Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 - 12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100047631

6. **Project Number and Title of Research Project:** 10 - Characterization and Application of a Novel Drosophila Model for CHARGE Syndrome

7. **Start and End Date of Research Project:** 1/1/2009 - 12/31/2009

8. **Name of Principal Investigator for the Research Project:** Daniel R. Marenda, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $105,159.00

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Melicharek	Laboratory Manager	100%	$36,000.00
Marenda	Principal Investigator	10%	$ 8,666.67

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Ramirez	Undergraduate Research Assistant	Part-time summer
Singh	Undergraduate Research Assistant	Part-time summer
Thompson	Undergraduate Research Assistant	Part-time summer

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No__X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Characterization of a novel Drosophila disease model for CHARGE Syndrome	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify: _____)	Submitted 7/14/2009 Award began 6/1/2010	$422,898	$422,898

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

The NIH has awarded an R21 based on this project for 2 years. Once these funds expire, I plan to submit an R01 grant based on this research that will extend for 5 years.

12. Future of Research Project. What are the future plans for this research project?

To utilize the model we have created to identify target genes involved in the neurological defects observed in our model. Ultimately, to identify drugs that mitigate or rescue these defects in this model.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____X____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	1			
Female	2			
Unknown				
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	1			
Non-Hispanic	1			
Unknown	1			
Total	**3**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black	1			
Asian	1			
Other				
Unknown				
Total	**3**			

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This research grant has allowed us to successfully compete and obtain an NIH funded research grant that will partly pay for the salary of a post-doctoral scientist that has been recruited to my lab at Drexel.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Project Goals: Our specific aim in this grant was to validate the effectiveness of utilizing our *Drosophila* CHARGE model towards further understanding the clinical symptoms and disease pathogenesis of CHARGE patients. We proposed to utilize the powerful behavior analyses capable of being performed in *Drosophila* to quantify any aberrant behavior in our CHARGE flies as compared to our control flies. In this respect, we have successfully determined that our CHARGE flies are capable of learning in a learning and memory task, but have defective immediate recall memory (as discussed further below).

We also proposed to determine how mutation in *kis*, *atonal*, and *daughterless* genes affect the neuronal morphology and arborization patterns of a specific subset of neurons in the brain through immunohistochemistry and confocal microscopy. We have successfully shown that mutation in each of these genes significantly affects the morphology of multiple different neuronal populations in specific ways (described in more detail below). Finally, we proposed to determine if the transcriptional regulation of potential target genes identified in our preliminary data is conserved in human cells. We have shown that this transcriptional regulation is indeed conserved in humans cells when the homolog of *kismet* is knocked down in these cells. Thus, while we have not completed each of the specific aims proposed in the grant application, we have successfully completed a significant number of them.

Detailed Report of Findings:

Adult flies with decreased kismet expression display numerous phenotypes

Null mutations in *kis* are embryonic lethal, presumably due to the fact that *kis* gene function is required to maintain states of homeotic gene transcription in early embryogenesis [1]. We hypothesized that if we were to partially knock down *kis* gene function in specific tissues, we could allow flies to overcome the requirement for *kis* in the embryo, while sufficiently hindering *kis* gene function in later stages of development, and generate viable adults with prominent phenotypes. To accomplish this, we utilized the Gal4/UAS system [2]. We expressed two different publicly available *kis RNAi* strains from the Vienna *Drosophila* RNAi Center (*kis RNAi.a*, and *kis RNAi.b*). To examine the extent to which we are able to knock down Kis protein expression with these constructs *in vivo*, we first expressed them in the posterior compartment of developing wing imaginal discs by using the *engrailed-Gal4* driver (Figure 1). *engrailed* is expressed only in the posterior compartment of tissues [3, 4], leaving the anterior compartment as a critical internal control for this assay, and allowing us to estimate the amount of knock down these constructs have *in vivo*. The Kis protein is normally expressed ubiquitously in the developing wing imaginal disc, with roughly equivalent expression levels in the posterior and anterior compartment (Figures 1, A-B). We found that the two different *kis RNAi* constructs knock down Kis protein expression differentially. *kis RNAi.a* shows a 60% knockdown (Figures 1, C-D), while *kis RNAi.b* shows a very strong 90% knockdown (Figures 1, E-F). These results are consistent with recently published data that also show significant knockdown of Kis protein using these constructs [5].

In order to generate viable adult flies with decreased Kis expression, we first expressed both of these *kis RNAi* constructs ubiquitously with the *daughterless-Gal4* driver (*da-Gal4*). Expression of *kis RNAi.b* with *da-Gal4* at 25ºC leads to late pupal lethality, suggesting that Kis is required for some critical function during pupal development. However, expression of *kis RNAi.a* with *da-Gal4* at 25ºC produces viable adults with obvious morphological defects (Figure 2). These flies are unable to fly, and exhibit a prominent postural defect where they

hold their wings apart and below their bodies with 100% penetrance (Figures 2, C and E). This postural defect is reminiscent of defects associated in muscle cells, and is also observed in mutants for other chromatin remodeling factors in flies [6, 7]. Further, this phenotype is consistent with hypotonia related posture problems observed in CHARGE patients [8].

To initially assess *kis* function in the developing nervous system, we expressed both of the RNAi constructs using *ELAV-Gal4*, a pan-neural driver. Expression of *kis RNAi.b* at 25ºC also leads to late pupal lethality with this driver, suggesting that the cause of the lethality observed at this stage with the *da-Gal4* driver may be due to loss of Kis protein within the nervous system. Expression of *kis RNAi.a* with *ELAV-Gal4* also produces viable flies with various morphological defects (Figure 2). These flies exhibit duplicated bristles on the adult thorax (Figure 2G, 24% penetrance [n=50] compared to sibling controls [n=50]). This is a defect in the development of peripheral nervous system structures, and has been previously observed in other regulators of chromatin structure in *Drosophila* [9, 10]. These flies also display extra vein differentiation in adult wings (Figure 2I). These defects were associated mostly near wing veins L2 (45% [n=50] compared to 0% in sibling controls [n=70]), and L5 (24% [n=50] compared to 0% in sibling controls [n=70]). This is also consistent with previously reported loss-of-function *kis* effects in wing vein differentiation [11].

