THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

# IDENTIFICATION AND ANALYSIS OF *DNAJ-2* (CG10565) AS A REGULATOR OF SYNAPSE DEVELOPMENT IN THE *DROSOPHILA* LARVAL NEUROMUSCULAR JUNCTION

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# ABSTRACT

The *Drosophila* larval neuromuscular junction (NMJ) is a powerful model system for the study of synaptic development generally and the assembly of post-synaptic membrane specialization specifically. Earlier work demonstrated that decreased expression of *Akt1* in muscle tissue causes specific mislocalization of one particular glutamate receptor Subunit, GluRIIA.[1] To search for additional signaling components affecting GluRIIA delivery and assembly into a functional glutamate receptor, an RNA interference (RNAi) genetic screen of ~500 genes was performed. The organization of the post-synaptic membranes of the test animals was assayed using fluorescence confocal microscopy and immune-detection of GluRIIA. From the screen, a previously uncharacterized gene, *DnaJ-2* (CG10565), was identified as a potential regulator of GluRIIA localization at the NMJ. This gene encodes a protein belonging to the J-protein chaperone family. In order to further study *DnaJ-2*, we have generated mutants via imprecise P-element excision. The putative mutants were screened by PCR genotyping. After the initial round of mutagenesis crosses, the genetic cross scheme and assaying methods were optimized in order to increase the frequency of the imprecise P-element excision detection. At present we have identified 4 excision mutants that disrupt the *DnaJ-2* sequence, ranging from a 67 bp deletion to a 1.8kb deletion. Additionally, constructs containing specific mutations of the *DnaJ-2* sequence were generated and placed into transgenic flies. These lines as well as the mutant will be useful for future study of *DnaJ-2*.

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# ACKNOWLEDGEMENTS

I would like to thank the members of my lab for supporting my research and for always providing assistance. I would also like to thank my graduate student mentor, Na Zhao, who guided me through most of the work presented in this thesis. Finally, I would like to thank my research supervisor, Dr. Scott Selleck, for introducing me to scientific research and for his continued support and mentorship.

# Chapter 1

# Introduction

## 1.1 *Drosophila melanogaster* as a Model Organism

Our understanding of physiology and biological mechanisms has greatly improved over the past century. However, the nervous system still remains largely a mystery. The complexity and importance of the nervous system makes it a relevant yet difficult subject for research. As with many complex systems, it is useful to study the nervous system in simpler, more easily manipulated model organisms, such as *Drosophila melanogaster*. *Drosophila* is an excellent model organism for genetic studies largely due to longstanding tradition of *Drosophila* research. T.H. Morgan was the first to study the genetics of *Drosophila* in the early 1900s and *Drosophila* has since been established as one of the most well-studied organisms on the planet. The popularity of *Drosophila* as a model organism is largely due to its short life cycle, simple maintenance requirements, and small number of chromosomes allowing for simple genetic manipulation[2]. Additionally, scientists have developed many tools that increase the power of *Drosophila* as a model system. In 1918, H. J. Muller first described balancer chromosomes, chromosomes containing several inversions, that allowed for stable maintenance of lethal mutants as heterozygotes[3]. These chromosomes are now used extensively in *Drosophila* genetics to prevent undesirable homologous recombination. Decades of study have also yielded numerous mutant lines readily available to

researchers. In 1981, the first transgenic flies were generated using transposable element vectors and since then libraries of transgenic flies have been established[4]. In recent years, sophisticated biological technologies such as targeted gene expression, RNA interference (RNAi), and next generation sequencing have further expanded the repertoire of methods for *Drosophila* research.

### 1.2 UAS-GAL4 System for Targeted Gene Expression

The UAS-GAL4 system has been adopted from *Saccharomyces cerevisiae* in order to provide targeted expression of transgenes in *Drosophila*. GAL4 is a transcription factor present in yeast that recognizes the Upstream Activating Sequence (UAS) to direct the transcription of *GAL1* and *GAL10* genes upon exposure to galactose.[5] In *Drosophila*, the UAS can be linked to the promoter region of a gene, allowing for GAL4-responsive transcription(Figure 1).[6] Because the UAS-GAL4 system is not naturally present in *Drosophila*, GAL4 activated transcription is very specific to the target gene.

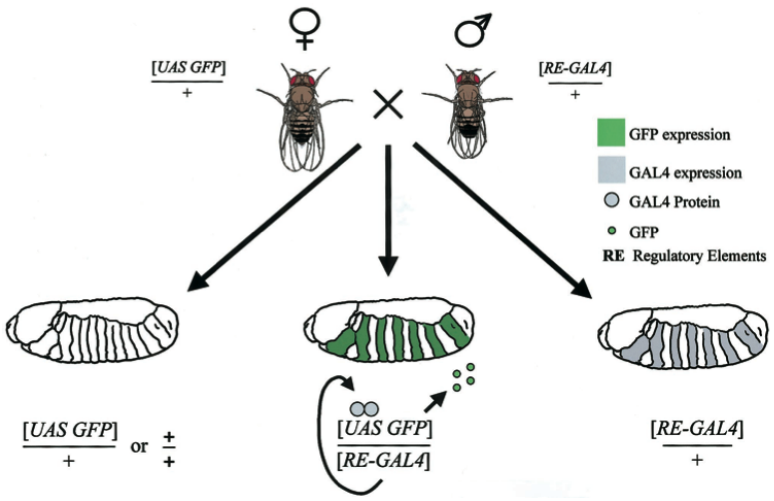


**Figure 1. UAS-GAL4 System in *Drosophila*[7]**

The UAS-GAL4 system is used to direct expression of GFP in specific segments designated by the regulatory elements (RE) that determine where GAL4 is expressed

Placing the *GAL4* gene under the control of tissue-specific regulatory sequences allows directed activation of the *UAS*-transgene construct. *GAL4* bearing constructs have also been inserted broadly throughout the genome using P-element-mediated transposition to "capture" neighboring regulatory sequences of hundreds of genes, conferring specific patterns of *GAL4* expression in specific cells and time windows during development.[8] Another useful feature of the UAS-GAL4 system is the temperature dependent activity of GAL4. GAL4 has minimal activity around 16°C and has optimal activity around 29°C.[7] This property of the GAL4 proteins allows researchers to control the level of expression of the target gene by adjusting the temperature at which the animals are bred. In the experiments presented below, muscle-specific Mef2-GAL4 was used to induce the expression of desired transgenes.

**1.3 RNA interference (RNAi) as a genetic tool**

RNAi has also been widely implemented in *Drosophila* as a research tool for targeted knockdown of gene expression. RNAi was first discovered in *Caenorhabditis elegans* as an adaptive immune response against pathogenic nucleic acids and as a gene regulatory mechanism.[9] RNAi utilizes small RNA molecules called small interfering RNAs (siRNAs) to direct degradation of specific mRNA molecules. In the RNAi pathway, a double stranded RNA (dsRNA) is first expressed from the genetic material present in the organism. In some experimental methods, dsRNAs can be manually introduced into the organism rather than having it expressed by the organism. The

dsRNA molecule is then cleaved by an endonuclease called Dicer into siRNAs that are recognized and bound by Ago. Ago is a part of the RNA-induced Silencing Complex (RISC) which degrades mRNA molecules that contain sequences that are complementary to the siRNAs.[10] The advent of next generation sequencing has led to the sequencing of the entire *Drosophila* genome. The availability of the full genome sequence allowed scientists to identify all of the genes in the *Drosophila* genome and then generate RNAi constructs for each gene.[11] Many researchers have taken advantage of the availability of RNAi lines to perform large-scale genetic screens. For the RNAi screen presented here, RNAi transgenes were placed under the control of a UAS promoter and then expressed in the muscle tissue using Mef-GAL4 to perform an RNAi-induced knockdown genetic screen.

### 1.4 The *Drosophila* Larval Neuromuscular Junction (NMJ) as a Model for Synapse Development

The *Drosophila* larval NMJ is one of the most well characterized and powerful models for synapse development available. Synapse function is evolutionarily highly conserved; thus the information gained from understanding the function of the NMJ is applicable to understanding synaptic mechanisms in other complex organisms, including humans. Conveniently, the NMJ can be easily manipulated for electrophysiology experiments and each individual cell of the synapse can be observed via light microscopy. The *Drosophila* larval body wall muscles are a segmental repeated and highly regular set of 30 muscles in each hemisegment (Figure 2).[12] For the experiments presented here, the synapse between muscles 6 and 7 was visualized.

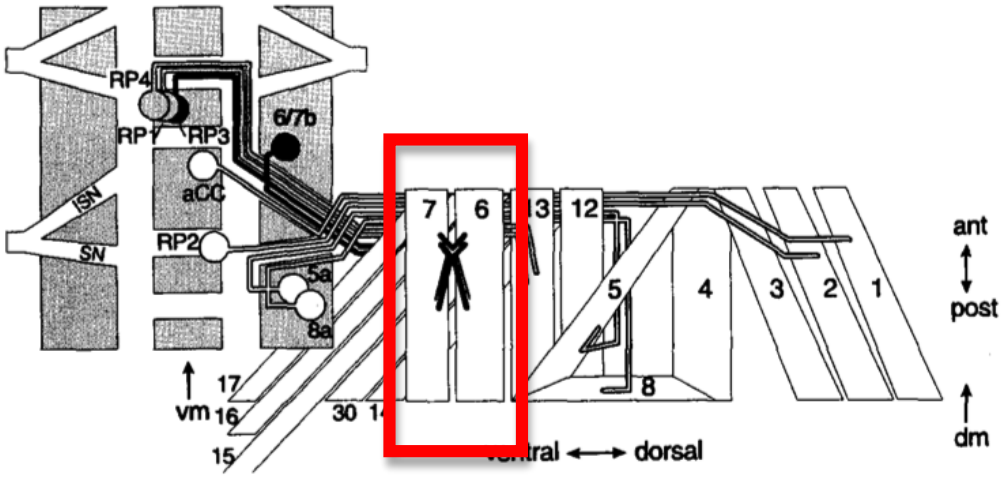


**Figure 2. Diagram of *Drosophila* Larval NMJ**[12]

One hemisegment of the larval muscle wall is shown above. The synapse between muscle 6 and 7 is boxed in red.

Glutamate is the excitatory neurotransmitter at the NMJ.[13] Glutamate also mediates most excitatory transmission in humans and the ionotropic glutamate receptors found in the NMJ have been highly conserved throughout evolution.[14] Ionotropic glutamate receptors are composed of four subunits. In *Drosophila*, there are five different subunits: GluRIIA, GluRIIB, GluRIID, GluRIIE, and GluRIII. GluRIIA and GluRIIB are redundant but GluRIID and GluRIIE are required for synaptic expression of the other subunits; therefore a functional receptor consists of GluRIID, GluRIIE, GluRII and either GluRIIA or GluRIIB.[15] In this project, the localization of GluRIIA at the synapse was observed via fluorescence confocal microscopy.

## 1.5 The *Akt1* Result

This project began as a result of previous work in the Selleck lab that demonstrated that muscle specific RNAi-induced knockdown of *Akt1* causes mislocalization of GluRIIA.[1] Normally, GluRIIA is localized to the synaptic boutons that surround the axonal projections of the neuron; however, in the *Akt1*$^{RNAi}$ animals, GluRIIA was expressed throughout the entire muscle in a banding pattern (Figure 3).

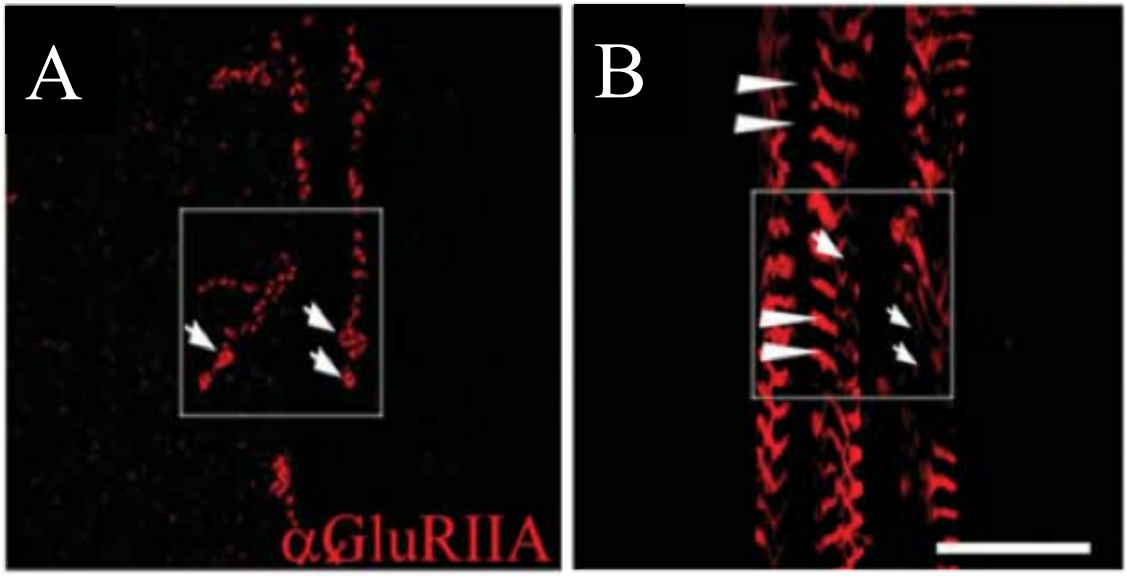


**Figure 3. GluRIIA mislocalization phenotype of muscle-specific RNAi-induced *Akt1* knockdown**[1]

Panel A shows the control NMJ (UAS-Akt1$^{RNAi}$/+), GluRIIA is localized to the synaptic boutons. Panel B shows that muscle-specific knockdown of *Akt1* (24B-Gal4>UAS-Akt1$^{RNAi}$ causes GluRIIA to mislocalize in a characteristic, banding pattern.

