Integrative Analysis of Huntington's Pathophysiology: Genetic Interactions and Molecular Profiling in *Drosophila*

URECA proposal: (Student Name)

Mentor: Dr. Kiel Ormerod

Summer 2024

Introduction: Huntington disease (HD) is an autosomal dominant, neurodegenerative disorder that effects roughly one in every 10-20 thousand people in the United States (Nopoulos, 2022). HD affects the brain and is characterized by the progressive degeneration of nerve cells in certain brain areas (e.x. Caudate nucleus, and Putamen), which can lead to a wide range of physical, cognitive, and psychiatric symptoms, ultimately leading to death (Ehrlich, 2012). Typically, onset of symptoms is in middle-aged individuals (40+ years), but the disorder can occur at any time between infancy and senescence (Walker, 2007). Based on registry date, two studies found that the mean age at death was 56 years in a population of individuals with HD from Southern Italy and in the United States (Solberg, 2018). Recent scientific and medical advancements have enabled earlier detection of HD with the onset of genetic testing and streamlined protocols for behavioral assessment and diagnosis (Paulsen, 2010). The classic behavioral sign of HD is chorea that gradually spreads to all muscles, which causes all psychomotor processes to become severely damaged (Roos, 2009). Diagnosis can be made clinically in a patient with motor and/or cognitive and behavioral disturbances with a parent diagnosed with HD and can be confirmed by DNA determination (Ajitkumar, 2023). Unfortunately, there is no cure for the disease and affected patients tend to be entirely dependent on their caregiver as the disease progresses (Ajitkumar, 2023). Over the past 150 years of HD research since George Huntington published his description, a plethora of pathogenic mechanisms have been proposed with key themes including excitotoxicity, dopaminergic imbalance, mitochondrial dysfunction, metabolic defects, disruption of proteostasis, transcriptional dysregulation, and neuroinflammation (Jiang, 2023).

Unlike other neurodegenerative disorders, HD research has focused mostly on one gene, the Huntingtin gene (htt), as mutations in this gene are highly associated with the pathophysiology of the disease. htt interacts with a large number of proteins, and also functions in transcription and

molecular trafficking processes that can alter the processing and localization of many others, thus loss of normal htt function may have even wider ranging impact on cell physiology than currently appreciated (Schultz and Littleton, 2011). The Huntingtin gene encodes a protein which the specific function of the protein is yet to be elucidated (Fiorille, 2021) but the scientific literature strongly suggests a role in transcription and molecular trafficking of organelles (Caviston 2009). Furthermore, Huntingtin protein is known to intersect with numerous proteins which can alter the function and localization thus loss of normal htt function may have even wider ranging impact on cell physiology that's currently appreciated (Schultz and Littleton, 2011). The gene, Htt, contains repeats of the triplet nucleotide sequence CAG, where normal individuals have between nine and thirty-five CAG repeats in their genes, affected individuals have forty or more repeats with an average of forty-six repeats (Walker, 2007). The triplet CAG codes for amino acid glutamine, which in mutant forms of the huntingtin protein have stretches of forty or more glutamines in the protein (Krench and Littleton, 2013). The severity of the disease and the age of onset have been shown to correlate with the degree of expansion with the PolyQ region of htt where an increase in glutamine repeats increases the pathogenicity and reduces age of onset (Arrasate and Finkbeiner, 2012).

While many models of HD have been created; in the invertebrate realm, a *Drosophila Melanogaster* (fruit fly) model of HD was created using the first exon of human htt which includes the PolyQ region. In this model, the PolyQ region was altered to include 15 or 138 glutamine repeats (htt-Q15 and htt-Q138; French and Littleton, 2013). Of note, these transgenic lines included an RFP tag for fluorescent imaging. Using the UAS/Gal4 system to express these transgenes specifically and selectively in motor neurons (Elav-gal4), the Ormerod lab demonstrated that htt-positive aggregates accumulate in the axons of motor neurons and at the

neuromuscular junction (NMJ) in third-instar larvae, which significantly reduces the intracellular trafficking of organelles like synaptic vesicles, mitochondria, and dense core vesicles (Hana et al, in submission). We are able to show that htt aggregation occurs in adults (Appendix E). Previously Weiss and Littleton (2012), demonstrated that the htt-Q138 when expressed in the nervous system causes lethality in adults after 21 days while control survived beyond 70 (Appendix F), and I have repeated the observation at 25 °C (Appendix C). Here, I aim to identify novel genetic enhancers and suppressors (Appendix B) by repeating the observation that expression of htt – Q138 in motor neurons significantly reduces adult viability (Appendix C).

