Figure 3.1

Table D1. Statistical analysis and comparison of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *ple-Gal4.*

D1.1. Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
ple-Gal4; +	371	72	0.0001	Yes
ple-Gal4; UAS-lacZ	362	70	N/A	N/A
ple-Gal4; UAS-GFP	305	70	0.3774	No
ple-Gal4; UAS-Hip1-RNAi^{106978}	296	78	<0.0001	Yes
ple-Gal4; UAS-Hip1-RNAi^{32504}	351	78	<0.0001	Yes
ple-Gal4; UAS-Hip1^{L2}	493	56	<0.0001	Yes
ple-Gal4; UAS-Hip1^{L6}	342	58	<0.0001	Yes
ple-Gal4; UAS-Hip1$^{S11.2}$	324	68	0.0003	Yes

Table D2. Statistical analysis and comparison of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *ple-Gal4*.

D2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
ple-Gal4; +	0.07465	0.008107	0.06013 to 0.09088	0.6987	0.0021	Yes
ple-Gal4; UAS-lacZ	0.07167	0.01303	0.0415 to 0.1143	0.4255	N/A	N/A
ple-Gal4; UAS-GFP	0.04549	0.005235	0.03502 to 0.05596	0.6057	0.2612	No
ple-Gal4; UAS-Hip1-RNAi^{106978}	0.05054	0.007991	0.03434 to 0.06673	0.5142	0.1441	No
ple-Gal4; UAS-Hip1-RNAi^{32504}	0.04469	0.004311	0.03606 to 0.05332	0.7715	<0.0001	Yes
ple-Gal4; UAS-Hip1^{L2}	0.03066	0.004132	0.02238 to 0.03894	0.516	0.0002	Yes
ple-Gal4; UAS-Hip1^{L6}	0.05639	0.00587	0.04462 to 0.06817	0.7123	0.0001	Yes
ple-Gal4; UAS-Hip1$^{S11.2}$	0.03877	0.005484	0.02777 to 0.04976	0.521	<0.0001	Yes

Appendix E – Supplemental data for Figure 3.2

Table E1. Statistical analysis of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$.

E1.1 Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
Ddc-Gal4$^{HL4.3D}$*; +*	394	74	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-lacZ*	692	54	N/A	N/A
Ddc-Gal4$^{HL4.3D}$*; UAS-GFP*	416	58	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1-RNAi*^{106978}	504	62	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1-RNAi*^{32504}	453	70	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*^{L2}	862	58	0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*^{L6}	377	44	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*$^{S11.2}$	612	58	<0.0001	Yes

Table E2. Statistical analysis of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$.

E2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
Ddc-Gal4$^{HL4.3D}$*; +*	0.04345	0.00719	0.02889 to 0.05801	0.5692	0.229	No
Ddc-Gal4$^{HL4.3D}$*; UAS-lacZ*	0.05996	0.01053	0.03851 to 0.08142	0.585	N/A	N/A
Ddc-Gal4$^{HL4.3D}$*; UAS-GFP*	0.0374	0.01345	0.007436 to 0.06736	0.4777	0.0965	No
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1-RNAi*^{106978}	0.03083	0.0069	0.01672 to 0.04494	0.3903	0.0466	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1-RNAi*^{32504}	0.03821	0.00492	0.02822 to 0.04821	0.639	0.0047	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*^{L2}	0.03338	0.007502	0.01804 to 0.04872	0.3467	0.0167	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*^{L6}	0.04813	0.009068	0.02959 to 0.06668	0.4892	0.0002	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-Hip1*$^{S11.2}$	0.02529	0.003785	0.01762 to 0.03295	0.5246	<0.0001	Yes

Appendix F – Supplemental data for Figure 3.3

Table F1. Statistical analysis of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4* $^{HL4.36}$.