Akt, also known as Protein Kinase B (PKB) is a serine/threonine kinase that is central to many growth signaling pathways and has been implicated in many diseases such as cancer and diabetes.[16] The RNAi screen presented below was initiated in order to find genes that could potentially be linked to Akt in regulating the localization of glutamate

receptor subunits to the NMJ. The goal of this project was to identify new pathways related to Akt1 that regulate post-synaptic membrane development.

# Chapter 2

# Materials and Methods

## 2.1 RNAi Genetic Screen

Approximately 500 genes were selected for the genetic screen based on availability of RNAi transgenic flies and literature review. Transgenic flies with the RNAi constructs under control of a UAS promoter were acquired from the Vienna *Drosophila* RNAi Center for each of the selected genes. The Oregon-R wild type stock was used for control experiments. Males containing the RNAi transgenes were crossed with female virgins containing the muscle specific Mef2-Gal4 driver and a ubiquitous GFP-tagged, mouse-membrane protein mCD8. mCD8-GFP was included to allow visualization of membrane structures. The crosses were incubated at 30°C for 5-7 days until third instar larvae appeared for maximum efficiency of the GAL4 driver. Third instar larvae were dissected in ice-cold $Ca^{2+}$ free HL-3 media and fixed for 5 minutes in Bouin's fixative solution. The larvae were then washed in PBST (0.5% triton X-100 in PBS) and blocked with 10% normal goat serum (NGS) in PBST before antibody application. The samples were then immunostained with a mouse primary antibody for GluRIIA (1:1000 dilution in 10% NGS blocking solution), followed by mouse, Alexa Fluor 546 secondary antibody (1:2000 dilution in 10% NGS blocking solution). The NMJs were imaged on an Olympus FluoView FV1000 laser scanning confocal

microscope. Knockdown lines that displayed a GluRIIA mislocalization phenotype similar to the *Akt1* knockdown phenotype were selected as candidates for further study.

This genetic screen was performed by myself at three other undergraduate researchers under the guidance of Hyun Gwan Lee (post-doc mentor) and Na Zhao (graduate student mentor).

### 2.2 *DnaJ-2* Imprecise P-element Excision Mutagenesis

Mutagenesis of *DnaJ-2* was performed using a stock line with an enhancer promoter (EP) P-element insertion in the 5' untranslated region (UTR) of the endogenous *DnaJ-2* sequence on the third chromosome. The full genotype of this line is y[1] w[*];+; P{w[+mC]=EP}CG10565[G4964]/TM3, Sb[1];+ and will be referred to from this point on as EP-*DnaJ-2*/Tm3Sb. As indicated by the genetic scheme in Figure 4a, EP-*DnaJ-2*/Tm3Sb males were crossed to female virgins containing a transgene for Δ2-3 transposase in order to induce removal of the P-element. A mosaic-eye phenotype was used as an indicator of transposase activity because removal of the w[+mC] gene contained within the P-element by the transposase in some somatic cells causes mosaic pigmentation in the eyes. Thus mosaic-eye, flies from the F1 generation were selected for the next set of crosses with a balancer line. Single males with white eyes or mosaic eyes, indicating some loss of the P-element, were then selected as possible *DnaJ-2* mutants. These flies were then expanded by crossing with balancer lines and then assayed for imprecise excision of the P-element resulting in loss of endogenous *DnaJ-2* sequence.

After the initial mutagenesis attempt, the genetic scheme was modified to use males from the F1 generation instead of females in order to eliminate the possibility of rare recombination events and only white-eye single males were selected for screening in order to increase the rate of imprecise P-element excision detection. The modified scheme is shown in Figure 4b.

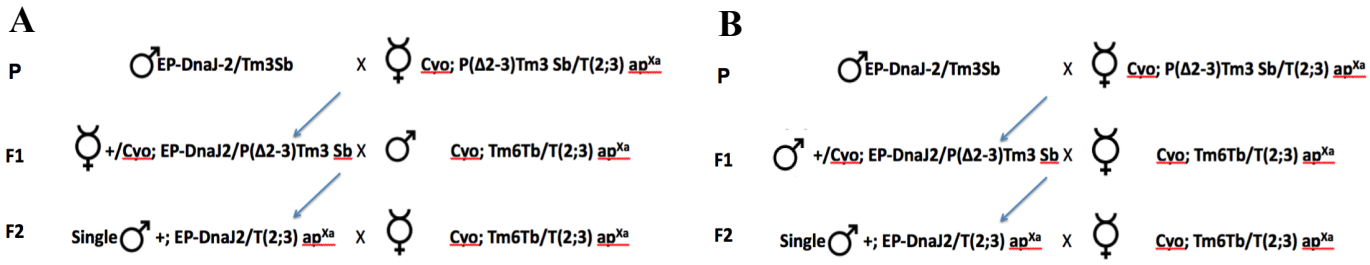


**Figure 4. Mutagenesis Scheme**
(A) Original mutagenesis genetic scheme (B) Modified mutagenesis scheme.

Potential mutants were screened by genotyping via PCR. Figure 5 shows the primer sets used to detect possible deletions in the endogenous *DnaJ-2* sequence. Candidates that were identified by the PCR primer sets 1 and 2 were then verified by sequencing the amplification product of primer set 3 for potential small mutations and primer set 5 for potential large mutations.

## 2.3 PCR Screening for Potential Mutants

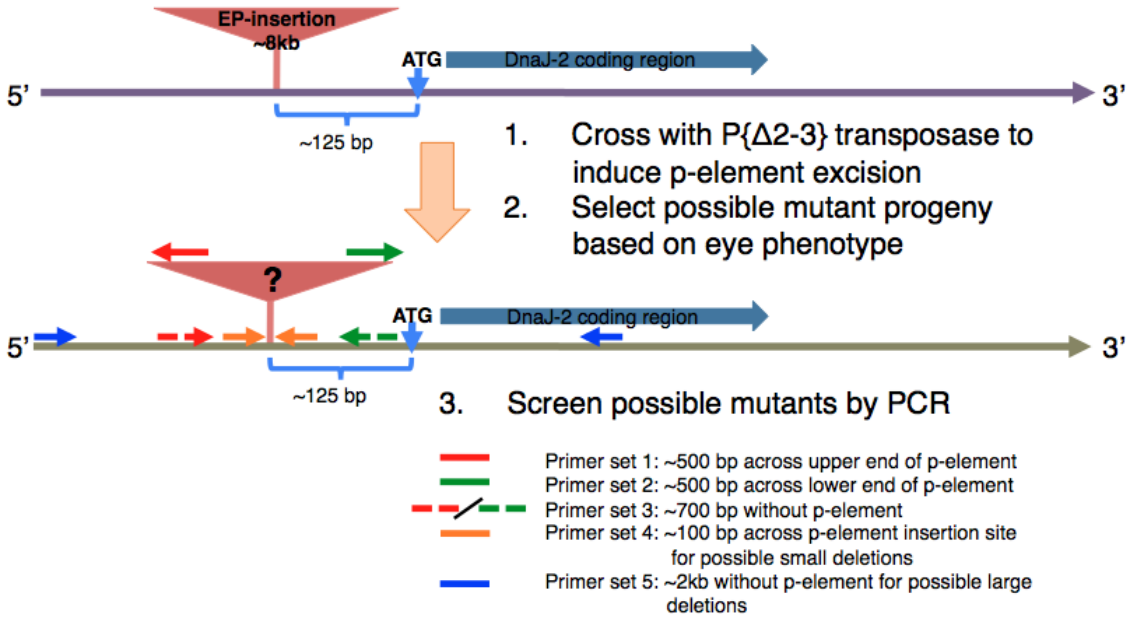


**Figure 5. Primer design for genotyping PCR of potential mutants**.
Primer set 1 and 2 provided the means of mapping the upstream and downstream ends of the P-element insertion. Primer set 3 examined the extent of the excision event if both set 1 and 2 amplification products were absent. Primer set 3 provided the tools to detect small insertion or deletion mutations near the P-element insertion site. Primer set 5 could detect large deletions including part of the upstream, endogenous DnaJ-2 coding sequence

Upon initial screening, it was discovered that all white-eye potential mutants were missing the p-element, eliminating the need to screen using primer sets 1-4. Therefore, further screening was performed using only primer set 5 to detect potential large deletions.

The initial round of mutagenesis was completed independently with guidance from Na Zhao. Primers were designed and provided by Na Zhao.

# Chapter 3

# Results

## 3.1 Identification of *DnaJ-2* RNAi Screen

The RNAi screen results revealed many genes that produced interesting phenotypes when their expression was decreased in muscle tissue. The phenotype of interest for the goals of the screen was the banding pattern of GluRIIA mislocalization seen in *Akt1*$^{RNAi}$ animals and 24 of genes surveyed replicated this phenotype with varying degrees of penetrance. The full results of the RNAi screen are presented in Appendix A. From this screen, *DnaJ-2* was selected for further study because RNAi –induced, muscle-specific knockdown of *DnaJ-2* displayed the same pattern of GluRIIA mislocalization at the post-synaptic membrane as the *Akt1* knockdown animals (Figure 6).

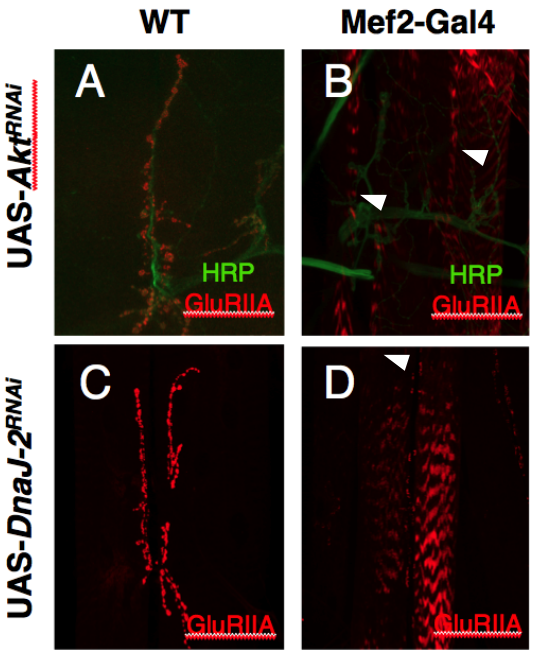


**Figure 6. GluRIIA Mislocalization Phenotype**

(A-C) Control NMJs between muscle 6 and 7. Anti-HRP antibodies recognize neuronal membrane and Anti-GluRIIA recognize GluRIIA at the post synaptic membrane. (B) Muscle specific RNAi knockdown of *Akt1*. (D) Muscle specific RNAi knockdown of *DnaJ-2*.

In control animals, GluRIIA is concentrated at the synaptic boutons as indicated by the colocalization of the HRP staining for neuronal membrane and the GluRIIA staining at the post-synaptic membrane in control panel A. Control panel C also shows the wildtype GluRIIA localization pattern at the NMJ. In contrast, both the *Akt* knockdown and *DnaJ-2* knockdown animals have the GluRIIA distributed across the entire muscle membrane in a banding pattern. This indicates that both *Akt1* and *DnaJ-2* are involved in regulating the localization of GluRIIA to the post-synaptic site of the NMJ. In follow-up experiments, the localization of GluRIIB in $DnaJ2^{RNAi}$ animals appeared to be normal; therefore, the effect of *DnaJ2* knockdown is specific to GluRIIA localization. This result provides more evidence for the potential relationship between *Akt1* and *DnaJ-2* because the $Akt1^{RNAi}$ phenotype was also specific to GluRIIA.

### 3.2 Generation of *DnaJ-2* Mutants by Imprecise P-element excision

In the initial mutagenesis attempt, approximately 200 animals were selected based on eye phenotype and screened by genotyping PCR. Of these, most appeared to have precise P-element excisions events or incomplete p-element excision. One potential mutant line, labeled as 28M, produced a ~500bp PCR product for primer set 5 for which the wildtype product is 2.2kb (Figure 7a). This result was verified by sequencing which showed a 1.8kb deletion including the start codon and part of the upstream coding sequence of *DnaJ-2* (Figure 7b). The full sequence of *DnaJ-2* annotated with the 28M deletion is provided in Appendix B. This result proved that the imprecise P-element

excision method was capable of producing large deletions in the endogenous *DnaJ-2* sequence.