Here we aim to target 50 different genes that are known to associate with HD pathology or Huntington protein function using RNA interference (RNAi) and screening for changes in adult viability (Appendix B). RNAi is a molecular mechanism that uses highly specific RNA-guided machinery to target the mRNA of a gene of interest. Upon interaction with the target mRNA transcript, the RNAi impairs translation ultimately significantly reducing protein expression of your targeted gene product (Heigwer 2018). In *Drosophila*, RNAi has been applied in cultured cells or in *vivo* to perturb the function of single genes or to systematically probe gene function on a genome-wide scale (Heigwer 2018). In my list of 50 targeted genes, half were identified from a Genome-Wide Associative Sequencing (GWAS) data set from a small human population with high incidents rates of HD (Collaborator of Dr. O). The remaining targeted genes were identified from previous scientific literature, with a focus on synaptic and autophagy pathways. Previous work in our lab has demonstrated that pharmacologically targeting the autophagy pathway using rapamycin can partially rescue the impact of htt-Q138 on adult lifespan (Appendix F). Any gene that is identified as an enhancer or suppressor of HD pathophysiology will subsequently be subjected to

our adult htt-protein aggregation assay. This will directly test is these genetic interactions correlate with changes in htt-aggregation at the molecular level.

The results from our genetic screen can help to identify novel therapeutic targets for treatments of Huntington Disease. By identifying therapeutic targets, we will be focusing on enhancers and suppressors of gene htt-Q138 which can elucidate the factors that exacerbate the symptoms of HD, providing insights into potential therapeutic interventions. Enhancers and suppressors may exert their effects through various molecular pathways implicated in HD pathogenesis. Studying genetic interactions in *Drosophila* can shed light on the underlying disease mechanisms, including protein aggregation, mitochondrial dysfunction, and neurotoxicity, among others.

Hypothesis: Huntington disease is not solely attributable to mutations in Huntingtin numerous additional genetic factors contribute to HD pathology.

Methodology: I have repeated the observation for the lifespan assay observation at 25°C and adult fruit fluids were no longer viable after 36 days. I tracked the viability of just female cross males crossed with females both come from the first filial generation of (Elav – GAL4 x htt – Q138). An intermediate "balancer line" was created to ensure that we can keep important traits, prevent mix-ups, and stay consistent across generations of fruit flies. Using the established protocols in the Ormerod Lab, I will cross 50 transgenic UAS-RNAi genes to knockdown the expression of our candidate genes). Novel hits from my screen will be subjected to our adult htt-aggregation assay to determine if any correlation exists between effects on lifespan htt cellular pathology.

Responsibilities: In the Ormerod Lab, I will be responsible for fly husbandry which includes making food and transferring fly populations on fresh food biweekly. Additionally, I will be responsible for conducting all the genetic crosses using RNAi to produce progeny of the candidate genes in htt background using the GAL4-UAS system. I will monitor the lifespan assay of the candidate genes I knock down with RNAi. I will also be responsible for microscopy, data management, compilation, analysis, and depiction.

Mentor's role: Dr. Ormerod and I meet every Wednesday at 2pm to discuss the next step for the progression of this project. Dr. Ormerod works with me directly to help guide my training in every theoretical and technical aspect of the project. He will guide along the way of checking my screening and train me directly on completing the wing analysis as it is a new tool for me. He will also train me on fluorescence microscopy, data analysis and compilation, running statistical tests, making figures, and helping me to present my work both visually and verbally.

Significance to your academic development: As a future healthcare provider targeting genetic screen for enhancers and suppressors of lifespan in a model of Huntington's disease in Drosophila has the potential to greatly contribute to our understanding of the disease process and provide avenues for therapeutic interventions and strategies to improve the quality and longevity of human life. As a biology student I'm learning about developing research, enhancing my microscopy skills, collecting and analyzing greater data, genetics of the Huntington's Disease.

Difference between previous URECA project: We are in critical time in the lab we submitted manuscript to describe and characterize in the larval model and now we can move forward and take advantage of the work we've done to identify novel targets