kismet function is required in muscles for climbing behavior

Patients with CHARGE display delayed or impaired motor coordination and adaptive motor skills, musculoskeletal anomalies, and hypotonia [8, 12]. To specifically test motor control of our CS flies, we utilized a simple, yet powerful behavioral assay based on climbing ability [13]. Flies with ubiquitous expression of *UAS:kis RNAi.a* by *Da-Gal4* exhibit a significant decrease in climbing behavior within 10 days after eclosion (Figure 3A) compared to controls. To more precisely define the tissue(s) in which loss of *kis* function affects this behavior, we expressed the *UAS:kis RNAi* constructs with a number of different tissue specific Gal4 drivers (Figure 3, Table 1). While expression of *kis RNAi.b* leads to late pupal lethality with the pan-neural *ELAV-Gal4* (see above), expression of *kis RNAi.a* with this driver has no significant effect on climbing behavior (Table 1). Further, strong and specific knockdown of Kis protein within motor neurons, dopaminergic neurons, glutamatergic neurons, cholinergic neurons, mushroom body neurons, and glial cells all have no significant effect on climbing behavior (Table 1). However, knockdown of Kis within muscles shows a strong effect (Figure 3B, Table 1), reducing climbing frequency by nearly half compared to control flies. These data suggest that *kis* function is critically required in *Drosophila* muscles for coordinated motor function. This is particularly of interest when we consider that knockdown of *kis* function in motor neurons, as well as pan-neural knockdown of *kis* function has no effect on climbing behavior. Thus, *kis* may either be regulating the expression of critical post-synaptic target gene(s) in muscle cells required to facilitate synaptic transmission and muscle coordination, and/or may be required for the morphology and/or development of the muscle cells themselves. Because of the similarity of these phenotypes to the hypotonia and impaired motor coordination observed in CHARGE patients, these results may suggest that there is a similar requirement for *Chd7* function for muscles in vertebrates.

In addition to identifying a requirement for *kis* function in muscles, we also observe a strong decrease in climbing ability by simultaneously reducing *kis* function in the ventral nerve cord, Kenyon neurons (neurons involved in learning and memory), and pars

intercerebralis (the major neuroendocrine gland in flies) (Figure 3C, Table 1). Because reduction of *kis* in the Kenyon neurons alone has no effect on this behavior (Table 1), and because ubiquitous knockdown of *kis* produces flies with wings held out abnormally at their sides, we analyzed Kis knockdown in the giant fiber circuit with the *OK307-Gla4* reagent. The giant fiber system in *Drosophila* is a pair of symmetrical inter-neurons that mediate the flight escape response by relaying sensory visual information to the thoracic flight muscles and leg extensor muscles of the thorax [14-16]. When we knock down Kis protein in these inter-neurons, we observe that roughly half of these flies also exhibit abnormal postural defects where they hold their wings out to the side of their bodies (53%, n=62). Further, these flies also exhibit a small but significant decrease in climbing behavior within the first 10 days after eclosion (Figure 3D, Table 1). Taken together, these data show that Kis function is also required outside of muscles in these inter-neuronal populations within the brain and ventral nerve cord to control climbing behavior and wing posture.

Kismet protein is broadly expressed in the developing fly brain

To begin to explore Kis protein function in the fly brain, we first analyzed Kis protein expression in developing central nervous system. Because we had identified *kis* as a regulator of *atonal* and *daughterless* proneural gene expression in the retina [17], we compared Kis protein expression in the brain to the expression pattern of both these proneural proteins. We find that Kis protein is strongly expressed in many areas of the larval brain, including the cortex, the brain lobes, and the ventral nerve cord (Figure 4), and strongly co-localized with both Atonal and Daughterless protein expression in this tissue. Our analysis of Ato protein expression is consistent with what has been previously reported in this tissue [18]. Ato is expressed in the inner proliferation center of the optic lobe (lower arrow in Figure 4A), as well as in a group of 20-30 cells in the dorso-lateral region of the central brain (upper arrow in Figure 4A). This group of 20-30 cells is known as the dorsal cluster nuerons (DCN), which send out bundles of axons ventrally and then contra-laterally towards the opposite optic lobe [18]. We find that the Da protein is strongly co-localized to both the Ato expressing neurons in the optic lobe as well as in the central brain (Figures 4 A-F). Da protein is also significantly expressed in neurons outside of the Ato-specific neurons, within regions of higher expression in both the cortex and central brain (Figure 4B). Similarly, Kis protein expression is also co-localized to sites of Ato expression (Figure 4A). Like Da protein expression, Kis is also significantly expressed outside of the Ato-specific expression domains, but unlike Da, Kis expression is more uniform in the brain (Figure 4C). Kis protein is also expressed within the neurons of the Kenyon cells (Figures 4, G-I), which are required for learning and memory in the fly.

kismet function is required for immediate recall memory

Based on the expression pattern of Kis in the developing brain, and the fact that patients with CHARGE Syndrome often suffer from intellectual disability, we tested the learning and memory ability of adult flies with reduced *kismet* function. To test for deficits in learning and memory in these flies, we performed the conditioned courtship suppression assay [19]. This assay is an associative conditioning procedure that is ethologically based and capable of measuring both learning and memory in individual flies [20]. The conditioning aspect of this assay is based on the observation that male courtship behavior is modified by exposure to a previously mated female that is unreceptive to courting [19, 21]. Thus, after 1 hour of

unsuccessful courting of a mated female, wild type males suppress their courtship behavior, even towards subsequent receptive virgin females, for 1-3 hours by 40% or more [19, 22-24].

In order to knock down Kis protein in this assay, we expressed each of the *UAS:kis RNAi* constructs continuously during development with the *OK107-Gal4* driver. This driver is expressed in discrete populations of neurons during development in the fly brain, including the Kenyon cells [25], neurons of the Ventral Ganglion [26], and early in the optic lobes, central brain regions, and protocerebrum [27]. To determine effects on learning, male flies were placed in a courtship chamber with a previously mated (unreceptive) wild type female for 60 minutes. The amount of time the male spent performing courtship behavior was assessed during the first 10 minutes of this training, and compared to the last 10 minutes of the training period. Wild type (Canton S) control flies show a significant drop in courtship behavior in the last 10 minutes of training as compared to the first 10 minutes (Figure 5A), indicative of an appropriate learning response. Similarly, out-crossed control flies (Figures 5, A-B), and Kis knockdown flies (Figure 5C) also show a significant decrease in courtship behavior in the last 10 minutes of the training period compared to the first 10. Importantly, this indicates that our Kis knockdown flies are capable of successful perception and interpretation of the sensory stimuli required in this assay, and that these flies are able to alter their behavior appropriately (learn) in response to this training.