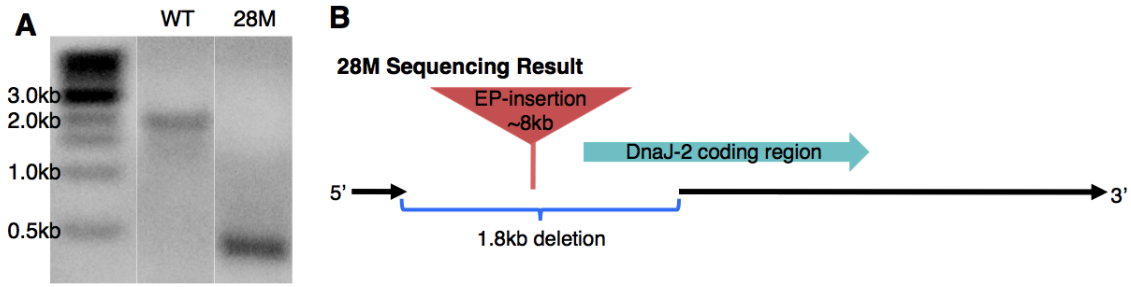


**Figure 7. Identification of 28M Deletion**

(A) Primer set 5 PCR of 28M sample. WT control shows expected ~2kb band and 28M shows >5kb band. (B) Sequencing of primer set 5 PCR product from 28M shows 1.8kb deletion that spans the 5' UTR to the upstream end of *DnaJ-2* coding sequence.

The rate of imprecise P-element excision was approximately 1 in 200 for the first round of mutagenesis. In order to optimize the mutagenesis and increase the rate of imprecise P-element excision the genetic schema and phenotypic selection methods were modified as previously mentioned. With these changes, the rate of imprecise p-element detection increased to approximately 1 in 40. The mutagenesis experiment is on-going in order to generate a variety of mutant alleles for *DnaJ-2*. Thus far, three additional mutants have been verified by sequencing results from the second round of mutagenesis. Figure 8 shows a schematic of these mutants and the precise locations of the deletions can be viewed in the sequences shown in Appendix B. These mutations are likely to have different effects on the expression of *DnaJ-2*. The availability of a range of hypomorphic and null alleles will be useful for future study.

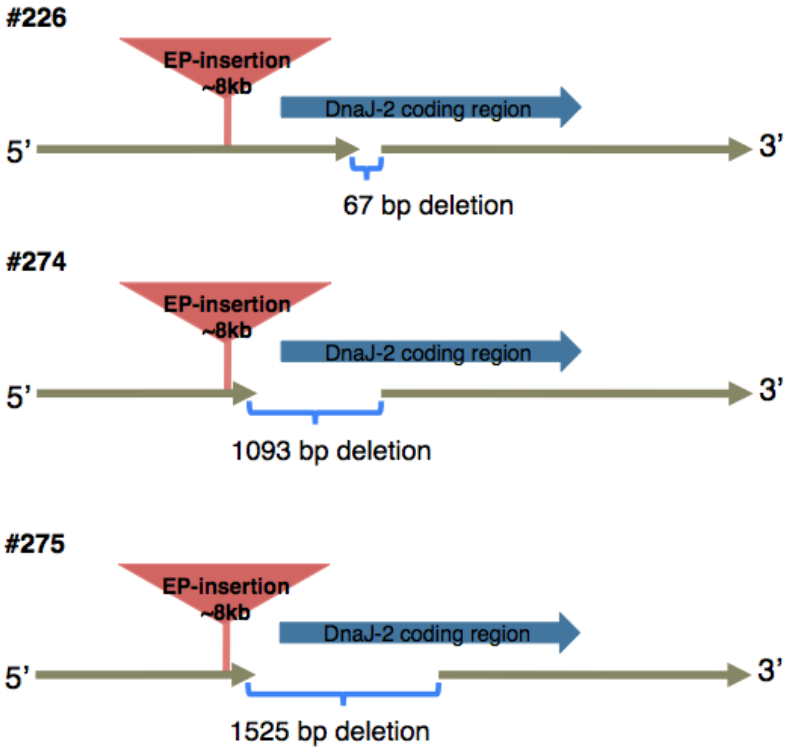


**Figure 8. Additional Mutants from Second Round of Mutagenesis**

The deletions in the three additional mutants (#226, #274, #275) are shown. All deletions are downstream of the EP insertion site. In #274 and #275, the start codon is lost in the deletion.

# Chapter 3

# Discussion

## 4.1 Potential Importance of *DnaJ-2*

*DnaJ-2* has not been studied; therefore information about its function and structure was inferred from its publicly available sequence. Sequence analysis from FlyBase indicated that *DnaJ*-2 is a member of the DnaJ or J-protein family. Members of the J-protein family contain a conserved 70 amino acid region called the J-domain; however, outside the J-domain, DnaJ-like proteins can be extremely structurally diverse.[17] J-proteins have diverse functions but all act as molecular chaperones by interacting with Hsp70 via the J-domain region. Most J-proteins have the J-domain located near the N-terminus, as is the case for the protein of interest in this study.[18] As shown in Figure 9 the J-domain consists of 4 α helices with a highly conserved HPD motif in the loop region.[19] The HPD motif is necessary for the J-protein to regulate the ATPase activity of Hsp70.[20] ATP hydrolysis changes the conformational state of Hsp70 from an open to a closed state which then stabilizes the substrate binding to Hsp70.[21] When associated with Hsp70, J-proteins work to refold misfolded proteins and also prevent aggregation of misfolded proteins.[22]

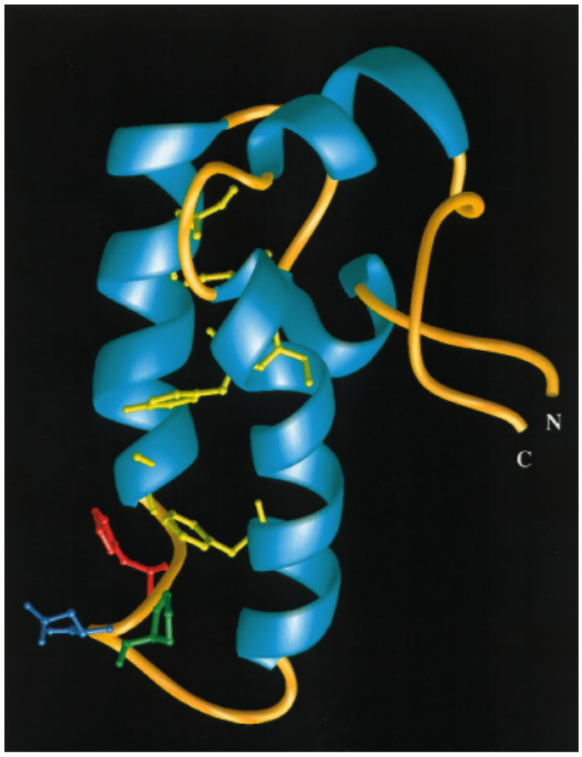


**Figure 9. Structure of the J-domain**[19]
The four main α helices are shown in cyan. The HPD motif is shown in red (H), green (P), and blue (D)

In addition to the primary protein chaperone activity, research has shown that members of the J-protein family play roles in regulating protein translocation. The yeast homolog of DnaJ, YDJ1, was shown to function in polypeptide translocation across mitochondrial membrane and endoplasmic reticulum membrane.[23] Similarly, *DnaJ-2* has been implicated in endocytosis and exocytosis. During endocytosis, J proteins have been shown to be necessary for the dissociation of clathrin from clathrin-coated vesicles by associating with Hsc70.[24] In other cases, J-proteins have been found on the cytoplasmic face of synaptic vesicles and were identified as mediators of synaptic vesicle fusion, suggesting that they may have a role in regulating exocytosis as well as endocytosis.[25]

Due to the importance of DnaJ proteins in regulating protein folding and assembly, they have been implicated in many neurodegenerative diseases, which are often caused by neuronal death as a result of accumulation of misfolded proteins.[26] For example, a J-protein was shown to suppress polyglutamine toxicity in a *Drosophila* model for polyglutamine diseases such as Huntington's disease.[27]

This connection to neurodegeneration and the many known functions of J-proteins make *DnaJ-2* an interesting candidate for study. The mislocalization of GluRIIA phenotype seen in *DnaJ-2* knockdown animals could be a result of misfolding of the receptor subunit, an error in exocytotic or endocytotic mechanisms that regulated post-synaptic membrane development, or some other unknown function of this particular J-protein. Also, sequence analysis of *DnaJ-2* showed that it contains a DNA binding domain near the C-terminus (Figure 10).


**Figure 10. *DnaJ-2* sequence**
The conserved J-domain that interacts with Hsp70 is located near the N-terminus and a DNA binding domain (DBD) is located near the C-terminus.

Based on this information, mutant constructs of *DnaJ-2* with the J-domain deleted, the HPD motif altered to QPD, and the DNA binding domain deleted were generated to investigate which functional regions are responsible for the mislocalization of GluRIIA. These constructs will be utilized in future experiments.

## 4.2 Alterations to the Mutagenesis Scheme and Continued Search for Mutant Alleles

The initial genetic scheme and PCR screening methods for the imprecise P-element mutagenesis were modified after the first mutagenesis attempt for several

reasons. First, the rate of imprecise excision was approximately 1 in every 200 identified excision events whereas the predicted rate of imprecise excision was ~1%.[28] Second, the mosaic-eye phenotype, which should have been lost concurrently with the removal of the Δ2-3 transposase after F1 cross, persisted in subsequent generations when mosaic-eye F2 males were chosen for the further screening. This could have been due to rare recombination events in the F1 females or potential reinsertion of the Δ2-3 construct at a different locus.

During the first round of mutagenesis females from the F1 generation were used to cross to the balancer line because P-element excision occurs slightly more efficiently in females.[29] However, upon reexamining the literature it was found that mosaic eyed males from the F1 generation were most commonly used instead of females in order to prevent the possibility of rare recombination events that could occur in the germline of females.[28] Thus, the genetic scheme was adjusted to use F1 males.

Additionally, in the first round of mutagenesis both mosaic-eyed and white-eyed male progeny were selected as potential mutants. PCR screening showed that an excision event had occurred in all white-eyed males but only in some mosaic-eyed males. Also, when mosaic-eyed males were chosen for F2 single male crosses, the mosaic-eyed phenotype, which should have already been lost, persisted in future generations as mentioned before. Therefore, in order to eliminate the possibility of the presence of Δ2-3 transposase in later generations and to increase the frequency of imprecise excision detection, only white-eyed males from the F2 generation were selected for screening after the initial mutagenesis attempt. Following these changes, the frequency of imprecise excision detection has increased to 1 in 40 of all lines screened.

**4.3 Future Experiments**

With the information presented above, it is necessary to conduct further experiment to better understand the role of *DnaJ-2* in regulating development and localization of GluRIIA at the post-synaptic site of the *Drosophila* larval NMJ. The mutagenesis of *DnaJ-2* is ongoing in order to generate a variety of mutant alleles with a range of effects on the expression and function of *DnaJ-2*. These mutants need to be sequenced to determine the extent of the mutation and then characterized via quantitative methods such as qRT-PCR or Western blot. The antibody for the DnaJ-2 protein is not yet available but the Selleck Lab is working towards generating an antibody suitable for immunohistochemistry and Western blotting. When a null mutant is generated, the various constructs of *DnaJ-2* with specific mutations generated under UAS promoter control can be placed in the mutant background in order to identify the regions of the protein necessary for its function. Genetic interaction assays to analyze the possible relationship between *Akt1* and *DnaJ-2* are also underway. Previous work in the Selleck lab has determined that overexpression of the *Akt1* cannot rescue the GluRIIA mislocalization phenotype of RNAi induced knockdown of *DnaJ-2* in the muscle tissue; therefore, further experiments are being conducted to test whether or not overexpression of *DnaJ-2* can rescue the GluRIIA mislocalization phenotype of the *Akt1* knockdown animals. A rescue of the *Akt1* knockdown phenotype by overexpression of *DnaJ-2* would provide evidence to suggest that *DnaJ-2* is downstream of *Akt1* in the *Akt1* mediated pathway for regulating GluRIIA localization. In the absence of rescue, the more likely conclusion is that the two genes are in parallel pathways and do not closely interact with

each other. Continued study of *DnaJ-2* may lead to new understanding of how J-proteins are involved in synapse development and discovery of a new regulatory pathway for neurotransmitter localization to the post-synaptic membrane.

# Chapter 4

## Conclusion

Even with the advanced medical technology available today, neurological disorders are still a largely unsolved public health issue. Scientists have only just begun to understand the workings of the nervous system. For neurodegenerative disorders such as Alzheimer's , Parkinson's and Huntington's, there are almost no available treatment options. The molecular basis for psychiatric disorders such as Schizophrenia, depression, and bipolar disorder is still not well understood.

Model organisms provide great opportunities to better understand the development and regulation of synaptic function. The *Drosophila* larval NMJ is a powerful model system to study glutamatergic synapses, which are also the principal excitatory synapses in the human brain. Additionally, activation of glutamate receptors have been implicated in cell death processes that lead to chronic neurodegenerative disorders.[30] Therefore, knowledge gained from studying the *Drosophila* model for glutamatergic synapses is highly relevant to understanding human neurological disorders.