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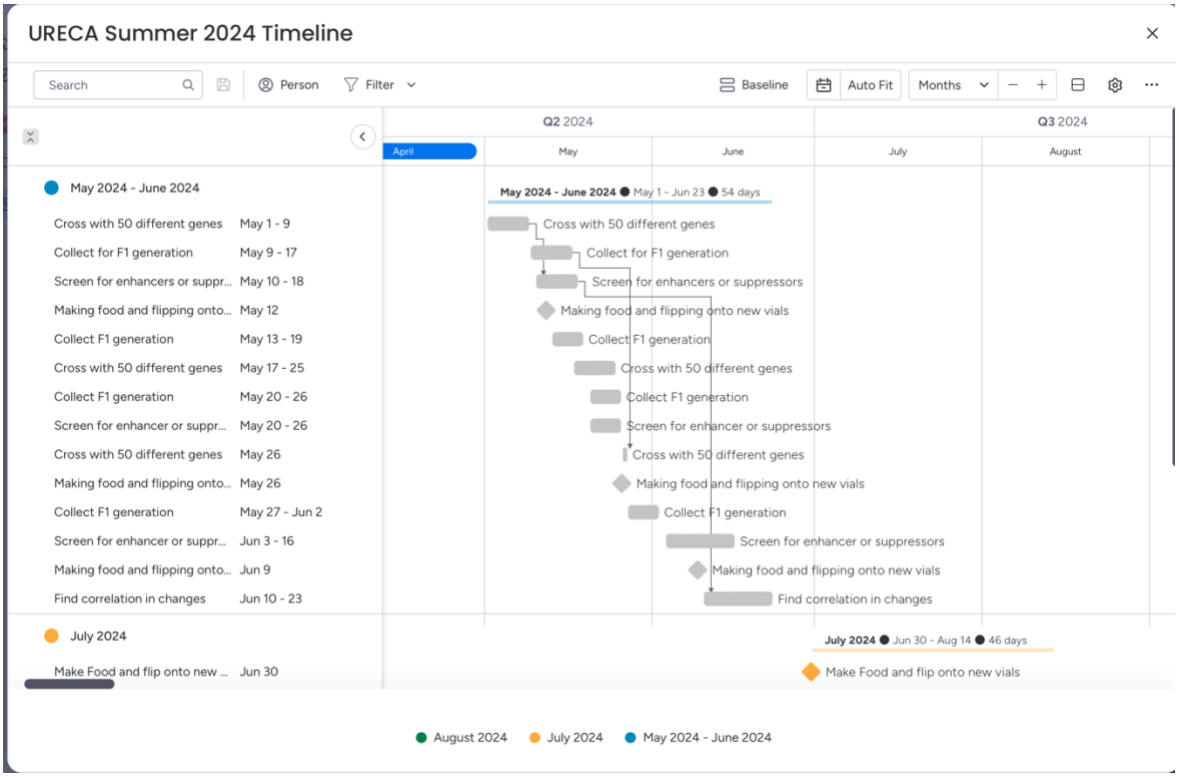
Timeline:

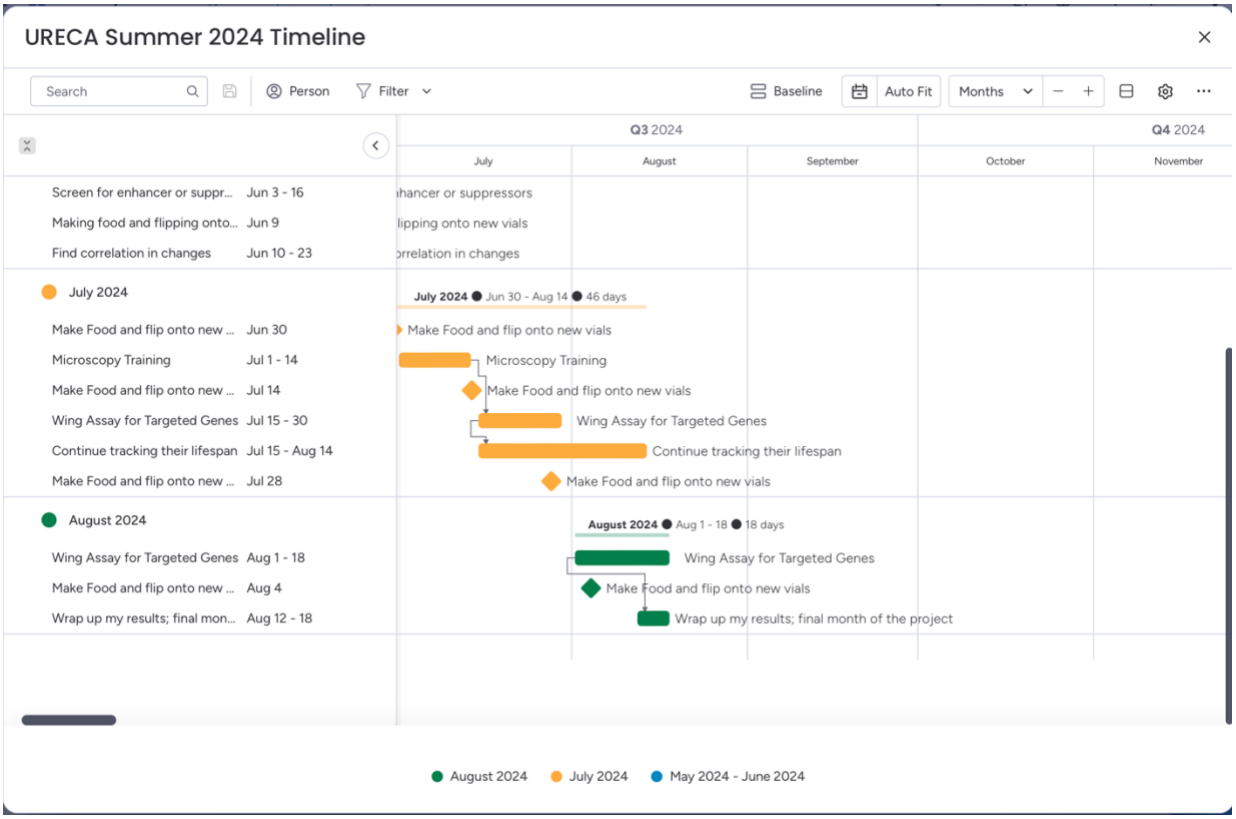
May 2024 – June 2024: I will continue to cross and screen all of the 50 transgenic UAS-RNAi genes to knockdown the expression of candidate genes. Identify the genes as enhancers or suppressors and find any correlation with changes in lifespan in our previous studies.