There have been five phases of memory defined in *Drosophila*, immediate recall (0-2 minutes post-training), short term memory (out to 1 hour post-training), medium term memory (out to six hours), anesthesia-resistant memory (out to two days), and long term memory (out to 9 days) [28, 29]. In order to test the earliest phase of memory first, we assayed Kis knockdown flies for their immediate recall memory by transferring trained male flies to clean mating chambers with a receptive virgin female within 2 minutes of training, and assaying their courtship behavior for 10 minutes. Trained wild type males show a clear decrease in courtship activity as compared to parallel sham trained wild type flies (Figure 5D), indicating a change in behavior consistent with normal immediate recall memory of training. Similarly, out-crossed *Gal4* and *UAS* control flies also show a significant decrease in their courtship activity compared to genotype equivalent out-crossed sham trained flies (Figures 5, D-E). However, both Kis knockdown flies show no significant decrease in their courtship activity within 2 minutes of training (Figure 5F). Because these flies are capable of successful perception, and experience-dependant alteration of their behavior in the learning component of this assay, their inability to suppress their courtship frequency in the second component of this assay indicates that these flies are defective in their immediate recall memory of this learning.

kismet mutants display abnormal axonal pruning and migration during development of Kenyon cells

Because the *kis* gene encodes for a transcription factor, these results suggest that *kis* may be regulating the gene expression of some critical factor(s) important for immediate recall memory formation during development. This could either be through the control of neurogenesis of appropriate neurons in this memory circuit, control of circuit connectivity once these neurons have already differentiated, and/or control of the development and/or morphology of these neuronal target tissues. To begin to address these questions, we first analyzed the effect of loss-of-function in *kis* on neuronal development and morphology of the

Kenyon neurons, as these neurons are associated with learning and memory in multiple experimental paradigms in *Drosophila* [30-34]. We utilized the MARCM technique [35] to create clones of neurons that are homozygous mutant for kis^{LM27}, a protein null mutant [17]. The population of Kenyon cells consist of three distinct groups of neurons, each with its own characteristic developmental morphology [25]. During larval development, the γ subset of Kenyon neurons are differentiated first, and these neurons undergo highly stereotyped dendritic and axonal pruning during pupal development [25, 36]. Pruning of these neurons occurs by localized degeneration [37], and is evident by 18 hours after puparium formation in wild type neurons (Figure 6A, arrow shows remnants of γ axonal projects into the α lobes). While γ neurons mutant for *kis* undergo proper dendritic pruning, they display defects in their axonal pruning (arrow in Figure 6B), and continue to display un-pruned γ axons projecting into α lobes 40% of the time (Table 2).

The final adult structure of the mushroom body axonal projections consist of 5 distinct projections to the α, α', β, β', and γ lobes (Figure 6C). To determine if structures other than γ lobes are affected by mutations in *kis*, we created MARCM clones mutant for kis^{LM27} using the *OK107-Gal4* driver, which is expressed in the α, β, and γ neurons. While Kenyon cell dendrite morphology was normal in these *kis* mutant neurons, we did observe axonal migration defects in these cells where axons cross the midline of the brain to migrate into the opposite brain lobe (arrow in Figure 6D, Table 2).

kismet mutants display abnormal axon extension and migration in DC neurons and photoreceptor axons

The Kis protein is widely expressed in the fly brain (Figure 4). Given that mutations in *kis* affect axonal pruning and axonal migration in developing Kenyon neurons, we analyzed the effect of *kis* loss-of-function in other neuron populations in the developing and adult brain to determine if the Kis protein may play a broader role in regulating neuronal morphology. Based on the co-localization of Kis and Atonal proteins in the developing larval brain, and that *kis* function is known to regulate *atonal* transcription in the developing retina [17], we created MARCM clones mutant for *kis* function in the Dorsal Cluster (DC) neurons, a subset of Atonal-expressing neurons in the larval and adult brain [18]. These neurons form a highly stereotypical pattern of connections in the fly brain that innervate the optic lobes [18, 38]. The regular array of these neuronal connections has been extremely useful to determine how genes function to regulate neurite extension, outgrowth, neuronal morphology, and arborization patterns [38, 39]. During larval development, DC neuron soma form a distinct cluster of roughly 20-30 cells in the dorso-lateral region of the central brain [18]. These neurons then extend a bundle of neurites posteriorly, and then form a bundle of commissural axons that migrate contralaterally to innervate targets of the opposite brain lobe (Figure 7A). Developing larval DC neurons mutant for kis^{LM27} exhibit several morphological abnormalities indicative of abnormal axon development (Table 2). These neurons display abnormal commissural axon projections (arrow in Figure 7B, Table 2), consistent with the defects in axonal migration observed in *kis* mutant Kenyon neurons. *kis* mutant cells also display abnormal positioning within the brain, appearing laterally in more than half of the clones observed (left arrow in Figure 7B, Table 2). Interestingly, these neurons appear to send their initial neurite bundles to the proper location for initial branching however (Figure 7B). Additionally, we occasionally observe groups of DC neuron soma that are not part of the larger soma cluster (arrowhead in Figure 7B, Table 2).

The defects observed in *kis* mutant DC neurons suggest that *kis* functions in DC neuron migration as well as axonal morphology. It is interesting to note that abnormal positioning of larval DC soma is also observed in larval brains mutant for *ato* gene function [18]. As *kis* regulates *ato* transcription in the larval retina [17], this neuron migration defect may be due to *kis* mediated regulation of *ato* transcription in these cells as well. Interestingly, Bajpai et al. recently showed that *Chd7* (the *kis* ortholog) is required for neural crest cell formation and migration in humans and *Xenopus* [40]. These results are consistent with what we observe in DC neurons, and may suggest a broad role for *kis* and orthologous genes in cell migration during development.