*DnaJ-2* is also interesting because it is a member of the J protein family of chaperones. Aggregation of misfolded proteins is the major cause of neuronal cell death in many neurodegenerative diseases; therefore chaperone proteins, such as J-proteins, are of great relevance to these diseases. Additionally, J-proteins, such as nerve-specific auxilin (DnaJC6), are involved in endocytotic processes that may regulate membrane structure at synapses.[31] There are 40 identified J-proteins in humans and the homolog of

the *DnaJ-2* in humans is DNAJC2 (aka Zuo1, MPP11).[32] DNAJC2 encodes an M-phase phoshoprotein (MPP) and, like *DnaJ-2*, contains a J-domain and a Myb DNA-binding domain. DNAJC2 is also known to associate with ribosomes to guide the proper folding of nascent polypetide chains.[33] New knowledge gained from studying J-proteins in the *Drosophila* model could easily provide understanding of how they function in more complex systems. The phenotype of RNAi mediated *DnaJ-2* knockdown provides strong evidence that this gene is a regulator of post-synaptic development. Continued study of *DnaJ-2* has potential to provide new understanding of synapse development and the role of J-proteins in synaptic development.

# Appendix A

## RNAi Screen

### Table 1. Results of RNAi Screen

All RNAi lines were expressed under muscle-specific Mef2Gal4-UAS control. mCD8-GFP selectively expressed in muscle tissue was used as a membrane marker to visualize post-synaptic membrane structure. αGluRIIA antibody was used to visualize the localization of GluRIIA at the post-synaptic site. *DnaJ-2* is serial# 67 and is highlighted in the table.

| Serial # | RNAi line Transformant ID # | CG # | Post-synaptic membrane structure (mCD8-GFP membrane marker) | dGluRIIA localization |
|---|---|---|---|---|
| 1 | 100011 | CG10545 | normal | normal |
| 2 | 100033 | CG10232 | normal | normal |
| 3 | 100081 | CG10217 | normal | normal |
| 4 | 100143 | CG10246 | normal | normal |
| 5 | 100180 | CG10269 | decreased signal | decreased signal |
| 7 | 100212 | CG10694 | normal | patches |
| 8 | 100225 | CG10725 | normal | decreased signal |
| 9 | 100226 | CG10203 | normal | normal |
| 10 | 100252 | CG10345 | normal | normal |
| 11 | 100272 | CG10038 | normal | normal |
| 13 | 100842 | CG10702 | normal | normal |
| 14 | 101360 | CG10723 | normal | normal |
| 15 | 101365 | CG1086 | patches | banding |
| 16 | 101391 | CG10652 | decreased signal | decreased signal |
| 17 | 101393 | CG10214 | decreased signal | decreased signal |
| 18 | 101460 | CG10375 | normal | normal |
| 19 | 101620 | CG10683 | normal | normal |
| 20 | 101643 | CG10175 | decreased signal | decreased signal |
| 21 | 101663 | CG10712 | patches | decreased signal or absent; slight banding |
| 22 | 101764 | CG10036 | normal | normal |
| 23 | 101774 | CG1028 | decreased signal | decreased signal |
| 26 | 101818 | CG10834 | normal | normal |
| 27 | 102405 | CG10016 | clumping | clumping |
| 28 | 102407 | CG10473 | decreased signal | decreased signal |
| 29 | 102478 | CG1076 | decreased signal | decreased signal |
| 30 | 102572 | CG10472 | normal | decreased signal |
| 32 | 102619 | CG10303 | normal | normal |
| 33 | 102660 | CG10140 | normal | normal |
| 34 | 102671 | CG10247 | normal | normal |
| 35 | 102698 | CG10134 | normal | normal |
| 36 | 102737 | CG10527 | normal | normal |
| 37 | 102744 | CG10508 | normal | normal |
| 38 | 102818 | CG10129 | normal | normal |
| 39 | 102824 | CG10014 | normal | normal |
| 41 | 103246 | CG10045 | normal | normal |
| 42 | 103288 | CG10598 | normal | normal |

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|---|---|---|---|---|
| 43 | 103329 | CG10528 | normal | normal |
| 44 | 103376 | CG10657 | normal | decreased signal; slight banding |
| 45 | 103421 | CG10286 | normal | decreased signal; slight banding |
| 46 | 103433 | CG10863 | underdeveloped | |
| 47 | 103438 | CG10031 | normal | normal |
| 48 | 103490 | CG10477 | normal | decreased signal or absent |
| 50 | 103645 | CG10743 | normal | normal |
| 51 | 103655 | CG10447 | normal | normal |
| 52 | 103749 | CG10082 | normal | decreased signal |
| 53 | 103985 | CG10706 | normal | normal |
| 54 | 104016 | CG10120 | normal | normal |
| 55 | 104038 | CG10789 | normal | normal |
| 57 | 104421 | CG10693 | normal | decreased signal |
| 58 | 104432 | CG10225 | normal | normal |
| 59 | 104451 | CG10474 | normal | normal |
| 61 | 104488 | CG10184 | normal | normal |
| 62 | 104526 | CG10407 | normal | normal |
| 63 | 104538 | CG10176 | normal | normal |
| 64 | 105054 | CG10719 | normal | slight banding |
| 65 | 105120 | CG10566 | normal | normal |
| 67 | 105149 | CG10565 | patches | banding |
| 68 | 105155 | CG10626 | normal | normal |
| 69 | 105179 | CG10654 | decreased signal | decreased signal |
| 71 | 105202 | CG00000 | normal | normal |
| 72 | 105533 | CG10307 | underdeveloped | |
| 73 | 105596 | CG10746 | normal | normal |
| 73 | 105843 | CG10838 | normal | normal |
| 74 | 105746 | CG10699 | normal | normal |
| 75 | 105760 | CG10751 | normal | normal |
| 76 | 105770 | CG10338 | normal | normal |
| 77 | 105781 | CG10243 | normal | normal |
| 79 | 105902 | CG10616 | normal | normal |
| 80 | 105950 | CG10084 | normal | decreased signal |
| 81 | 105952 | CG10144 | normal | normal |
| 82 | 106002 | CG10209 | normal | normal |
| 83 | 106017 | CG10713 | underdeveloped | |
| 84 | 106051 | CG10061 | normal | normal |
| 85 | 106064 | CG10550 | normal | normal |
| 87 | 106141 | CG10610 | normal | normal |
| 88 | 106150 | CG1031 | normal | normal |
| 89 | 106185 | CG10052 | normal | normal |
| 90 | 106212 | CG10170 | normal | normal |
| 91 | 106226 | CG10479 | normal | normal |
| 92 | 106590 | CG10264 | normal | normal |
| 93 | 106599 | CG10803 | elongated boutons | decreased signal |
| 96 | 106729 | CG10207 | normal | normal |
| 97 | 106737 | CG10553 | normal | normal |
| 98 | 106784 | CG10366 | normal | normal |
| 99 | 107131 | CG10465 | normal | normal |
| 100 | 107140 | CG10576 | normal | normal |
| 101 | 107143 | CG1024 | increase | normal |
| 102 | 107187 | CG10572 | normal | decreased signal |
| 103 | 107329 | CG10674 | decreased signal | decreased signal |
| 104 | 107394 | CG10700 | normal | normal |
| 105 | 107784 | CG10130 | normal | increased |
| 106 | 107842 | CG10505 | underdeveloped | |
| 109 | 107903 | CG10486 | normal | normal |

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|---|---|---|---|---|
| 110 | 107908 | CG10171 | normal | normal |
| 111 | 107922 | CG10659 | normal | normal |
| 114 | 108196 | CG10226 | blurry | decreased signal |
| 115 | 108216 | CG10210 | normal | normal |
| 116 | 108219 | CG10191 | normal | normal |
| 117 | 108255 | CG10034 | elongated boutons | banding |
| 118 | 108259 | CG10603 | normal | decreased signal |
| 119 | 108261 | CG10763 | normal | normal |
| 121 | 108354 | CG10420 | normal | normal |
| 122 | 108388 | CG10237 | patches | normal |
| 123 | 108425 | CG10814 | underdeveloped | |
| 124 | 108572 | CG10581 | normal | normal |
| 125 | 108599 | CG1064 | normal | normal |
| 126 | 108617 | CG10367 | underdeveloped | |
| 127 | 108634 | CG10850 | normal | decreased signal; patches |
| 128 | 108635 | CG10069 | normal | normal |
| 129 | 108648 | CG10001 | normal | normal |
| 130 | 108657 | CG10254 | normal | normal |
| 131 | 108848 | CG10133 | normal | normal |
| 132 | 108861 | CG1066 | normal | normal |
| 133 | 108866 | CG10050 | normal | normal |
| 134 | 108941 | CG10582 | underdeveloped | |
| 135 | 108948 | CG10078 | patches | normal |
| 136 | 109000 | CG10233 | normal | normal |
| 137 | 109001 | CG10277 | patches | decreased signal |
| 138 | 109012 | CG10262 | patches | decreased signal |
| 139 | 109631 | CG10844 | underdeveloped | |
| 140 | 109718 | CG10623 | normal | normal |
| 141 | 109727 | CG10151 | normal | normal |
| 142 | 109730 | CG10112 | normal | normal |
| 143 | 109767 | CG10732 | normal | normal |
| 146 | 109883 | CG10495 | decreased signal | decreased signal |
| 147 | 109895 | CG10671 | normal | normal |
| 148 | 110063 | CG10041 | normal | normal |
| 149 | 110079 | CG10252 | normal | banding |
| 150 | 110321 | CG10480 | normal | normal |
| 151 | 110388 | CG10341 | normal | normal |
| 152 | 110419 | CG10215 | normal | decreased signal |
| 153 | 110429 | CG10042 | normal | decreased signal |
| 156 | 110564 | CG10153 | normal | normal |
| 157 | 110657 | CG1009 | normal | normal |
| 158 | 110679 | CG10590 | normal | mislocalization |
| 160 | 110717 | CG10392 | normal | normal |
| 162 | 110731 | CG10721 | normal | decreased signal at NMJ; increased across muscle |
| 163 | 102362 | CG10861 | normal | normal |
| 164 | 107547 | CG10855 | underdeveloped | |
| 165 | 110669 | CG10080 | underdeveloped | |
| 166 | 110640 | CG10268 | decreased signal | normal |
| 167 | 110571 | CG10110 | normal | decreased signal, patches |
| 168 | 110232 | CG10274 | underdeveloped | |
| 169 | 110225 | CG10281 | decreased signal | decreased signal |
| 170 | 110223 | CG10185 | normal | normal |
| 171 | 110196 | CG10585 | normal | normal |
| 172 | 110190 | CG10160 | underdeveloped | |
| 173 | 110172 | CG10399 | normal | normal |
| 174 | 110164 | CG1074 | normal | normal |

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|---|---|---|---|---|
| 175 | 109881 | CG10697 | normal | normal |
| 176 | 109507 | CG10637 | normal | normal |
| 177 | 109472 | CG10300 | decreased signal | decrease signal |
| 178 | 109469 | CG10749 | normal | normal |
| 179 | 109465 | CG10777 | normal | normal |
| 180 | 109422 | CG10068 | normal | normal |
| 181 | 109407 | CG10538 | normal | normal |
| 182 | 109402 | CG10535 | normal | normal |
| 183 | 109311 | CG10672 | normal | normal |
| 184 | 109284 | CG10425 | normal | normal |
| 185 | 109279 | CG10198 | normal | normal |
| 186 | 109043 | CG10533 | underdeveloped | |
| 187 | 109027 | CG10121 | underdeveloped | |
| 188 | 108982 | CG10839 | normal | normal |
| 189 | 108937 | CG10295 | decreased signal | normal |
| 190 | 108879 | CG10118 | increase in boutons | normal |
| 191 | 108608 | CG10023 | normal | decreased signal |
| 192 | 108582 | CG10484 | decreased signal | banding |
| 193 | 108506 | CG10698 | underdeveloped | |
| 194 | 108431 | CG10104 | normal | normal |
| 195 | 108426 | CG10311 | normal | normal |
| 196 | 108409 | CG1072 | underdeveloped | |
| 197 | 108293 | CG10033 | normal | normal |
| 198 | 108193 | CG10360 | underdeveloped | |
| 199 | 108163 | CG10086 | underdeveloped | |
| 200 | 108046 | CG10764 | normal | normal |
| 201 | 107996 | CG10443 | normal | decreased signal |
| 202 | 107988 | CG10335 | normal | normal |
| 203 | 107744 | CG10257 | underdeveloped | |
| 204 | 107721 | CG10194 | normal | normal |
| 205 | 107720 | CG10682 | normal | normal |
| 206 | 107674 | CG10113 | normal | normal |
| 207 | 107595 | CG10124 | normal | normal |
| 208 | 107561 | CG10734 | underdeveloped | |
| 209 | 107452 | CG10026 | normal | normal |
| 210 | 107417 | CG10711 | normal | normal |
| 211 | 107335 | CG10076 | normal | normal |
| 212 | 107008 | CG10444 | decreased signal | normal |
| 213 | 106891 | CG10374 | normal | normal |
| 214 | 106565 | CG10852 | normal | normal |
| 215 | 106487 | CG10229 | normal | decreased signal, patches |
| 216 | 106383 | CG10737 | underdeveloped | |
| 217 | 106237 | CG10583 | normal | decreased signal |
| 218 | 106172 | CG10064 | normal | normal |
| 219 | 105940 | CG10418 | patches | patches; increased signal |
| 220 | 105919 | CG10621 | normal | normal |
| 221 | 105916 | CG10317 | normal | normal |
| 222 | 105900 | CG10051 | normal | normal |
| 223 | 105871 | CG10501 | normal | normal |
| 224 | 105614 | CG10260 | normal | normal |
| 225 | 105463 | CG1058 | normal | normal |
| 226 | 105440 | CG10628 | underdeveloped | |
| 227 | 105380 | CG10354 | normal | banding |
| 228 | 105317 | CG10822 | normal | normal |
| 229 | 105314 | CG10382 | normal | decreased signal |
| 230 | 105266 | CG10658 | underdeveloped | |
| 231 | 105263 | CG10123 | underdeveloped | |