F1.1 Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
Ddc-Gal4$^{HL4.36;}$ +	404	58	0.165	No
Ddc-Gal4$^{HL4.36;}$ *UAS-lacZ*	366	64	N/A	N/A
Ddc-Gal4$^{HL4.36;}$ *UAS-GFP*	328	71	0.0036	Yes
Ddc-Gal4$^{HL4.36;}$ *UAS-Hip1-RNAi*^{106978}	399	60	0.0038	Yes
Ddc-Gal4$^{HL4.36;}$ *UAS-Hip1-RNAi*^{32504}	324	76	<0.0001	Yes
Ddc-Gal4$^{HL4.36;}$ *UAS-Hip1*^{L2}	333	72	<0.0001	Yes
Ddc-Gal4$^{HL4.36;}$ *UAS-Hip1*^{L6}	324	60	0.0175	Yes
Ddc-Gal4$^{HL4.36;}$ *UAS-Hip1*$^{S11.2}$	388	62	0.918	No

Table F2. Statistical analysis of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.36}$.

F2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
Ddc-Gal4$^{HL4.36}$*; +*	0.04906	0.004264	0.04053 to 0.05759	0.8096	<0.0001	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-lacZ*	0.03101	0.005868	0.01921 to 0.04280	0.3459	N/A	N/A
Ddc-Gal4$^{HL4.36}$*; UAS-GFP*	0.03623	0.007135	0.02194 to 0.05051	0.3295	0.0503	No
Ddc-Gal4$^{HL4.36}$*; UAS-Hip1-RNAi*^{106978}	0.02434	0.004102	0.01603 to 0.03266	0.3697	0.2717	No
Ddc-Gal4$^{HL4.36}$*; UAS-Hip1-RNAi*^{32504}	0.04579	0.005176	0.03544 to 0.05613	0.6938	<0.0001	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-Hip1*^{L2}	0.02945	0.00345	0.02253 to 0.03637	0.5891	<0.0001	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-Hip1*^{L6}	0.0624	0.00849	0.04535 to 0.07945	0.6841	<0.0001	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-Hip1*$^{S11.2}$	0.03695	0.004766	0.02739 to 0.04651	0.5984	<0.0001	Yes

Appendix G – Supplemental data for Figure 3.4

Table G1. Statistical analysis of longevity between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$ plus *Ddc-Gal4*$^{HL4.36}$.

G1.1 Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; +	354	70	0.1223	No
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-lacZ*	408	63	N/A	N/A
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-GFP*	371	68	0.5221	No
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{106978}	359	60	0.0051	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{32504}	345	76	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*^{L2}	363	52	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*^{L6}	322	53	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*$^{S11.2}$	326	60	0.0083	Yes

Table G2. Statistical analysis of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$ plus *Ddc-Gal4*$^{HL4.36}$.

G2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; +	0.0379	0.003417	0.03105 to 0.04474	0.7887	0.0002	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-lacZ*	0.05062	0.005911	0.03876 to 0.06248	0.7196	N/A	N/A
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-GFP*	0.05519	0.006878	0.04137 to 0.069	0.6203	0.2844	No
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{106978}	0.04804	0.004997	0.03801 to 0.05806	0.6913	0.5548	No
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1-RNAi*^{32504}	0.01956	0.002482	0.01459 to 0.02453	0.5752	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*^{L2}	0.02547	0.004542	0.01628 to 0.03466	0.4673	0.0002	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*^{L6}	0.0438	0.01304	0.01723 to 0.07037	0.2855	0.3946	No
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$; *UAS-Hip1*$^{S11.2}$	0.02968	0.004912	0.01974 to 0.03961	0.4981	0.0527	Yes

Appendix H – Supplemental data for Figure 4.1

Table H1. Statistical analysis of longevity between the *UAS-lacZ* control and reduced *Hippi* expression in *D. melanogaster* expressed through *elav-Gal4* and *GawB*$^{l(3)31\text{-}1}$.