July 2024: For the first two weeks I will train on my microscopy skills and will begin a wing analysis. The wing analysis will take place for about 6 weeks.

August 2024: I will finish the wing analysis and will conclude my project by comparing the results.

Appendix A : Timeline




URECA Summer 2024 Timeline
Search
Person
Filter
Baseline
Auto Fit
Months
Q3 2024
Q4 2024
July
August
September
October
November
Screen for enhancer or suppr... Jun 3 - 16
Making food and flipping onto... Jun 9
Find correlation in changes Jun 10 - 23
July 2024
July 2024 Jun 30 - Aug 14 46 days
Make Food and flip onto new ... Jun 30
Make Food and flip onto new vials
Microscopy Training Jul 1 - 14
Microscopy Training
Make Food and flip onto new ... Jul 14
Make Food and flip onto new vials
Wing Assay for Targeted Genes Jul 15 - 30
Wing Assay for Targeted Genes
Continue tracking their lifespan Jul 15 - Aug 14
Continue tracking their lifespan
Make Food and flip onto new ... Jul 28
Make Food and flip onto new vials
August 2024
August 2024 Aug 1 - 18 18 days
Wing Assay for Targeted Genes Aug 1 - 18
Wing Assay for Targeted Genes
Make Food and flip onto new ... Aug 4
Make Food and flip onto new vials
Wrap up my results; final mon... Aug 12 - 18
Wrap up my results; final month of the project
August 2024
July 2024
May 2024 - June 2024

May 2024 - June 2024

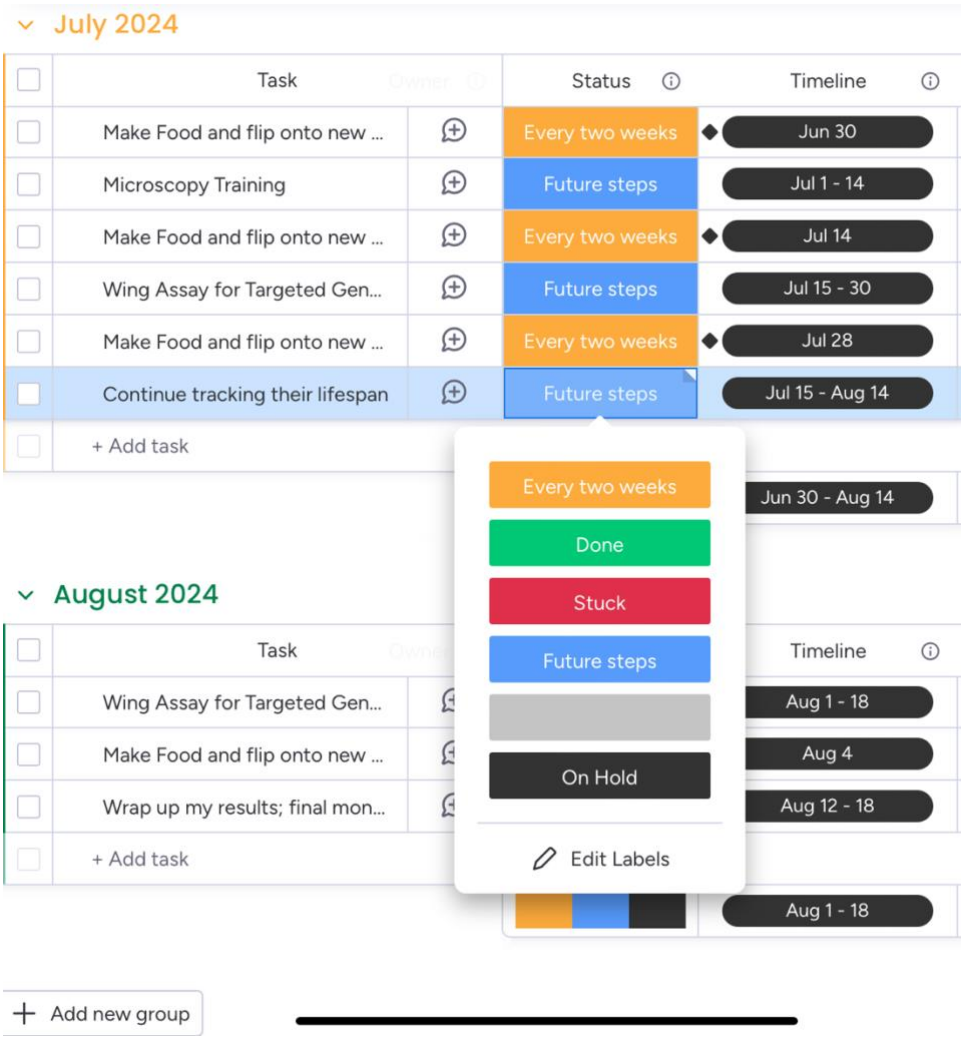
Task	Owner	Status	Timeline	Duration
Cross with 50 different ge... 2		Every two weeks	May 1 - 9	9 days
Collect for F1 generation		Future steps	May 9 - 17	9 days
Cross with 50 different genes		Future steps	May 26	1 days
Making food and flipping onto...		Every two weeks	May 12	0 days
Collect F1 generation		Future steps	May 13 - 19	7 days
Screen for enhancers or supp...		Future steps	May 10 - 18	9 days
Cross with 50 different genes		Future steps	May 17 - 25	9 days
Making food and flipping onto...		Every two weeks	May 26	0 days
Collect F1 generation		Future steps	May 20 - 26	7 days
Screen for enhancer or suppr...		Future steps	May 20 - 26	7 days
Collect F1 generation		Future steps	May 27 - Jun 2	7 days
Making food and flipping onto...		Every two weeks	Jun 9	0 days
Screen for enhancer or suppr...		Future steps	Jun 3 - 16	14 days
Find correlation in changes		On Hold	Jun 10 - 23	14 days
+ Add task				
			May 1 - Jun 23	93 days sum