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|---|---|---|---|---|
| 232 | 105093 | CG10043 | normal | normal |
| 233 | 104941 | CG10384 | normal | normal |
| 234 | 104910 | CG10063 | normal | normal |
| 235 | 104790 | CG10326 | normal | normal |
| 236 | 104774 | CG10371 | normal | normal |
| 237 | 104764 | CG18635 | underdeveloped | |
| 238 | 104759 | CG1014 | normal | normal |
| 240 | 104658 | CG10352 | decreased signal | decreased signal |
| 241 | 104618 | CG10578 | normal | normal |
| 242 | 104431 | CG10336 | normal | normal |
| 243 | 104414 | CG10157 | normal | normal |
| 244 | 104401 | CG10327 | normal | normal |
| 245 | 104188 | CG10778 | few patches | decreased signal |
| 246 | 104160 | CG1034 | normal | normal |
| 247 | 104138 | CG10669 | normal | normal |
| 248 | 103837 | CG10197 | normal | normal |
| 249 | 103798 | CG10091 | normal | normal |
| 250 | 103781 | CG10681 | normal | normal |
| 251 | 103780 | CG10413 | normal | normal |
| 252 | 103744 | CG10098 | normal | normal |
| 253 | 103714 | CG10428 | underdeveloped | |
| 254 | 103585 | CG10619 | normal | normal |
| 255 | 103524 | CG10107 | underdeveloped | |
| 256 | 102914 | CG10638 | normal | decreased signal; patches |
| 257 | 102670 | CG10359 | normal | normal |
| 258 | 102518 | CG10287 | underdeveloped | |
| 259 | 102435 | CG10126 | normal | decreased signal; patches |
| 260 | 102318 | CG10589 | normal | normal |
| 261 | 102105 | CG1056 | normal | normal |
| 262 | 102047 | CG1079 | normal | normal |
| 263 | 101903 | CG10571 | normal | normal |
| 264 | 101609 | CG10013 | normal | normal |
| 265 | 101599 | CG10163 | normal | normal |
| 266 | 101569 | CG10353 | underdeveloped | |
| 267 | 101555 | CG10377 | underdeveloped | |
| 268 | 101554 | CG10622 | patches | decreased signal |
| 269 | 101547 | CG10574 | normal | normal |
| 270 | 101500 | CG10847 | normal | normal |
| 271 | 101493 | CG10009 | patches | decreased signal |
| 272 | 101488 | CG1057 | normal | normal |
| 273 | 101358 | CG1044 | normal | normal |
| 274 | 101259 | CG10655 | normal | normal |
| 275 | 101178 | CG10845 | normal | normal |
| 276 | 101174 | CG10369 | normal | increased |
| 277 | 101168 | CG10283 | underdeveloped | |
| 278 | 101159 | CG10631 | normal | normal |
| 279 | 101139 | CG10090 | normal | normal |
| 280 | 101133 | CG10154 | underdeveloped | |
| 281 | 100990 | CG1071 | underdeveloped | |
| 282 | 100982 | CG10093 | normal | normal |
| 283 | 100959 | CG10739 | normal | normal |
| 284 | 100895 | CG10301 | normal | normal |
| 285 | 100830 | CG10346 | normal | normal |
| 286 | 100805 | CG10128 | normal | normal |
| 287 | 100789 | CG10808 | underdeveloped | |
| 288 | 100773 | CG10540 | normal | normal |
| 289 | 100704 | CG10189 | normal | normal |

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|---|---|---|---|---|
| 290 | 100623 | CG10075 | underdeveloped | |
| 291 | 100617 | CG10340 | increased | increased |
| 292 | 100587 | CG1007 | normal | normal |
| 293 | 100583 | CG10043 | normal | normal |
| 294 | 100446 | CG10841 | normal | normal |
| 296 | 100400 | CG10804 | normal | normal |
| 297 | 100397 | CG10801 | normal | normal |
| 298 | 32929 | CG1743 | normal | normal |
| 299 | 23483 | CG9734 | normal | decreased signal |
| 300 | 29275 | CG9779 | normal | normal |
| 301 | 100271 | CG11324 | normal | normal |
| 302 | 100428 | CG11008 | normal | absent |
| 303 | 100518 | CG11251 | increased | increased |
| 304 | 100526 | CG1089 | normal | normal |
| 305 | 100527 | CG11570 | normal | normal |
| 306 | 100612 | CG11785 | decreased signal | decreased signal |
| 307 | 100666 | CG11635 | normal | normal |
| 308 | 100671 | CG11218 | increased quantity of boutons | decreased signal |
| 309 | 100721 | CG11326 | normal | normal |
| 310 | 100742 | CG11328 | normal | decreased signal |
| 311 | 100763 | CG11143 | normal | normal |
| 312 | 100776 | CG11611 | normal | decreased signal |
| 313 | 100845 | CG11291 | normal | normal |
| 314 | 100862 | CG1132 | normal | normal |
| 315 | 100869 | CG10973 | normal | normal |
| 316 | 100896 | CG11786 | underdeveloped | |
| 317 | 100907 | CG11254 | normal | normal |
| 318 | 100942 | CG1108 | normal | normal |
| 319 | 100944 | CG11064 | underdeveloped | |
| 320 | 101032 | CG10956 | normal | normal |
| 321 | 101080 | CG11069 | normal | normal |
| 322 | 101081 | CG11415 | normal | normal |
| 323 | 101239 | CG1113 | normal | normal |
| 324 | 101266 | CG11313 | normal | decreased signal |
| 325 | 101282 | CG11101 | normal | normal |
| 327 | 101397 | CG11753 | normal | normal |
| 328 | 101491 | CG11699 | normal | normal |
| 329 | 101601 | CG11147 | normal | normal |
| 330 | 101666 | CG11629 | normal | normal |
| 331 | 101668 | CG10981 | normal | normal |
| 332 | 101758 | CG1098 | normal | normal |
| 333 | 101895 | CG11175 | normal | decreased signal |
| 335 | 101952 | CG11750 | decreased signal | decreased signal |
| 336 | 101998 | CG11138 | normal | normal |
| 337 | 102021 | CG10930 | decreased signal | decreased signal |
| 338 | 102040 | CG10946 | elongation of boutons | mislocalization |
| 339 | 102058 | CG1128 | normal | normal |
| 340 | 102059 | CG11398 | patches | normal |
| 334 | 101929 | CG11671 | normal | decreased signal |
| 341 | 102061 | CG11000 | normal | normal |
| 342 | 102062 | CG11455 | underdeveloped | |
| 343 | 102112 | CG11816 | normal | decreased signal |
| 344 | 102170 | CG11406 | decreased signal | absent or decreased signal |
| 345 | 102191 | CG10959 | patches | normal |
| 346 | 102252 | CG11422 | normal | increased |
| 347 | 102282 | CG11019 | underdeveloped | |
| 348 | 102331 | CG11381 | normal | normal |

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|---|---|---|---|---|
| 349 | 102353 | CG11165 | underdeveloped | |
| 350 | 102359 | CG11637 | normal | normal |
| 352 | 102363 | CG1139 | normal | normal |
| 353 | 102365 | CG11562 | | |
| 354 | 102428 | CG11742 | normal | decreased signal |
| 355 | 102485 | CG11298 | normal | normal |
| 357 | 103012 | CG10883 | normal | decreased signal |
| 358 | 103207 | CG11529 | normal | normal |
| 359 | 103212 | CG11581 | increased | increased |
| 360 | 103232 | CG11085 | normal | normal |
| 361 | 103262 | CG11598 | normal | decreased signal |
| 364 | 103457 | CG11516 | normal | absent |
| 367 | 103736 | CG11144 | normal | normal |
| 368 | 103931 | CG11516 | underdeveloped | |
| 370 | 104169 | CG11228 | normal | normal |
| 371 | 104245 | CG11106 | normal | normal |
| 372 | 104286 | CG11306 | patches | decreased signal |
| 373 | 104359 | CG11586 | underdeveloped | |
| 374 | 104375 | CG11268 | underdeveloped | |
| 375 | 104377 | CG11077 | normal | normal |
| 376 | 104378 | CG11208 | normal | normal |
| 377 | 104435 | CG11777 | normal | normal |
| 378 | 104443 | CG11722 | underdeveloped | |
| 379 | 104456 | CG10919 | normal | decreased signal |
| 380 | 104761 | CG10975 | normal | normal |
| 381 | 104878 | CG1116 | normal | normal |
| 382 | 104926 | CG11294 | decreased signal | decreased signal |
| 383 | 104938 | CG10899 | normal | patches |
| 384 | 104997 | CG11555 | abnormal shape | banding |
| 385 | 105036 | CG11405 | normal | normal |
| 386 | 105063 | CG1171 | normal | patches |
| 387 | 105073 | CG11668 | normal | normal |
| 388 | 105079 | CG11508 | normal | normal |
| 389 | 105392 | CG11388 | normal | normal |
| 391 | 105416 | CG11025 | normal | decreased signal |
| 392 | 105417 | CG11814 | decreased signal | decreased signal |
| 393 | 105487 | CG11594 | normal | normal |
| 394 | 105547 | CG11456 | normal | decreased signal |
| 396 | 105586 | CG1154 | normal | normal |
| 397 | 105588 | CG11664 | normal | normal |
| 398 | 105683 | CG11076 | normal | decreased signal |
| 399 | 105859 | CG11411 | decreased signal | decreased signal |
| 400 | 105865 | CG11158 | normal | normal |
| 401 | 105975 | CG10997 | normal | decreased signal and banding |
| 402 | 105976 | CG11622 | normal | normal |
| 403 | 105985 | CG11148 | normal | normal |
| 405 | 106008 | CG11213 | decreased signal | decreased signal |
| 406 | 106030 | CG11650 | normal | normal |
| 407 | 106531 | CG11760 | normal | normal |
| 408 | 106566 | CG10910 | normal | increased |
| 409 | 106921 | CG11210 | normal | normal |
| 411 | 106969 | CG1093 | normal | banding |
| 412 | 106978 | CG10971 | normal | normal |
| 413 | 107054 | CG11372 | normal | decreased signal |
| 414 | 107110 | CG11140 | normal | normal |
| 415 | 107113 | CG11145 | normal | normal |
| 417 | 107148 | CG11139 | normal | decrease |

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|---|---|---|---|---|
| 419 | 107344 | CG11579 | normal | normal |
| 420 | 107713 | CG11661 | normal | decreased signal |
| 421 | 107724 | CG11755 | normal | normal |
| 422 | 107754 | CG11686 | normal | normal |
| 424 | 108017 | CG1135 | normal | decreased signal or absent |
| 425 | 108034 | CG1149 | normal | decreased signal |
| 426 | 108081 | CG11678 | patches | decreased signal |
| 427 | 108188 | CG11771 | normal | normal |
| 428 | 108277 | CG11656 | normal | normal |
| 429 | 108307 | CG11793 | normal | normal |
| 431 | 108337 | CG11340 | decreased signal | decreased signal |
| 432 | 108456 | CG11761 | normal | normal |
| 433 | 108467 | CG11357 | normal | decreased signal |
| 434 | 108488 | CG1165 | normal | patches |
| 435 | 108505 | CG11516 | normal | normal |
| 437 | 108663 | CG11062 | normal | normal |
| 438 | 108695 | CG11798 | underdeveloped | |
| 440 | 108735 | CG10914 | normal | normal |
| 441 | 108781 | CG11719 | normal | absent |
| 442 | 108830 | CG1109 | normal | normal |
| 443 | 108852 | CG10923 | normal | decreased signal |
| 444 | 108869 | CG11593 | normal | normal |
| 446 | 108919 | CG11762 | normal | normal |
| 447 | 108938 | CG11534 | normal | normal |
| 449 | 109010 | CG11400 | normal | normal |
| 450 | 109111 | CG11100 | patches | banding |
| 451 | 109295 | CG11033 | normal | normal |
| 453 | 109331 | CG11133 | normal | normal |
| 454 | 109336 | CG11055 | normal | normal |
| 456 | 109445 | CG11739 | normal | normal |
| 457 | 109610 | CG10903 | normal | banding |
| 458 | 109672 | CG11334 | normal | normal |
| 459 | 109733 | CG11606 | decreased signal | normal |
| 460 | 110064 | CG11698 | normal | normal |
| 461 | 110192 | CG11301 | normal | decreased signal; lots of noise |
| 464 | 110656 | CG10979 | normal | normal |
| 465 | 110651 | CG11241 | normal | normal |
| 466 | 110587 | CG11018 | slightly blurry | normal |
| 468 | 110476 | CG11360 | normal | normal |
| 470 | 110392 | CG10955 | normal | banding |
| 471 | 110357 | CG10948 | dispersion of bouton structure | normal |
| 473 | 110342 | CG10895 | normal | normal |
| 475 | 109898 | CG11440 | normal | decreased signal and banding |
| 476 | 109866 | CG1168 | dispersiong of bouton structure | normal |
| 478 | 109781 | CG10936 | normal | decreased signal |
| 479 | 109778 | CG11369 | normal | normal |
| 480 | 108803 | CG11037 | normal | decreased signal |
| 482 | 108618 | CG11375 | normal | normal |
| 488 | 108372 | CG11356 | normal | normal |
| 489 | 108361 | CG1152 | normal | normal |
| 490 | 108194 | CG11233 | decreased signal | decreased signal |
| 491 | 108158 | CG11337 | normal | normal |
| 496 | 107883 | CG11280 | normal | normal |
| 497 | 107858 | CG10864 | normal | normal |
| 500 | 107547 | CG10855 | normal | normal |
| 501 | 107546 | CG10901 | normal | normal |
| 503 | 107529 | CG11068 | normal | normal |