In adults, the *atonal* expressing neurons form highly complex dendritic and axonal fields (Figures 7C and E) [18, 39]. Adult DC neurons project neurite bundles that branch ipsilaterally to form the dendritic field (Figure 7C), and also project a large bundle of commissural axons contralaterally to innervate the lobula and medulla in a stereotypical fan-like pattern (Figure 7E) [18, 38, 39]. Adult DC neurons homozygous mutant for *kis* do not display abnormal dendrite formation (Figure 7D). However, *kis* mutant DC neurons show a severe reduction in the number of axons extending into the lobulla, and subsequently extending from the lobulla into the medulla (arrow Figure 7F, Table 2). Wild type MARCM clones exhibit 14.25±.95 neurites extending from the lobulla into the medulla, while *kis* mutant MARCM clones exhibit 6.0±3.47 neurites extending from the lobulla to the medulla, a significantly lower number (P=0.0055). These results are similar to those observed in Kenyon cells, in that the dendrites develop normally, while the axons do not.

To further validate the axonal defects we observe in *kis* mutant neurons, we analyzed the axonal projections of photoreceptor cells into the optic lobe of developing larval brains, a standard technique for analyzing photoreceptor axonal guidance [41, 42]. We dissected third instar retina-brain complexes from developing larvae, and stained these tissues for the MAP1B-like protein Futsch [43]. Wild type photoreceptor cells extend their axons from retinal cells away from retinal tissue towards the optic stalk where the axons then fasciculate into a bundle. These axons then migrate to innervate the lamina and medulla of the larval brain in a highly stereotypical fashion (Figure 8A). We then created clones of kis^{LM27} homozygous mutant cells in the developing retina, and analyzed migration of photoreceptor axons into the brain. In these retinas, photoreceptor axons exhibit a severe defect in axonal migration into the optic lobe (Figure 8B), with photoreceptor axons extending beyond their normal targets and radiating out into the surrounding brain areas. These axons also show abnormal defasciculation of axonal bundles in the optic stalk (arrow in Figure 8B). Interestingly, the photoreceptor cells migrate normally into their proper positions in the ommatidial clusters in *kis* mutant retinal tissue (Figure 8C, non-green tissue) and initially send their axons to properly bundle in the optic stalk. This is consistent with what we observe in adult Kenyon cell and DC axons, that also initially send their axon bundles to their proper locations, but then extend their axons abnormally.

Concluding remarks

In summary, the data presented here describe a requirement for *kis* gene function in adult *Drosophila* climbing behavior and memory. Our results suggest that reduced climbing ability observed in *kis* mutants is a result of decreased *kis* gene function in the post-synaptic muscle cells, as opposed to a requirement for *kis* in pre-synaptic motor neurons, and may help to elucidate a possible mechanism for the hypotonia and motor coordination problems often

observed in CHARGE patients. Further, our behavioral analyses suggest that decreased *kis* function does not alter the fly's ability to learn, although it does have an effect on immediate recall memory. In analyzing the morphology of a number of neuronal populations mutant for *kis*, we consistently observe defects in axon morphology and positioning. These defects may be due to abnormal axonal pruning observed in some *kis* mutant neurons, or may be due to defective axonal migration, or defects in axonal extension and/or retraction. In each of the neuronal populations studied, neuronal differentiation into the appropriate cell types was not affected in *kis* mutant cells, nor was dendritic development. When taken together, these data suggest that *kis* may function to regulate the expression of target gene(s) normally required for axon morphology and connectivity, as opposed to dendritic morphology, at least in the neuronal populations studied here. We suggest that the analyses presented here can complement and expand upon the studies done in cell culture and vertebrate model organisms towards a better understanding of the role of *kis* in neural developmental, and *Chd7* in CHARGE Syndrome pathogenesis.

Materials and Methods

Drosophila Stocks and Genetics: All flies were maintained at 25°C in a 12:12 light:dark cycle at 60% humidity. All crosses were carried out at 25°C. Normal food consisted of a standard cornmeal, yeast, molasses recipe. BL# refers to Bloomington Stock Center stock number (http://flystocks.bio.indiana.edu/bloomhome.htm). VDRC# refers to the Vienna Drosophila Resource Center stock number (http://stockcenter.vdrc.at/control/main). Wild type flies used were Canton S. Stocks used are described: kis^{LM27} [17], *UAS:kis RNAi.a* (VDRC #10762), and *UAS:kis RNAi.b* (VDRC #46685).

All Gal4 stocks were obtained from the *Drosophila* Bloomington Stock Center. The tissues affected and Bloomington Stock number are listed in Table I.

Clones in developing retinas to analyze axonal guidance were generated using *ey:FLP* as previously described [41]. MARCM mosaic analysis was performed as previously described [35, 44]. Genotypes for MARCM analysis were:

1) y^-,w^-*,hs:Flp, UAS:CD8-GFP; Gal80,Frt40A/Cyo; +/+; OK107-Gal4*
2) y^-,w^-*,hs:Flp, UAS:CD8-GFP; Gal80,Frt40A, 201Y-Gal4*
3) y^-,w^-*,hs:Flp, UAS:CD8-GFP; Gal80,Frt40A/Cyo; Ato-Gal4(14a)/TM6B*
4) w^-*;* kis^{LM27}*,Frt40A/Cyo*
5) w^-*; Frt40A*

Immunohistochemistry and Antibodies: Kis antibody was a kind gift from J. Tamkun, and is described in [45]. Daughterless antibody was a kind gift from C. Cronmiller, and is described in [46]. Futsch antibody was obtained from the Iowa Developmental Hybridoma Bank (22C10). Secondary antibodies for immunohistochemistry used were goat anti-mouse TRITC (# 115-116-072, 1:150), goat anti-rabbit TRITC (# 111-116-144, 1:250), goat anti-rabbit Cy5 (#111-176-144, 1:1000), goat anti-mouse Cy5 (# 115-176-072, 1:500). All secondary antibodies were from Jackson ImmunoResearch.

Adult and larval brains were dissected, fixed and prepared essentially as described [47]. Adult and larval brains were dissected directly in fix. Brains were mounted in vectashield (Vector Labs, H-1000). All fluorescent imaging was done using an Olympus FluoView FV1000 laser scanning confocal microscope.

Behavioral testing and training: For climbing assays, a modified version of Le Bourg and Lints was used [13]. Flies were collected between 0-8 hours after eclosion and assayed every two days. Groups of 10 or fewer flies were transferred to a clean, empty vial and given 18 seconds to climb 5 cm. The number of flies that successfully reach the 5 cm line were recorded.