July 2024

Task	Status	Timeline	Duration	Dependent On
Make Food and flip onto new ...	Every two weeks	Jun 30	0 days	
Microscopy Training	Future steps	Jul 1 - 14	14 days	
Make Food and flip onto new ...	Every two weeks	Jul 14	0 days	
Wing Assay for Targeted Gen...	Future steps	Jul 15 - 30	16 days	Microscopy Training
Make Food and flip onto new ...	Every two weeks	Jul 28	0 days	
Continue tracking their lifespan	Future steps	Jul 15 - Aug 14	31 days	Wing... SS
+ Add task				
		Jun 30 - Aug 14	61 days sum	

August 2024

Task	Status	Timeline	Duration	Dependent On
Wing Assay for Targeted Gen...	Future steps	Aug 1 - 18	18 days	
Make Food and flip onto new ...	Every two weeks	Aug 4	0 days	
Wrap up my results; final mon...	On Hold	Aug 12 - 18	7 days	Wing... SS
+ Add task				
		Aug 1 - 18	25 days sum	


July 2024
Task
Status
Timeline
Make Food and flip onto new ...
Every two weeks
Jun 30
Microscopy Training
Future steps
Jul 1 - 14
Make Food and flip onto new ...
Every two weeks
Jul 14
Wing Assay for Targeted Gen...
Future steps
Jul 15 - 30
Make Food and flip onto new ...
Every two weeks
Jul 28
Continue tracking their lifespan
Future steps
Jul 15 - Aug 14
+ Add task
Jun 30 - Aug 14
Every two weeks
Done
Stuck
Future steps
On Hold
Edit Labels
August 2024
Task
Timeline
Wing Assay for Targeted Gen...
Aug 1 - 18
Make Food and flip onto new ...
Aug 4
Wrap up my results; final mon...
Aug 12 - 18
+ Add task
Aug 1 - 18
Add new group

Appendix B:

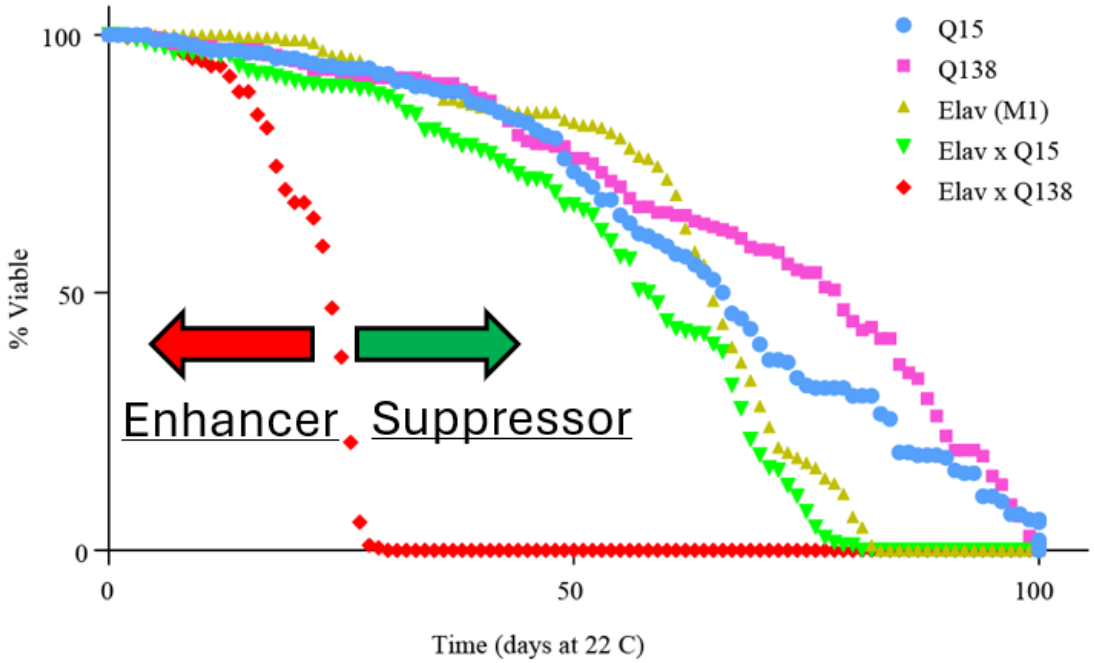


Figure 1: This illustrates the viability of *Drosophila Melanogaster* at 22°C. Employing five distinct expressions of htt genes in motor neurons, we observed significant variations in the reduction of adult viability. The gene we are focused on ELAV x htt – Q138 will show the enhancers or suppressors.

Appendix C:

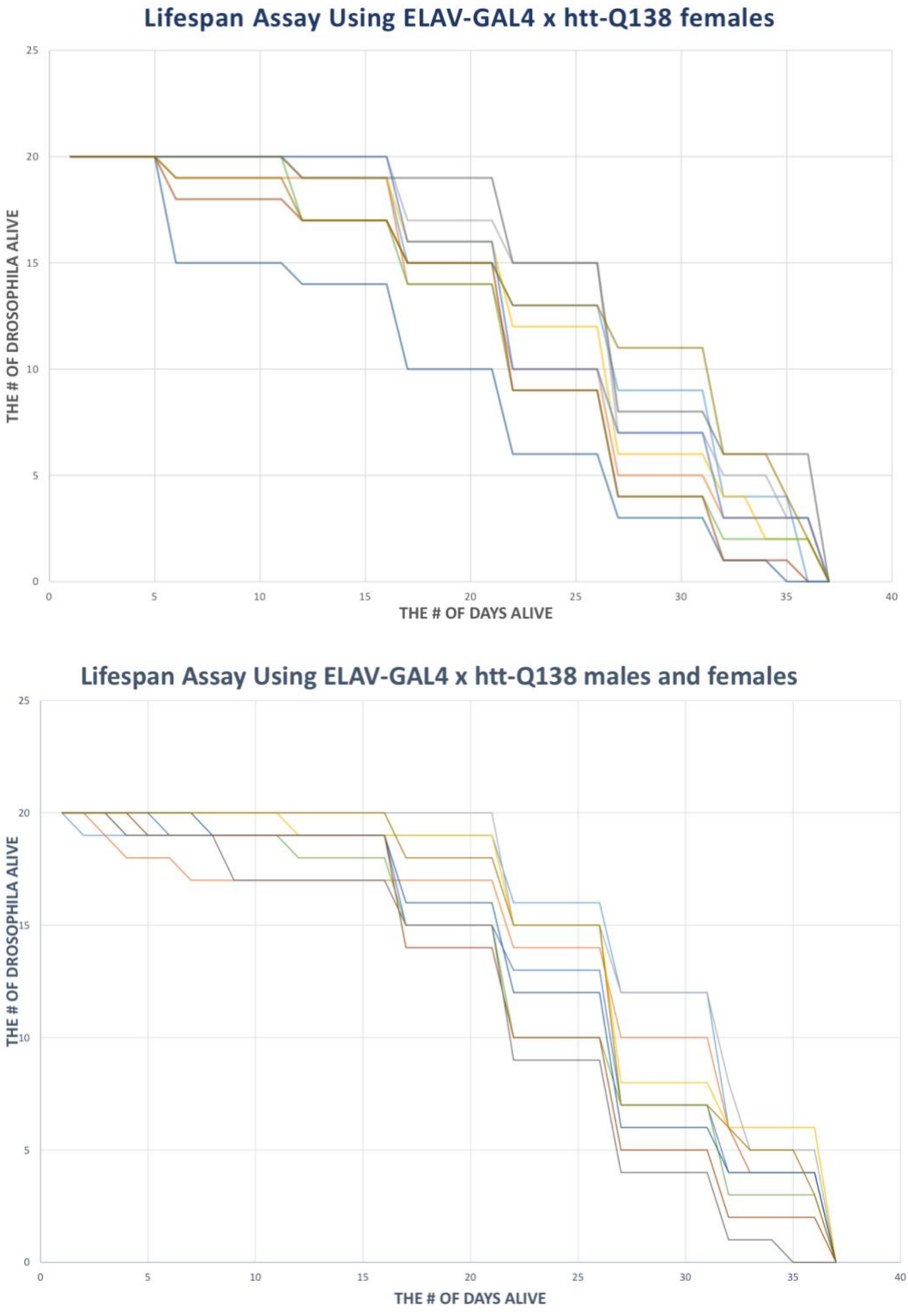


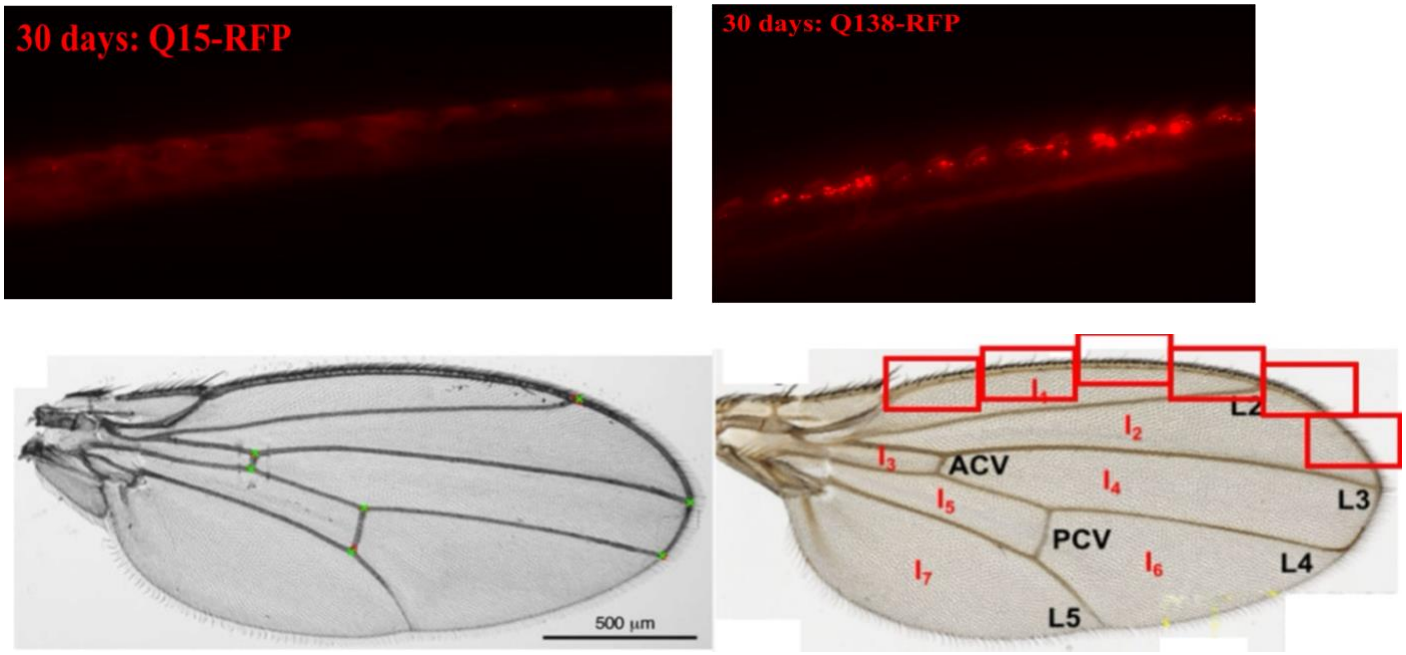
Figure 2: This illustrates the results of the repeated observation at 25°C

Appendix D

Chromosome 2	BDSC stock #	Chromosome 3	BDSC stock #	Chromosome 3	BDSC stock #	Chromosome 3	BDSC stock #	Chromosome 3	BDSC stock #
FANcd2-RNAi	53329	FANci - RNAi	32972	Frequinin-RNAi	27696	HIP1 - RNAi	32504	Pnut - RNAi	65157
RRM2B-RNAi	44022	FANcd2-RNAi	56141	APC2 - RNAi	28585	ATG1 - RNAi	26731	Nrx-1 - RNAi	27502
Myotubularin-RNAi	57298	Rnrl-RNAi	51418	ADD (China) - RNAi	35421	ATG8a - RNAi	34340	Alpha - snap - RNAi	29587
MRM2 - RNAi	43207	Dpr-20 - RNAi	32864	DRK-RNAi	27563	PSD - 95 - RNAi	25780	Arm - RNAi	31304
MARF - RNAi	55189	Contactin-RNAi	28923	ATG1- RNAi	44304	SVR - RNAi	44487	Dgkepsilon - RNAi	57750
WWOx - RNAi	44546	MTMR6 related prote	25864	Adapter 2 - RNAi	32866	gbb - RNAi	34898	Wwox	51747
TSEN2 - RNAi	55659	MTMR6 - RNAi	38340	AKT - RNAi	31701	SNAP25 - RNAi	27306	CaMKI - RNAi	35362
Hu Li Tai Shan - RNAi	38283	Myotubularin-RNAi	31552	CREB A* htt	27648	MGLuR - RNAi	25938	APC2 - RNAi	34875
ATG7 - RNAi	22707	Proteosome B1 -RNA	34824	CREB B* htt	29332	Inr - RNAi	31037	CREB A - RNAi	31900
Dgkepsilon - RNAi	57750	Diacyle Glycerol Kina	35340	SYT Beta - RNAi	27293	Six 1A - RNAI	25811	Syt beta - RNAi	41882