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|---|---|---|---|---|
| 504 | 107518 | CG10916 | decreased signal | decreased signal |
| 506 | 107435 | CG11335 | normal | normal |
| 507 | 107404 | CG11331 | normal | normal |
| 509 | 107368 | CG11416 | normal | normal |
| 510 | 107351 | CG11120 | normal | normal |
| 511 | 107331 | CG11419 | decreased signal | decreased signal |
| 512 | 107327 | CG11711 | dispersion of bouton structure | normal |
| 513 | 107251 | CG10949 | normal | normal |
| 514 | 107205 | CG11377 | normal | normal |
| 518 | 106779 | CG11466 | normal | banding |
| 521 | 106714 | CG11160 | dispersion of bouton structure | decreased signal |
| 523 | 106689 | CG1099 | normal | normal |
| 524 | 106415 | CG11286 | normal | normal |
| 526 | 106277 | CG11170 | dispersion of bouton structure | normal |
| 529 | 106063 | CG11494 | normal | normal |
| 530 | 105927 | CG11261 | normal | normal |
| 531 | 105800 | CG11756 | normal | normal |
| 534 | 105736 | CG11577 | normal | normal |
| 539 | 105103 | CG11115 | dispersion of bouton structure | normal |
| 540 | 104729 | CG11597 | normal | normal |
| 541 | 104667 | CG10988 | normal | normal |
| 543 | 104639 | CG11052 | normal | normal |
| 546 | 104471 | CG1101 | dispersion of bouton structure | decreased signal |
| 549 | 104151 | CG11608 | normal | normal |
| 551 | 104059 | CG11124 | normal | banding |
| 552 | 104037 | CG10991 | normal | normal |
| 554 | 103943 | CG11242 | normal | normal |
| 559 | 103608 | CG11164 | normal | normal |
| 560 | 103589 | CG11513 | normal | banding |
| 563 | 103546 | CG11396 | normal | normal |
| 564 | 103529 | CG1158 | patches | normal |
| 565 | 103495 | CG11073 | decreased signal | decreased signal |
| 566 | 103409 | CG11367 | decreased signal | decreased signal; banding |
| 567 | 103367 | CG11093 | normal | banding (2/6) |
| 579 | 101697 | CG11200 | normal | normal |
| 580 | 101480 | CG11084 | normal | banding |
| 581 | 101458 | CG11024 | normal | normal |
| 586 | 100338 | CG11784 | normal | normal |
| 593 | 100087 | CG11475 | normal | banding |
| 594 | 100055 | CG11284 | normal | decreased signal |

## Appendix B

### *DnaJ-2* Sequences

**Legend:**
XXX-mRNA
XXX-CDS
*XXX*- non-coding region
**XXX**start/stop codon
**XXX**-J-domain (HPD motif is underlined)
**XXX** – SANT DNA binding domain
.....- -Deletion Site
**The EP-Insertion site for the transgenic line used in the mutagenesis scheme is also indicated in the sequence below.

**Full *DnaJ-2* Sequence: Chomosome Region: 3L:21126350,21129453**

5'-CTTTCTTTTTTTAGTGGCCCATTATAACAAAAACTCGAATGTGGAATGTAT
TTAATAGTGTAGGATTTGTTATGTAATGAACGTGGATTAATAATTTGTTAAGA
CGGTTAACTAAATATATAAATCATAAATAACCATTAGTTGAGATACGGTCAC
ACTTTGGCGCCAAACCAATACAACAGTTGCCATTCGATTTTAGCTATAGCAGC
TGGCAGCACTGCCAATAAGTGCCAGCGATACATGCTTGCTGTTATCGATCAA
ATATAGAGTAGGAAGCCAATCGATTTTACCAGCGCCATAATCGATGATGGCA
TCGATGTTTCACCACCACTAGTCGAAACACATGCGGTGCTGCGGAAAA(EP
Insertion Site )GTGTGATTATTAATTGGATTTCGACGTTTTGAAGGCAGCGGAT
CGCCAAATTTTGAGTAACGTGAATCCACAGGAGTGGCGGTGAATGCCCATTA
ACCAGCGGACCTGACCAGAG**ATG**ACGAGCGGTACGGTAGCAACGGCGGTGG
AGGTGCAGCTGCCGCTCAAGGTCGTTCGCCGTAAAATCGAGCGCGTTGGCTT
CGCTTACTTTGCGCAACGGCGCCAATTTCTAGCTCCCGGCGGCGTGGAGCGC
AGCGAAAGCGATGAGAAATTGGAGGGCGTGGGCGAGGAGGTGGACATCAGC
TACCTAAAGTCGCTGGATCCTAAGGAGTGGAAGGACCAG**GATCATTACGCC**
**GTTCTTGGCTTGGGCAAGCTCCG***GTTAGTGCGACGGCAGCACGTGGTGTTCT*
*TCCCATGTGTTCGTTTCTATCAATATTTAATGGGACTTCCCCAACTAATACTGTATTG*
*TCTGACTAAATCACCCTTTAG***ATACGAGGCCAGCGAGGATGATGTTCGACGG**
**GCTTACAGGCGCATGGTTCTGCTGCACCATCCCGATAAGCGGAAAGCCA**
**AGGGCGAGGAAGTCATCCAGGACGATGATTACTTCACATGCATAACCAA**
**AGCTTACGAGATACTGG***GTGGGTGTCAAGAAAATGTCCTTGCTGATCCTTGGGT*
*GTCCTTCGGACTTCGCATTGCCCATGCAAATCGACAGATAAGAATATTTTGATATTA*
*ATTTTCAGAGCATTTGCTTAAGCTATATAAAATTATGATACCATACAATCCTGTTGAG*
*TATAATAATCAATTAAGATTTTAAATTATCTGAAACTAATTTATACTCGATCTAATTCA*
*TTGGTGTGCTGTTGCCTTTGTCTAAGCGCGCAGAACTTGAAGCTTTATTTTTGCATA*
*TCTAAAAATAGGACTAGTTGTTTGCTCTCACATTCTTTTAAACAAAATGCCAAATTAT*

*AACTTACTATTATACAACAGAATTTTTTTGGCTTTAAACACTTTGAATAATTGTAAACA*
*ATTGTACGAAATAGACAGGAAGATTTTAAACTATTTCCTCTTAAACCATCTAATACTC*
*CATCTTGTATTCCAG*GCACATCCAAGCCGCGCCGCAGCTTTGACTCAGTGGATC
CCGAGTTTGACGACTCGCTGCCCTCACAGAACGACATCGATAACGACTACTT
TGGCGTATTTAACAAATTTTTCACACTTAACGGGCGCTGGAGCGAAAAGCCG
CATGTTCCCTCCTTCGGGCAGGTGGACGCCAAACGCGAAGAGGTGGAGCGCT
TCTACAACTTCTGGTACGATTTTAAGTCATGGCGGGAATTCAGCTACTTGGAC
GAAGAGGACAAGGAGAAGGGCCAGGACCGCGACGAACGTCGCTGGATCGAA
AAGGAGAACAGGGCGGCTCGAATCAAGCGCAAGAAGGAGGAAATGTCTCGT
ATCCGGTCGCTCGTCGATTTAGCCTACAACAATGATAAGCGCATTCAGCGCTT
CAAGCAGGAGGAAAAGGATCGCAAGGCTGCTGCCAAGCGAGCCAAGATGGA
CGCCGCCCAGGCCCAGAAGGCAGAAGCTGATCGTGCCATCCGTGAAGCGGCT
TTGGCCAAGGAGAAGGCTGAAAAGGCCGAGCAGAAACGCATCGAGCAGATC
CGCATCGAACGCGAGCAGCAGAAAAAGCTGCTTAAGAAGGAGCGCAAAACG
CTGCGCGACAAGGTCAAGGACTGCAAGTATTATGCCAAGAACGACAAGGATC
AGCTGAAGCACATGGAGGGCACCGAGAAGATTTGCGAGACCTTCAATCTGGC
AGAGCTGCAAGCTCTAAATAAGGCAATGGAAAGCAAGGGCCGTGAGTCGTTT
GTTGCTGCCCTTCAAACGGCCGAGCAGAAGATAGCTGCCGAGTTGGAGGAGA
TTAACCAGACACAGGCCAAGAAGCTTGCCAGCTCAGCGGCCACGCCAAAAG
GCGTTAAGGAGGTCAAGAAGAATGAGCTCTGGAGCAATGAGAACGTGCAGT
TGCTTATCAAGGCGGTTAATCTGTTTCCCGCTGGCACTGCACAGCGTTGGGAT
GTCATAGCCACTTTCATTAACCAGCATAGCCCGGATAACACTGTTCTGGTCAA
TGCCAGGGATGTGCTCAACAAAGCCAAGGCGCTCCAGAACACAGATCACTCG
AAGAGCTCCCTGAAGACTCAAGCCAACGATGCGGCATTTGCCAGTTTCGAGA
AGTCCAAGAAAGATGTTCAGACGTGTAAGGATATAACCCTGGGCGAGGAGA
CGGCCCAGGCGAGCAAGGAGAACTTGAAACAAAACGGTGTAGATCATAAGG
CGAACAATCAGTCAACCAAGCAGAATGGCACGGCTCCAGCTCCAGCAAACCC
CACGGCTGCACCAGCCCCAGTTCCGGCCACAAATGGGTCTACGGGCGGCGGT
GCTGCCTCCAAAACCTGGACCAAG**GAGGAGCAAGCCCTGCTCGAACAGGC**
**CATTAAAACCTATCCAACGACGACACCCGATCGCTGGGATTGCATCGCC**
**GCCTGCATACCGAATCGCAGTAAAAAGGACTGCTTACGTCGCGTCAAAG**
**AGCTGGTCGAGCTG**GTTAACTCCAAGAAGGAGGCACAGGCGGCGGTCAAA
**TGA**GTGCGGCTCCTGCTCCACTTTACCTGCCTCTCGTCCGCCTCATCCACTTCT
AGCTGCTGTGTCCCATATAAATTTTATTTAAATAAAACATAAAAAGTTTTTGA
ACGTTTAGCGAAAAGTT-3’

**Amino Acid Sequence**

MTSGTVATAVEVQLPLKVVRRKIERVGFAYFAQRRQFLAPGGVERSESDEKLEG
VGEEVDISYLKSLDPKEWKDQDHYAVLGLGKLRYEASEDDVRRAYRRMVLLH
**HPD**KRKAKGEEVIQDDDYFTCITKAYEILGTSKPRRSFDSVDPEFDDSLPSQNDID
NDYFGVFNKFFTLNGRWSEKPHVPSFGQVDAKREEVERFYNFWYDFKSWREFS
YLDEEDKEKGQDRDERRWIEKENRAARIKRKKEEMSRIRSLVDLAYNNDKRIQR
FKQEEKDRKAAAKRAKMDAAQAQKAEADRAIREAALAKEKAEKAEQKRIEQIR
IEREQQKKLLKKERKTLRDKVKDCKYYAKNDKDQLKHMEGTEKICETFNLAEL

QALNKAMESKGRESFVAALQTAEQKIAAELEEINQTQAKKLASSAATPKGVKEV
KKNELWSNENVQLLIKAVNLFPAGTAQRWDVIATFINQHSPDNTVLVNARDVLN
KAKALQNTDHSKSSLKTQANDAAFASFEKSKKDVQTCKDITLGEETAQASKENL
KQNGVDHKANNQSTKQNGTAPAPANPTAAPAPVPATNGSTGGGAASKTWTKE
EQALLEQAIKTYPTTTPDRWDCIAACIPNRSKKDCLRRVKELVELVNSKKEAQAA
K