For courtship behavioral training, virgin male flies of the appropriate genotype were collected between 0 and 6 hours after eclosion and transferred to individual food vials. All flies were maintained at 25°C in a 12:12 light:dark cycle at 60% humidity. All behavioral tests were performed in a separate room maintained at 25°C and 60% humidity and illuminated under a constant 130 V white light Kodak Adjustable Safelight Lamp mounted above the courtship chambers. All behavior was digitally recorded using a Sony DCR-SR47 Handycam with Carl Zeiss optics. Subsequent digital video analysis of time spent performing courtship behavior was quantified using iMovies software (Apple). The total time that a male performed courtship activity was measured and scored. The Courtship Index (CI) was calculated as the total time observed performing courting behavior divided by the total time assayed, as described [19].

Virgin female wild type (Canton S) flies were collected and kept in normal food vials in groups of 10. Male flies were aged for 5 days before behavioral training and testing. All tests were performed during the relative light phase. Mated Cantons S females used for training were 5 days old, and observed to have mated with a Canton S male the evening prior to training. Virgin female Canton S targets used were 4 days old. Male flies were assigned to random groups the day of training, and assays were set up and scored blind. Male flies were transferred without anesthesia to one half of a partitioned mating chamber from Aktogen (http://www.aktogen.com) that contained a previously mated Canton S female in the other partitioned half. Males were allowed to acclimate for 1 minute, then the partition between the male and female was removed. Male flies were then trained for 60 minutes. After 60 minutes, male flies were transferred within 2 minutes without anesthesia to one half of a clean partitioned mating chamber that contained a virgin Canton S female in the other partitioned half. The partition was removed and the flies were recorded for 10 minutes.

Statistical Analysis: All statistical analyses were performed on SPSS version 17. To determine significance between multiple different genotypes, a one-way ANOVA analysis was performed with Tukey posthoc analysis. Genotype is the independent variable. To determine significance between different measures of the same genotype, a two-tailed paired Student's t-test was performed. An unpaired Student's t-test was performed between 2 groups of different genotypes. Significance was determined at the 95% confidence interval.

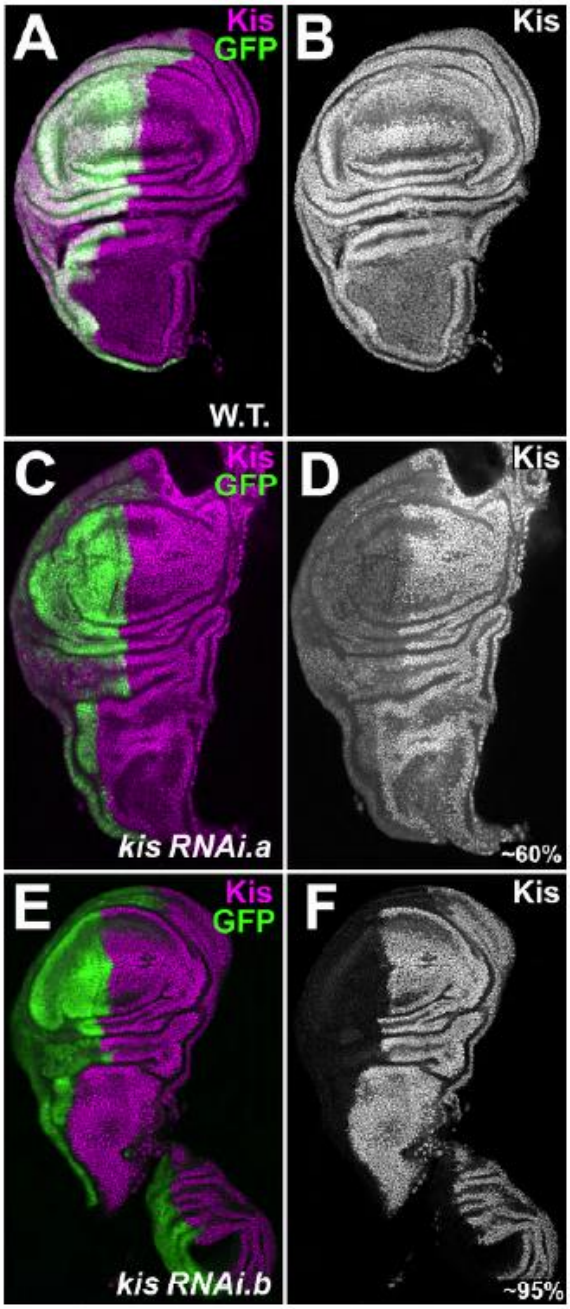

Figure 1. *UAS:kis RNAi* contstructs reduce Kis protein expression *in vivo*. (A-F) Third instar larval wing discs stained for Kis protein expression. Anterior right. (A-B) Wild type Kis protein expression (magenta) is uniform throughout wing. GFP (green) is expressed by *engrailed-Gal4*, and marks the posterior compartment of these discs. (*engrailed-Gal4 / +*). (B) Kis protein (white) from panel (A). (C-D) Expression of Kis protein (magenta) in wing discs expressing *UAS:kis RNAi.a* in the posterior compartment. (*engrailed-Gal4 / UAS:kis RNAi.a*). (D) Kis protein (white) from panel (C). Note decreased Kis protein expression in posterior compartment compared to anterior. (E-F) Expression of Kis protein (magenta) in wing discs expressing *UAS:kis RNAi.b* in the posterior compartment. (*engrailed-Gal4 / UAS:kis RNAi.b*). (F) Kis protein (white) from panel (E). Note decreased Kis protein expression in posterior compartment.