H1.1 Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	*p*-value compared to control	Significant
elav-Gal4; UAS-lacZ	315	70	N/A	N/A
elav -Gal4; UAS-Hippi-RNAi	332	64	<0.0001	Yes
GawB$^{l(3)31\text{-}1}$*; UAS-lacZ*	383	48	N/A	N/A
GawB$^{l(3)31\text{-}1}$*; UAS-Hippi-RNAi*	311	64	<0.0001	Yes

Table H2. Statistical analysis of locomotor ability between the *UAS-lacZ* control and reduced *Hippi* expression in *D. melanogaster* expressed through *elav-Gal4* and *GawB*$^{l(3)31\text{-}1}$.

H2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R^2	*p*-value	Significant
elav-Gal4; UAS-lacZ	0.06359	0.008349	0.04609 to 0.08364	0.6669	N/A	N/A
elav-Gal4; UAS-Hippi-RNAi	0.01175	0.001981	0.00779 to 0.01578	0.4311	<0.0001	Yes
GawB$^{l(3)31\text{-}1}$*; UAS-lacZ*	0.02342	0.002958	0.01751 to 0.02934	0.5906	N/A	N/A
GawB$^{l(3)31\text{-}1}$*; UAS-Hippi-RNAi*	0.06288	0.00704	0.04871 to 0.07705	0.7202	<0.0001	Yes

Appendix I – Supplemental data for Figure 4.2

Table I1. Statistical analysis of longevity between the *UAS-lacZ* control and altered *Hippi* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$, *Ddc-Gal4*$^{HL4.36}$ and *Ddc-Gal4*$^{HL4.3D}$ plus *Ddc-Gal4*$^{HL4.36}$.

I1.1 Longevity at 25°C

Genotype	Number of Flies (N)	Median Survival (Days)	p-value compared to control	Significant
ple-Gal4; UAS-lacZ	362	70	N/A	N/A
ple-Gal4; UAS-Hippi-RNAi	351	70	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-lacZ*	692	54	N/A	N/A
Ddc-Gal4$^{HL4.3D}$*; UAS-Hippi-RNAi*	509	50	0.0016	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-lacZ*	366	64	N/A	N/A
Ddc-Gal4$^{HL4.36}$*; UAS-Hippi-RNAi*	352	68	0.0008	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ*	408	63	N/A	N/A
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hippi-RNAi*	208	58	0.0034	Yes

Table I2. Statistical analysis of locomotor ability between the *UAS-lacZ* control and altered *Hip1* expression in *D. melanogaster* expressed through *Ddc-Gal4*$^{HL4.3D}$, *Ddc-Gal4*$^{HL4.36}$ and *Ddc-Gal4*$^{HL4.3D}$ plus *Ddc-Gal4*$^{HL4.36}$.

I2.1 Climbing ability at 25°C

Genotype	Slope (k)	Standard error	95% Confidence interval	R2	p-value	Significant
ple-Gal4; UAS-lacZ	0.07167	0.01303	0.0415 to 0.1143	0.4255	N/A	N/A
ple-Gal4; UAS-Hippi-RNAi	0.01924	0.004421	0.01039 to 0.02809	0.2312	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$*; UAS-lacZ*	0.05996	0.01053	0.03851 to 0.08142	0.585	N/A	N/A
Ddc-Gal4$^{HL4.3D}$*; UAS-Hippi-RNAi*	0.05671	0.005361	0.04577 to 0.06764	0.7887	0.0025	Yes
Ddc-Gal4$^{HL4.36}$*; UAS-lacZ*	0.03101	0.005868	0.01921 to 0.04280	0.3459	N/A	N/A
Ddc-Gal4$^{HL4.36}$*; UAS-Hippi-RNAi*	0.03139	0.002608	0.02616 to 0.03662	0.7756	<0.0001	Yes
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-lacZ*	0.05062	0.005911	0.03876 to 0.06248	0.7196	N/A	N/A
Ddc-Gal4$^{HL4.3D}$; *Ddc-Gal4*$^{HL4.36}$*; UAS-Hippi-RNAi*	0.0267	0.006957	0.01251 to 0.04089	0.347	<0.0001	Yes