**28M Mutant**

5'-……GATCCCGAGTTTGACGACTCGCTGCCCTCACAGAACGACATC
GATAACGACTACTTTGGCGTATTTAACAAATTTTTCACACTTAACGGGCGCTG
GAGCGAAAAGCCGCATGTTCCCTCCTTCGGGCAGGTGGACGCCAAACGCGAA
GAGGTGGAGCGCTTCTACAACTTCTGGTACGATTTTAAGTCATGGCGGGAATT
CAGCTACTTGGACGAAGAGGACAAGGAGAAGGGCCAGGACCGCGACGAACG
TCGCTGGATCGAAAAGGAGAACAGGGCGGCTCGAATCAAGCGCAAGAAGGA
GGAAATGTCTCGTATCCGGTCGCTCGTCGATTTAGCCTACAACAATGATAAGC
GCATTCAGCGCTTCAAGCAGGAGGAAAAGGATCGCAAGGCTGCTGCCAAGC
GAGCCAAGATGGACGCCGCCCAGGCCCAGAAGGCAGAAGCTGATCGTGCCA
TCCGTGAAGCGGCTTTGGCCAAGGAGAAGGCTGAAAAGGCCGAGCAGAAAC
GCATCGAGCAGATCCGCATCGAACGCGAGCAGCAGAAAAAGCTGCTTAAGA
AGGAGCGCAAAACGCTGCGCGACAAGGTCAAGGACTGCAAGTATTATGCCA
AGAACGACAAGGATCAGCTGAAGCACATGGAGGGCACCGAGAAGATTTGCG
AGACCTTCAATCTGGCAGAGCTGCAAGCTCTAAATAAGGCAATGGAAAGCAA
GGGCCGTGAGTCGTTTGTTGCTGCCCTTCAAACGGCCGAGCAGAAGATAGCT
GCCGAGTTGGAGGAGATTAACCAGACACAGGCCAAGAAGCTTGCCAGCTCA
GCGGCCACGCCAAAAGGCGTTAAGGAGGTCAAGAAGAATGAGCTCTGGAGC
AATGAGAACGTGCAGTTGCTTATCAAGGCGGTTAATCTGTTTCCCGCTGGCAC
TGCACAGCGTTGGGATGTCATAGCCACTTTCATTAACCAGCATAGCCCGGAT
AACACTGTTCTGGTCAATGCCAGGGATGTGCTCAACAAAGCCAAGGCGCTCC
AGAACACAGATCACTCGAAGAGCTCCCTGAAGACTCAAGCCAACGATGCGG
CATTTGCCAGTTTCGAGAAGTCCAAGAAAGATGTTCAGACGTGTAAGGATAT
AACCCTGGGCGAGGAGACGGCCCAGGCGAGCAAGGAGAACTTGAAACAAAA
CGGTGTAGATCATAAGGCGAACAATCAGTCAACCAAGCAGAATGGCACGGCT
CCAGCTCCAGCAAACCCCACGGCTGCACCAGCCCCAGTTCCGGCCACAAATG
GGTCTACGGGCGGCGGTGCTGCCTCCAAAACCTGGACCAAG**GAGGAGCAAG**
**CCCTGCTCGAACAGGCCATTAAAACCTATCCAACGACGACACCCGATCG**
**CTGGGATTGCATCGCCGCCTGCATACCGAATCGCAGTAAAAAGGACTGC**
**TTACGTCGCGTCAAAGAGCTGGTCGAGCTG**GTTAACTCCAAGAAGGAGGC
ACAGGCGGCGGTCAAA**TGA**GTGCGGCTCCTGCTCCACTTTACCTGCCTCTCGT
CCGCCTCATCCACTTCTAGCTGCTGTGTCCCATATAAATTTTATTTAAATAAA
ACATAAAAAGTTTTTGAACGTTTAGCGAAAAGTT-3'

**#226 Mutant**

5’-CTTTCTTTTTTTAGTGGCCCATTATAACAAAAACTCGAATGTGGAATGTATT
TAATAGTGTAGGATTTGTTATGTAATGAACGTGGATTAATAATTTGTTAAGAC
GGTTAACTAAATATATAAATCATAAATAACCATTAGTTGAGATACGGTCACA
CTTTGGCGCCAAACCAATACAACAGTTGCCATTCGATTTTAGCTATAGCAGCT
GGCAGCACTGCCAATAAGTGCCAGCGATACATGCTTGCTGTTATCGATCAAA
TATAGAGTAGGAAGCCAATCGATTTTACCAGCGCCATAATCGATGATGGCAT
CGATGTTTCACCACCACTAGTCGAAACACATGCGGTGCTGCGGAAAA(EP
Insertion Site )GTGTGATTATTAATTGGATTTCGACGTTTTGAAGGCAGCGGAT
CGCCAAATTTTGAGTAACGTGAATCCACAGGAGTGGCGGTGAATGCCCATTA
ACCAGCGGACCTGACCAGAG**ATG**ACGAGCGGTACGGTAGCAACGGCGGTGG
AGGTGCAGCTGCCGCTCAAGGTCGTTCGCCGTAAAATCGAGCGCGTTGGCTT
CGCTTACTTTGCGCAACGGCGCCAATTTCTAGCTCCCGGCGGCGTGGAGCGC
AGCGAAAGCGATGAGAAATTGGAGGGCGTGGGCGAGGAGGTGGACATCAGC
TACCTAAAGTCGCTGGATCCTAAGGAGTGGAAGGACCAG**GATCATTACGCC**
**GTTCTTGGCTTGGGCAAGCTCCG***GTTAGTGCGACGGCAGCACGTGGTGTTCT*
*TCCCATGTGTTCGTTTCTATCAATATTTAATGGGACTTCCCCAACTAATACTGTATTG*
*TCTGACTAAATCACCCTTTAG***ATACGAGGCCAGCGAGGATGATGTTCGACGG**
**GCTTACAGGCGCATGGTTCTGCTGCACCATCCCGATAAGCGGAAAGCCA**
**AGGGCGAGGAAGTCATCCAGGACGATGATTACTTCACATGCATAACCAA**
**AGCTTACGAGATACTGG***GTGGGTGTCAAGAAAATGTCCTTGCTGATCCTTGGGT*
*GTCCTTCGGACTTCGCATTGCCCATGCAAATCGACAGATAAGAATATTTTGATATTA*
*ATTTTCAGAGCATTTGCTTAAGCTATATAAAATTATGATACCATACAATCCT……*
*-GTGTGCTGTTGCCTTTGTCTAAGCGCGCAGAACTTGAAGCTTTATTTTTGCATATCT*
*AAAAATAGGACTAGTTGTTTGCTCTCACATTCTTTTAAACAAAATGCCAAATTATAAC*
*TTACTATTATACAACAGAATTTTTTTGGCTTTAAACACTTTGAATAATTGTAAACAATT*
*GTACGAAATAGACAGGAAGATTTTAAACTATTTCCTCTTAAACCATCTAATACTCCAT*
*CTTGTATTCCAG*GCACATCCAAGCCGCGCCGCAGCTTTGACTCAGTGGATCCC
GAGTTTGACGACTCGCTGCCCTCACAGAACGACATCGATAACGACTACTTTG
GCGTATTTAACAAATTTTTCACACTTAACGGGCGCTGGAGCGAAAAGCCGCA
TGTTCCCTCCTTCGGGCAGGTGGACGCCAAACGCGAAGAGGTGGAGCGCTTC
TACAACTTCTGGTACGATTTTAAGTCATGGCGGGAATTCAGCTACTTGGACGA
AGAGGACAAGGAGAAGGGCCAGGACCGCGACGAACGTCGCTGGATCGAAAA
GGAGAACAGGGCGGCTCGAATCAAGCGCAAGAAGGAGGAAATGTCTCGTAT
CCGGTCGCTCGTCGATTTAGCCTACAACAATGATAAGCGCATTCAGCGCTTCA
AGCAGGAGGAAAAGGATCGCAAGGCTGCTGCCAAGCGAGCCAAGATGGACG
CCGCCCAGGCCCAGAAGGCAGAAGCTGATCGTGCCATCCGTGAAGCGGCTTT
GGCCAAGGAGAAGGCTGAAAAGGCCGAGCAGAAACGCATCGAGCAGATCCG
CATCGAACGCGAGCAGCAGAAAAAGCTGCTTAAGAAGGAGCGCAAAACGCT
GCGCGACAAGGTCAAGGACTGCAAGTATTATGCCAAGAACGACAAGGATCA
GCTGAAGCACATGGAGGGCACCGAGAAGATTTGCGAGACCTTCAATCTGGCA
GAGCTGCAAGCTCTAAATAAGGCAATGGAAAGCAAGGGCCGTGAGTCGTTTG
TTGCTGCCCTTCAAACGGCCGAGCAGAAGATAGCTGCCGAGTTGGAGGAGAT
TAACCAGACACAGGCCAAGAAGCTTGCCAGCTCAGCGGCCACGCCAAAAGG

CGTTAAGGAGGTCAAGAAGAATGAGCTCTGGAGCAATGAGAACGTGCAGTT
GCTTATCAAGGCGGTTAATCTGTTTCCCGCTGGCACTGCACAGCGTTGGGATG
TCATAGCCACTTTCATTAACCAGCATAGCCCGGATAACACTGTTCTGGTCAAT
GCCAGGGATGTGCTCAACAAAGCCAAGGCGCTCCAGAACACAGATCACTCG
AAGAGCTCCCTGAAGACTCAAGCCAACGATGCGGCATTTGCCAGTTTCGAGA
AGTCCAAGAAAGATGTTCAGACGTGTAAGGATATAACCCTGGGCGAGGAGA
CGGCCCAGGCGAGCAAGGAGAACTTGAAACAAAACGGTGTAGATCATAAGG
CGAACAATCAGTCAACCAAGCAGAATGGCACGGCTCCAGCTCCAGCAAACCC
CACGGCTGCACCAGCCCCAGTTCCGGCCACAAATGGGTCTACGGGCGGCGGT
GCTGCCTCCAAAACCTGGACCAAG**GAGGAGCAAGCCCTGCTCGAACAGGC**
**CATTAAAACCTATCCAACGACGACACCCGATCGCTGGGATTGCATCGCC**
**GCCTGCATACCGAATCGCAGTAAAAAGGACTGCTTACGTCGCGTCAAAG**
**AGCTGGTCGAGCTG**GTTAACTCCAAGAAGGAGGCACAGGCGGCGGTCAAA
**TGA**GTGCGGCTCCTGCTCCACTTTACCTGCCTCTCGTCCGCCTCATCCACTTCT
AGCTGCTGTGTCCCATATAAATTTTATTTAAATAAAACATAAAAAGTTTTTGA
ACGTTTAGCGAAAAGTT-3’

**#274 Mutant**

5’-CTTTCTTTTTTTAGTGGCCCATTATAACAAAAACTCGAATGTGGAATGTATT
TAATAGTGTAGGATTTGTTATGTAATGAACGTGGATTAATAATTTGTTAAGAC
GGTTAACTAAATATATAAATCATAAATAACCATTAGTTGAGATACGGTCACA
CTTTGGCGCCAAACCAATACAACAGTTGCCATTCGATTTTAGCTATAGCAGCT
GGCAGCACTGCCAATAAGTGCCAGCGATACATGCTTGCTGTTATCGATCAAA
TATAGAGTAGGAAGCCAATCGATTTTACCAGCGCCATAATCGATGATGGCAT
CGATGTTTCACCACCACTAGTCGAAACACATGCGGTGCTGCGGAAAA**(EP**
**InsertionSite)**GTGTGATT……CGCCGCAGCTTTGACTCAGTGGATCCCGAG
TTTGACGACTCGCTGCCCTCACAGAACGACATCGATAACGACTACTTTGGCGT
ATTTAACAAATTTTTCACACTTAACGGGCGCTGGAGCGAAAAGCCGCATGTT
CCCTCCTTCGGGCAGGTGGACGCCAAACGCGAAGAGGTGGAGCGCTTCTACA
ACTTCTGGTACGATTTTAAGTCATGGCGGGAATTCAGCTACTTGGACGAAGA
GGACAAGGAGAAGGGCCAGGACCGCGACGAACGTCGCTGGATCGAAAAGGA
GAACAGGGCGGCTCGAATCAAGCGCAAGAAGGAGGAAATGTCTCGTATCCG
GTCGCTCGTCGATTTAGCCTACAACAATGATAAGCGCATTCAGCGCTTCAAGC
AGGAGGAAAAGGATCGCAAGGCTGCTGCCAAGCGAGCCAAGATGGACGCCG
CCCAGGCCCAGAAGGCAGAAGCTGATCGTGCCATCCGTGAAGCGGCTTTGGC
CAAGGAGAAGGCTGAAAAGGCCGAGCAGAAACGCATCGAGCAGATCCGCAT
CGAACGCGAGCAGCAGAAAAAGCTGCTTAAGAAGGAGCGCAAAACGCTGCG
CGACAAGGTCAAGGACTGCAAGTATTATGCCAAGAACGACAAGGATCAGCT
GAAGCACATGGAGGGCACCGAGAAGATTTGCGAGACCTTCAATCTGGCAGA
GCTGCAAGCTCTAAATAAGGCAATGGAAAGCAAGGGCCGTGAGTCGTTTGTT
GCTGCCCTTCAAACGGCCGAGCAGAAGATAGCTGCCGAGTTGGAGGAGATTA
ACCAGACACAGGCCAAGAAGCTTGCCAGCTCAGCGGCCACGCCAAAAGGCG
TTAAGGAGGTCAAGAAGAATGAGCTCTGGAGCAATGAGAACGTGCAGTTGCT
TATCAAGGCGGTTAATCTGTTTCCCGCTGGCACTGCACAGCGTTGGGATGTCA