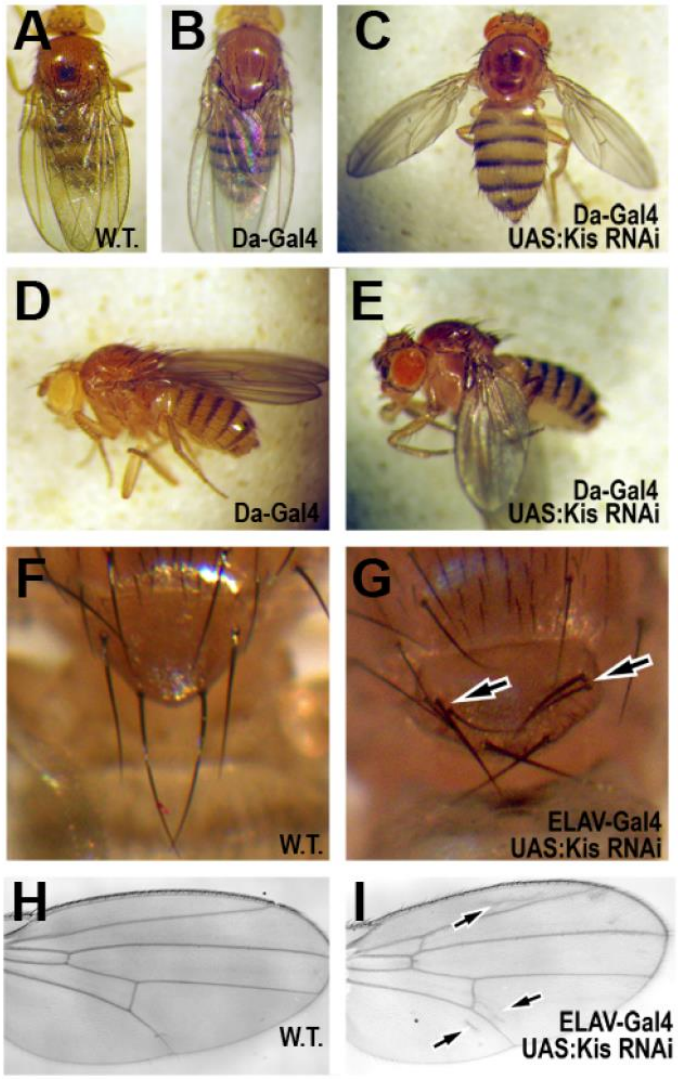

Figure 2. Adult phenotypes in Kismet knockdown flies. (A-E) Adult female flies. (A-C) Dorsal view. (D-E) Lateral view. (A) Wild type (Canton S) fly. Note the position of the adult wings. (B, D) A fly expressing only the *Daughterless-Gal4* reagent (*Da-Gal4/* +) holds its wings normally. (C, E) A fly where Kis protein is ubiquitously knocked down (*Da-Gal4*/*UAS:kis RNAi.a*) holds its wings abnormally apart from its body. (F-G) Dorsal view of bristles on scutellum in (F) wild type flies and (G) flies where Kis protein is knocked down in nervous tissue (*Elav-Gal4*/*UAS:kis RNAi.a*). Arrow notes duplication of bristles in Kis knockdown flies. (H-I) Adult wings from (H) wild type and (I) Kis knockdown flies (*Elav-Gal4*/*UAS:kis RNAi.a*). Arrows denote presence of extra vein tissue near wing vein L2 (top arrow), the posterior crossvein (middle arrow), and wing vein L5 (bottom arrow). In cases not shown, *Da-Gal4/* + flies show wild type phenotypes.

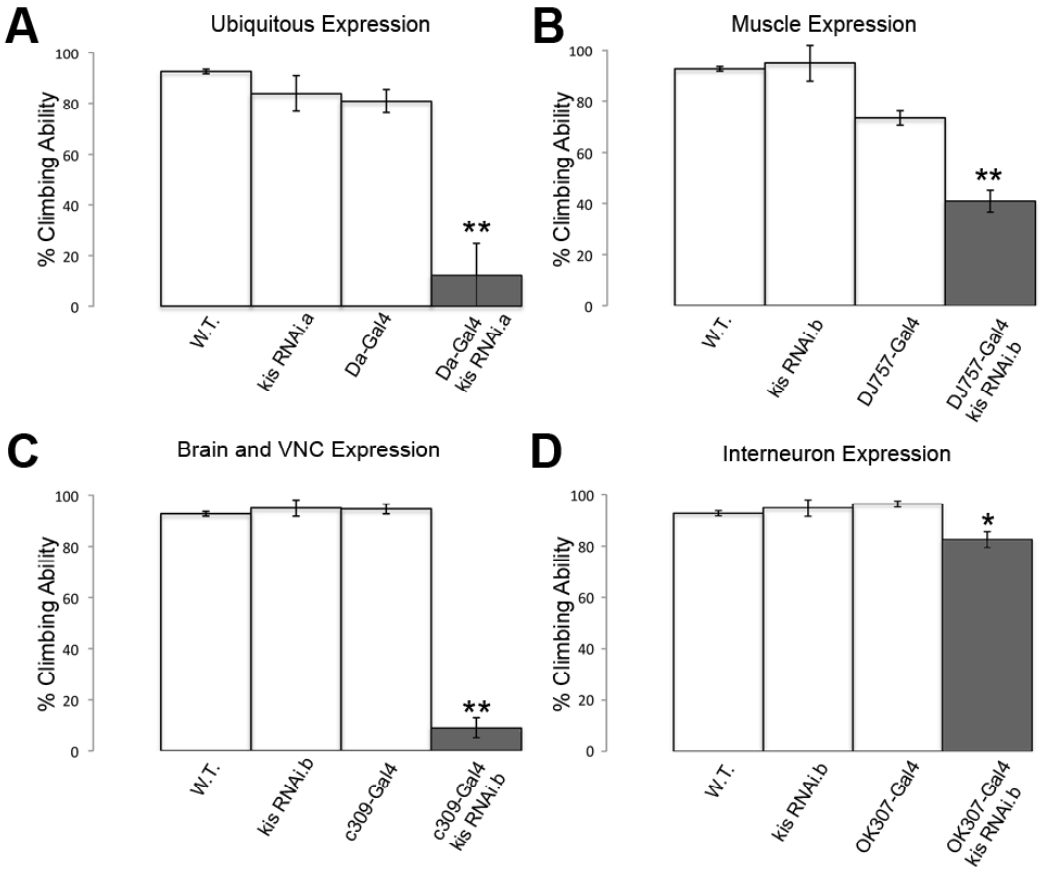

Figure 3. Kismet knockdown in muscles causes defective climbing behavior. (A-D) shows compiled climbing ability of wild type, control, and Kis knockdown flies at days 2-10. (A) Ubiquitous expression of *UAS:Kis RNAi.a* (*Da-Gal4*/*UAS:Kis RNAi.a*) shows severely reduced climbing ability compared to wild type, *Da-Gal4*/+, and *UAS:Kis RNAi.a*/+ outcrossed control flies. (B) Kis knockdown restricted to muscles (*DJ757-Gal4*/*UAS:Kis RNAi.b*) also shows a strong reduction in climbing ability compared to wild type, *DJ757-Gal4*/+, and *UAS:Kis RNAi.b*/+ outcrossed controls. (C) Kis knockdown in mushroom body neurons and the ventral nerve cord (*c309-Gal4*/*UAS:Kis RNAi.b*) shows a strong reduction in climbing ability compared to wild type, *c309-Gal4*/+, and *UAS:Kis RNAi.b*/+ outcrossed controls. (D) Kis knockdown in the giant fiber inter-neurons (*OK307-Gal4*/*UAS:Kis RNAi.b*) shows a small but significant effect compared to wild type, *OK307-Gal4*/+, and *UAS:Kis RNAi.b*/+ outcrossed controls. Error bars represent ± SEM. In all cases ** indicates P<.001 and * indicates P<.05 compared to controls.