Figure 3:The list of 50 targeted genes, half were identified from a Genome-Wide Associative Sequencing (GWAS) data set from a small human population with high incidents rates of HD (Collaborator of Dr. O)

Appendix E:



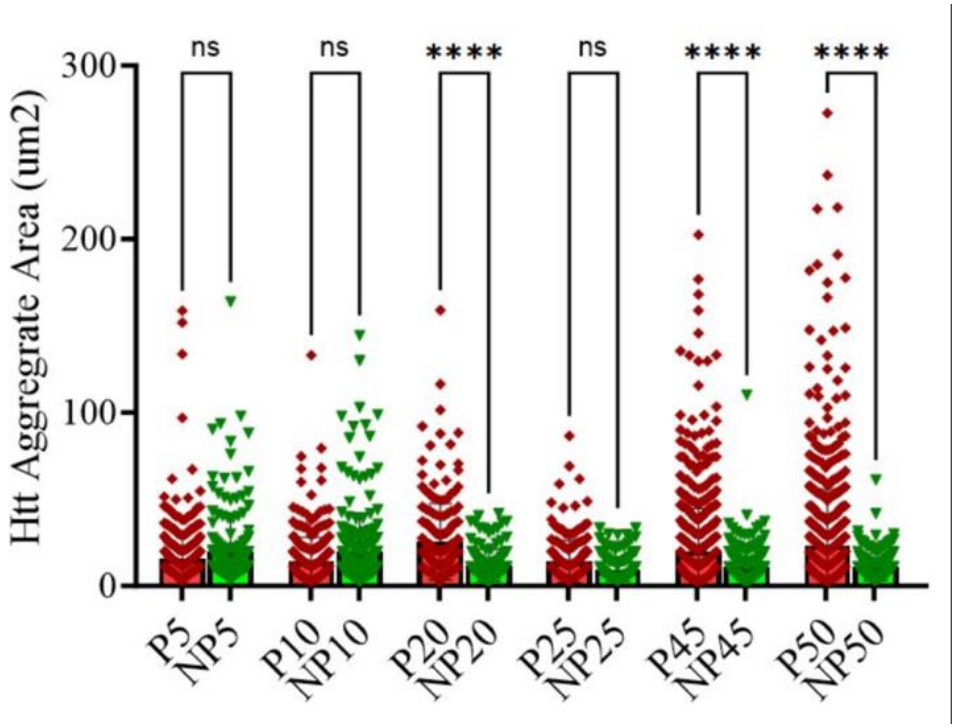


Figure 4: Identified wing – driver o examine Htt – aggregation in adult wings. Significant accumulation of the size and number of Htt -positive puncta in wings.

Appendix F:

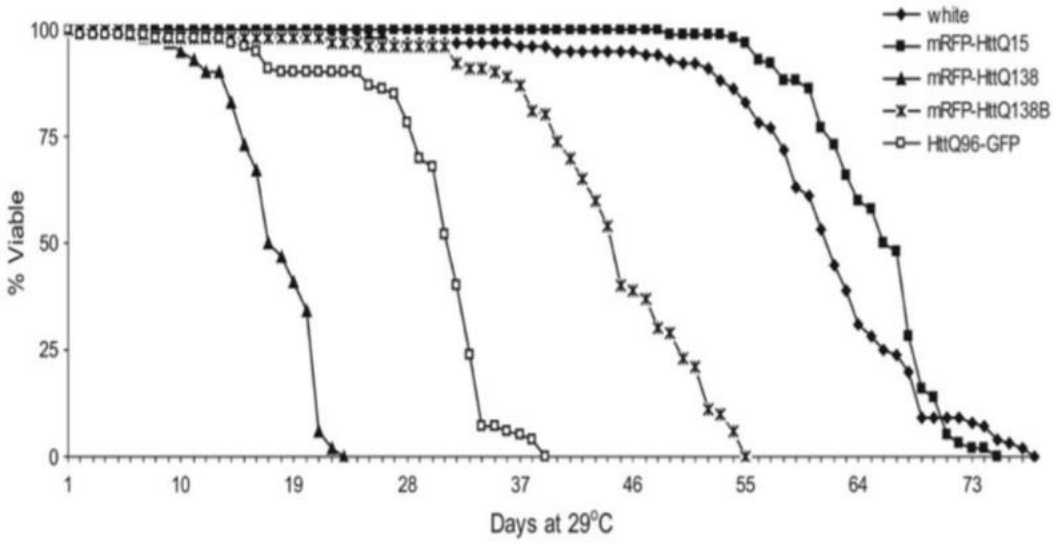


Figure 5: Data from Weiss et.al.(2012), reveal significant effects of polyp-expansions in Htt on adult viability.