TAGCCACTTTCATTAACCAGCATAGCCCGGATAACACTGTTCTGGTCAATGCC
AGGGATGTGCTCAACAAAGCCAAGGCGCTCCAGAACACAGATCACTCGAAG
AGCTCCCTGAAGACTCAAGCCAACGATGCGGCATTTGCCAGTTTCGAGAAGT
CCAAGAAAGATGTTCAGACGTGTAAGGATATAACCCTGGGCGAGGAGACGG
CCCAGGCGAGCAAGGAGAACTTGAAACAAAACGGTGTAGATCATAAGGCGA
ACAATCAGTCAACCAAGCAGAATGGCACGGCTCCAGCTCCAGCAAACCCCAC
GGCTGCACCAGCCCCAGTTCCGGCCACAAATGGGTCTACGGGCGGCGGTGCT
GCCTCCAAAACCTGGACCAAG**GAGGAGCAAGCCCTGCTCGAACAGGCCAT**
**TAAAACCTATCCAACGACGACACCCGATCGCTGGGATTGCATCGCCGCC**
**TGCATACCGAATCGCAGTAAAAAGGACTGCTTACGTCGCGTCAAAGAGC**
**TGGTCGAGCTG**GTTAACTCCAAGAAGGAGGCACAGGCGGCGGTCAAA**TGA**
GTGCGGCTCCTGCTCCACTTTACCTGCCTCTCGTCCGCCTCATCCACTTCTAGC
TGCTGTGTCCCATATAAATTTTATTTAAATAAAACATAAAAAGTTTTTGAACG
TTTAGCGAAAAGTT-3’

**#275 Mutant**

5’-CTTTCTTTTTTTAGTGGCCCATTATAACAAAAACTCGAATGTGGAATGTAT
TTAATAGTGTAGGATTTGTTATGTAATGAACGTGGATTAATAATTTGTTAAGA
CGGTTAACTAAATATATAAATCATAAATAACCATTAGTTGAGATACGGTCAC
ACTTTGGCGCCAAACCAATACAACAGTTGCCATTCGATTTTAGCTATAGCAGC
TGGCAGCACTGCCAATAAGTGCCAGCGATACATGCTTGCTGTTATCGATCAA
ATATAGAGTAGGAAGCCAATCGATTTTACCAGCGCCATAATCGATGATGGCA
TCGATGTTTCACCACCACTAGTCGAAACACATGCGGTGCTGCGGAAAA**(EP**
**InsertionSite)**GTGTGATT……ATCCGTGAAGCGGCTTTGGCCAAGGAGAA
GGCTGAAAAGGCCGAGCAGAAACGCATCGAGCAGATCCGCATCGAACGCGA
GCAGCAGAAAAAGCTGCTTAAGAAGGAGCGCAAAACGCTGCGCGACAAGGT
CAAGGACTGCAAGTATTATGCCAAGAACGACAAGGATCAGCTGAAGCACAT
GGAGGGCACCGAGAAGATTTGCGAGACCTTCAATCTGGCAGAGCTGCAAGCT
CTAAATAAGGCAATGGAAAGCAAGGGCCGTGAGTCGTTTGTTGCTGCCCTTC
AAACGGCCGAGCAGAAGATAGCTGCCGAGTTGGAGGAGATTAACCAGACAC
AGGCCAAGAAGCTTGCCAGCTCAGCGGCCACGCCAAAAGGCGTTAAGGAGG
TCAAGAAGAATGAGCTCTGGAGCAATGAGAACGTGCAGTTGCTTATCAAGGC
GGTTAATCTGTTTCCCGCTGGCACTGCACAGCGTTGGGATGTCATAGCCACTT
TCATTAACCAGCATAGCCCGGATAACACTGTTCTGGTCAATGCCAGGGATGT
GCTCAACAAAGCCAAGGCGCTCCAGAACACAGATCACTCGAAGAGCTCCCTG
AAGACTCAAGCCAACGATGCGGCATTTGCCAGTTTCGAGAAGTCCAAGAAAG
ATGTTCAGACGTGTAAGGATATAACCCTGGGCGAGGAGACGGCCCAGGCGA
GCAAGGAGAACTTGAAACAAAACGGTGTAGATCATAAGGCGAACAATCAGT
CAACCAAGCAGAATGGCACGGCTCCAGCTCCAGCAAACCCCACGGCTGCACC
AGCCCCAGTTCCGGCCACAAATGGGTCTACGGGCGGCGGTGCTGCCTCCAAA
ACCTGGACCAAG**GAGGAGCAAGCCCTGCTCGAACAGGCCATTAAAACCT**
**ATCCAACGACGACACCCGATCGCTGGGATTGCATCGCCGCCTGCATACC**
**GAATCGCAGTAAAAAGGACTGCTTACGTCGCGTCAAAGAGCTGGTCGAG**
**CTG**GTTAACTCCAAGAAGGAGGCACAGGCGGCGGTCAAA**TGA**GTGCGGCTC

CTGCTCCACTTTACCTGCCTCTCGTCCGCCTCATCCACTTCTAGCTGCTGTGTC
CCATATAAATTTTATTTAAATAAAACATAAAAAGTTTTTGAACGTTTAGCGAA
AAGTT-3’

## REFERENCES


1. Lee, H.-G., Zhao, N., Campion, B. K., Nguyen, M. M. & Selleck, S. B. Akt regulates glutamate receptor trafficking and postsynaptic membrane elaboration at the Drosophila neuromuscular junction. *Dev. Neurobiol.* **73,** 723–743 (2013).
2. Rubin, G. M. & Lewis, E. B. A Brief History of Drosophila's Contributions to Genome Research. *Science* **287,** 2216–2218 (2000).
3. Muller, H. J. Genetic Variability, Twin Hybrids and Constant Hybrids, in a Case of Balanced Lethal Factors. *Genetics* **3,** 422–499 (1918).
4. Rubin, G. M. & Spradling, A. C. Genetic transformation of Drosophila with transposable element vectors. *Science* **218,** 348–353 (1982).
5. Giniger, E., Varnum, S. M. & Ptashne, M. Specific DNA binding of GAL4, a positive regulatory protein of yeast. *Cell* **40,** 767–774 (1985).
6. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415 (1993).
7. Duffy, J. B. GAL4 system in drosophila: A fly geneticist's swiss army knife. *genesis* **34,** 1–15 (2002).
8. Elliott, D. A. & Brand, A. H. in *Drosophila* (Dahmann, D. C.) 79–95 (Humana Press, 2008). at <http://link.springer.com/protocol/10.1007/978-1-59745-583-1_5>
9. Hannon, G. J. RNA interference. *Nature* **418,** 244–251 (2002).
10. Kim, D. H. & Rossi, J. J. RNAi mechanisms and applications. *BioTechniques* **44,** 613–616 (2008).
11. Dietzl, G. *et al.* A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* **448,** 151–156 (2007).
12. Keshishian, H., Broadie, K., Chiba, A. & Bate, M. The Drosophila Neuromuscular Junction: A Model System for Studying Synaptic Development and Function. *Annu. Rev. Neurosci.* **19,** 545–575 (1996).
13. O'Kane, C. J. in *Mol. Funct. Models Neuropsychiatry* (Hagan, J. J.) **7,** 37–60 (Springer Berlin Heidelberg, 2011).
14. Littleton, J. T. & Ganetzky, B. Ion Channels and Synaptic Organization: Analysis of the Drosophila Genome. *Neuron* **26,** 35–43 (2000).
15. Qin, G. *et al.* Four Different Subunits Are Essential for Expressing the Synaptic Glutamate Receptor at Neuromuscular Junctions of Drosophila. *J. Neurosci.* **25,** 3209–3218 (2005).
16. Manning, B. D. & Cantley, L. C. AKT/PKB Signaling: Navigating Downstream. *Cell* **129,** 1261–1274 (2007).
17. Cheetham, M. E. & Caplan, A. J. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones* **3,** 28–36 (1998).

18. Qiu, X.-B., Shao, Y.-M., Miao, S. & Wang, L. The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell. Mol. Life Sci. CMLS* **63,** 2560–2570 (2006).
19. Qian, Y. Q., Patel, D., Hartl, F.-U. & McColl, D. J. Nuclear Magnetic Resonance Solution Structure of the Human Hsp40 (HDJ-1) J-domain. *J. Mol. Biol.* **260,** 224–235 (1996).
20. Tsai, J. & Douglas, M. G. A Conserved HPD Sequence of the J-domain Is Necessary for YDJ1 Stimulation of Hsp70 ATPase Activity at a Site Distinct from Substrate Binding. *J. Biol. Chem.* **271,** 9347–9354 (1996).
21. Bukau, B. & Horwich, A. L. The Hsp70 and Hsp60 Chaperone Machines. *Cell* **92,** 351–366 (1998).
22. Young, J. C., Agashe, V. R., Siegers, K. & Hartl, F. U. Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell Biol.* **5,** 781–791 (2004).
23. Caplan, A. J., Cyr, D. M. & Douglas, M. G. YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. *Cell* **71,** 1143–1155 (1992).
24. Ungewickell, E. J. & Hinrichsen, L. Endocytosis: clathrin-mediated membrane budding. *Curr. Opin. Cell Biol.* **19,** 417–425 (2007).
25. Zinsmaier, K. E., Eberle, K. K., Buchner, E., Walter, N. & Benzer, S. Paralysis and early death in cysteine string protein mutants of Drosophila. *Science* **263,** 977–980 (1994).
26. Muchowski, P. J. & Wacker, J. L. Modulation of neurodegeneration by molecular chaperones. *Nat. Rev. Neurosci.* **6,** 11–22 (2005).
27. Kazemi-Esfarjani, P. & Benzer, S. Genetic Suppression of Polyglutamine Toxicity in Drosophila. *Science* **287,** 1837–1840 (2000).
28. Hummel, T. & Klämbt, C. in *Drosophila* (Dahmann, D. C.) 97–117 (Humana Press, 2008). at <http://link.springer.com/protocol/10.1007/978-1-59745-583-1_6>
29. Zhang, P. & Spradling, A. C. Efficient and dispersed local P element transposition from Drosophila females. *Genetics* **133,** 361–373 (1993).
30. Meldrum, B. S. Glutamate as a Neurotransmitter in the Brain: Review of Physiology and Pathology. *J. Nutr.* **130,** 1007–1007 (2000).
31. Ungewickell, E. *et al.* Role of auxilin in uncoating clathrin-coated vesicles. *Nature* **378,** 632–635 (1995).
32. Zhao, X., Braun, A. P. & Braun, J. E. A. Biological Roles of Neural J Proteins. *Cell. Mol. Life Sci.* **65,** 2385–2396 (2008).
33. Hundley, H. A., Walter, W., Bairstow, S. & Craig, E. A. Human Mpp11 J Protein: Ribosome-Tethered Molecular Chaperones Are Ubiquitous. *Science* **308,** 1032–1034 (2005).

# ACADEMIC VITA

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## EDUCATION

**The Pennsylvania State University, University Park, PA**
B.S. in Biochemistry and Molecular Biology with minor in Chemistry (2014)

## RESEARCH EXPERIENCE

**Selleck Lab, BMB Department, Penn State-University Park, PA**
*Undergraduate Researcher (Fall 2011 – Present)*
Identify and analyze potential regulators of post-synaptic membrane development at the *Drosophila* larval neuromuscular junction (NMJ) as a model system for development of the nervous system

**Centyrex, Centocor R&D (Johnson&Johnson)-Radnor, PA**
*Laboratory Intern (Summer 2011)*
Optimized the transition from laboratory-scale production to large-scale production of scaffold proteins for clinical trial.

## TEACHING EXPERIENCE

**Dr. Bratoljub Milosavljevic, Chemistry Department, Penn State University-University Park, PA**
*Teaching Assistant (Fall 2013)*
Teaching Assistant for Chem 450: Physical Chemistry I: Thermodynamics.

**Summer Experience at Eberly College of Science (SEECoS)**
*Research Mentor (Summer 2012)*
Designed and led a novel research project for a group of four high school students from underprivileged areas of Pennsylvania for 5 weeks

## HONORS AND AWARDS

Honorable Mention-Goldwater Scholarship
Summer Discovery Grant
Braddock Scholarship
Speaker at Discovery-U: Where Genius Meets Imagination
Schreyer Honors Scholar
Penn State University's President's Freshmen Award
Morrow Family Endowed Prize for 2011-2012