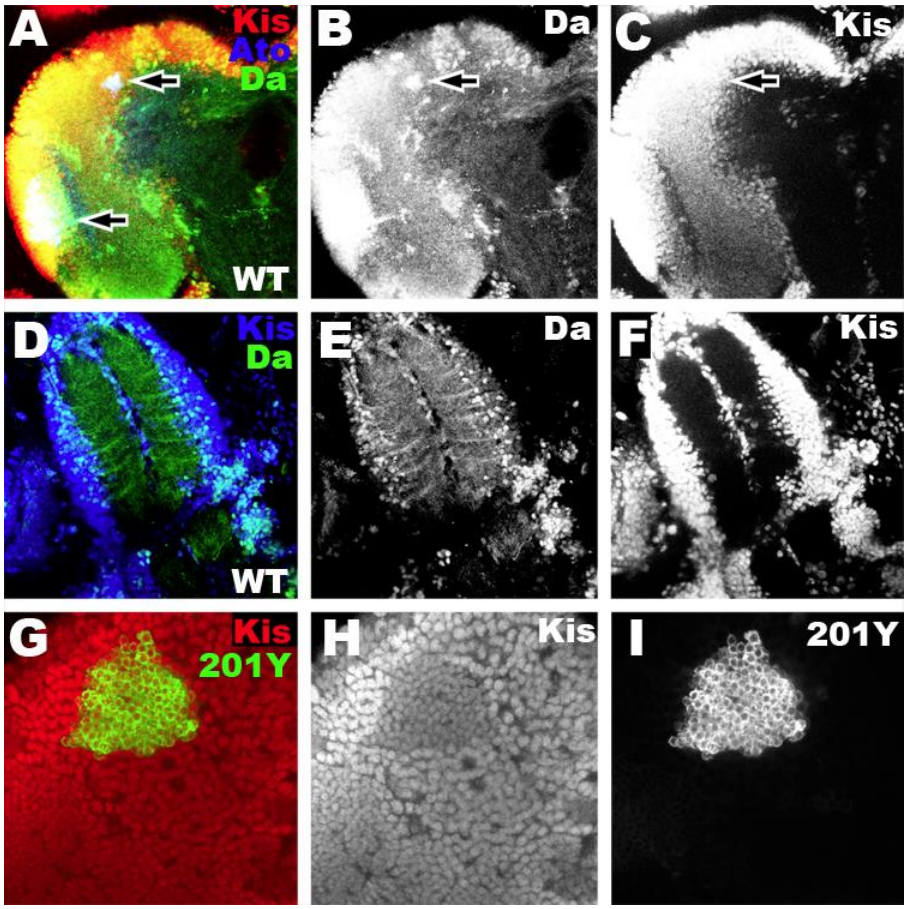

Figure 4. Kismet is widely expressed in the developing larval central nervous system. (A-C, G-I) Developing brain lobe of wild type late third instar central nervous system. (D-F) Developing ventral nerve cord of wild type late third instar larval central nervous system. (A) Kis expression (red), Atonal expression (blue), and Daughterless expression (green) in wild type brain lobe. Upper arrow denotes the Atonal expressing Dorsal Cluster (DC) neurons. Lower arrow denotes the developing optic lobe. (B) Daughterless expression (white) from panel (A). Arrow denotes Daughterless expression in DC neurons. (C) Kis expression (white) from panel (A). Arrow denotes Kis expression in DC neurons. (D) Kis expression (blue) and Daughterless expression (green) in developing ventral nerve cord. (E) Daughterless expression (white) from panel (D). (F) Kis expression (white) from panel (D). (G) GFP expression (green) in the membrane as driven from the *201Y-Gal4* driver (*201Y-Gal4*/*UAS:CD8-GFP*) to denote the location of the γ Kenyon neurons. Kis expression (red). Note strong co-localization. (H) Kis expression (white) from panel (G). (I) GFP expression (white) from panel (G).

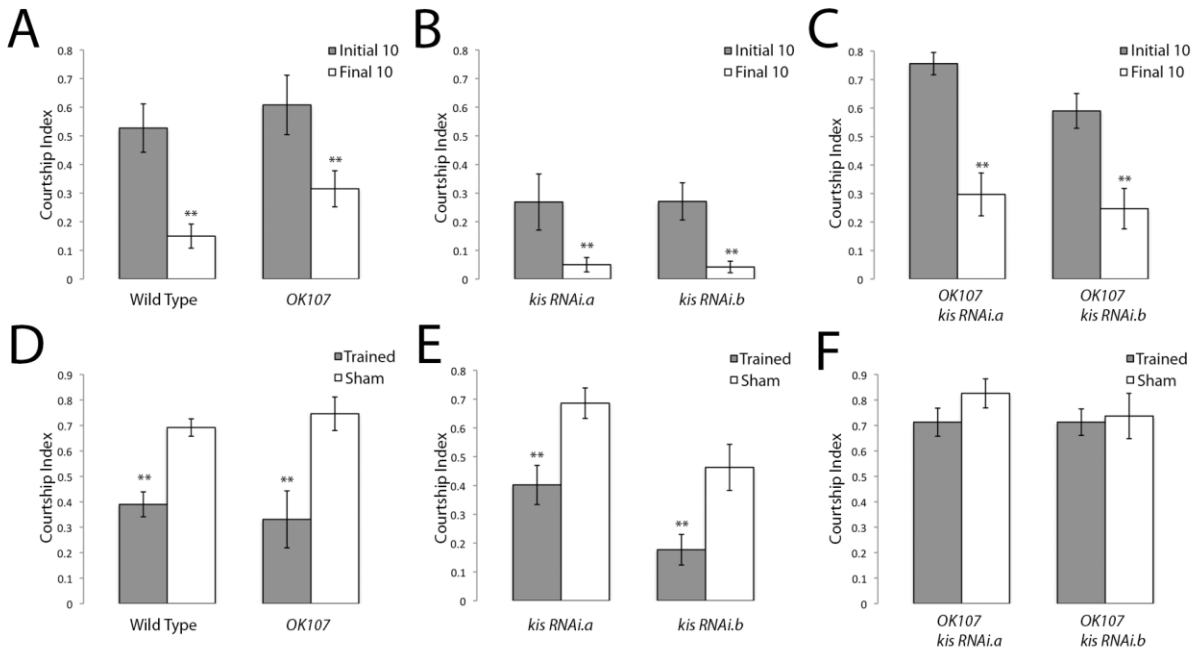

Figure 5. Kismet knockdown flies can learn, but are deficient in their immediate recall memory. (A-C) Panels denote learning during the first 10 (grey columns) and last 10 minutes (white columns) of the training phase during the courtship suppression assay. Genotypes are indicated. Note normal response of Kis knockdown mutants. (D-F) Panels denote immediate recall memory (0-2 minutes post-training) of trained flies (grey columns) as compared to sham trained matching genotypes (white columns). Kis knockdown showed no significant difference between trained and sham trained flies, indicative of no immediate recall memory of training. Error bars represent ± SEM. In all cases ** indicates P<.001 and * indicates P<.05 compared to controls.

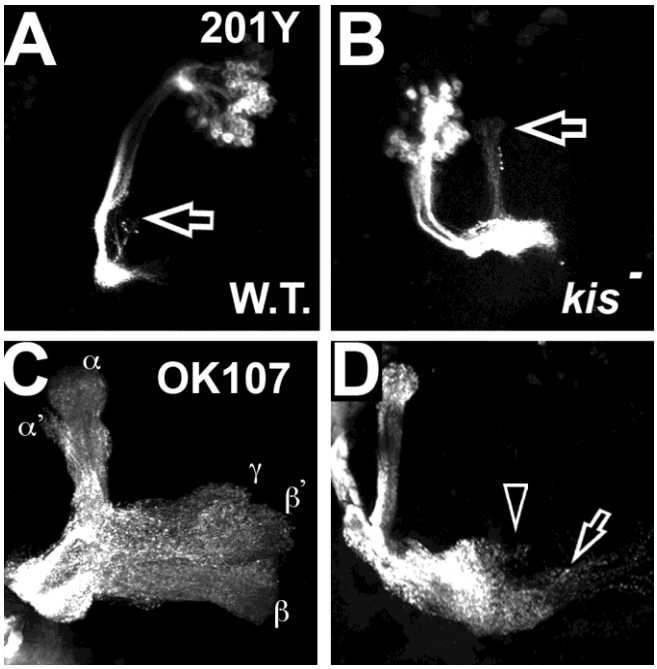

Figure 6. Kismet is required for proper axon pruning and axon migration in developing mushroom bodies. All panels show GFP in Kenyon neurons by MARCM analysis. (A-B) Pupal brains 18-20 hours after puparium formation (apf). (C-D) Adult brains 48 hours after eclosion. (A) Wild type MARCM clones (FRT 40A) in pupal brains driven by *201Y-Gal4* show remnants of proper axonal pruning of γ neurons that were previously populating the α lobes (arrow). (B) kis^{LM27} homozygous mutant MARCM clones display unpruned γ axons that continue to populate the α lobes (arrow). (C) Wild type MARCM clones (FRT 40A) in adult brains driven by *OK107-Gal4* show normal pattern of α, α', β, β' and γ axons innervating the mushroom body lobes, as labeled. (D) kis^{LM27} homozygous mutant MARCM clones in these adult brains often display abnormal axon migration beyond the midline (demarcated by the arrowhead).

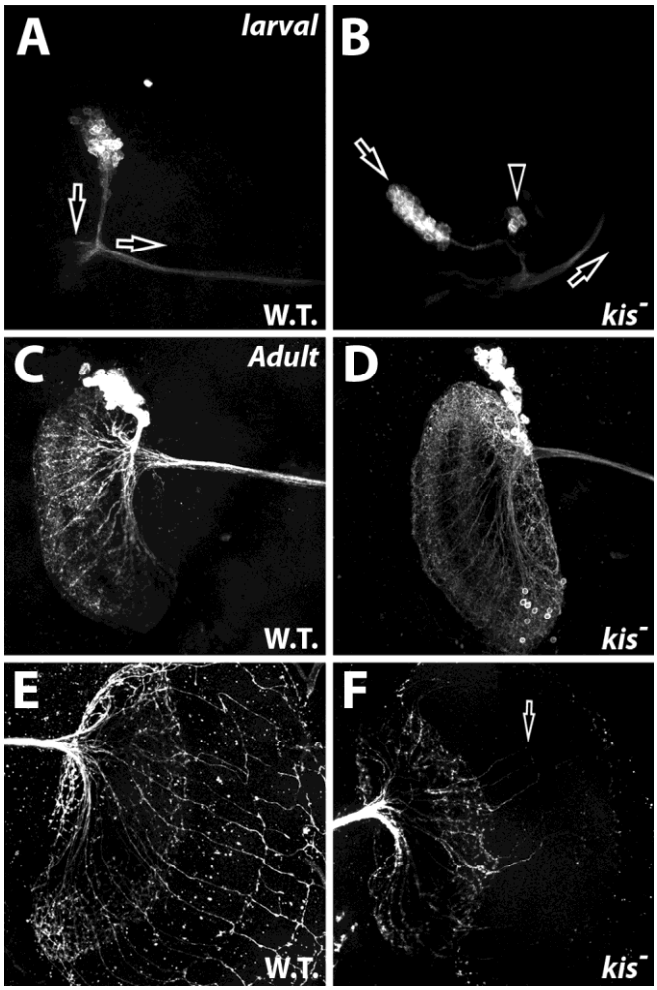

Figure 7. Kismet is required for proper cell and axon migration in developing Dorsal Cluster neurons. All panels show GFP in DC neurons by MARCM analysis. (A-B) Late third instar larval brains. (C-F) Adult brains 48 hours after eclosion. (A) Wild type MARCM clones (FRT 40A) in laval DC neurons. Note position of the soma cluster axon bundles (parallel to vertical arrow) compared to commissural axon bundles (below horizontal arrow). (B) *kis*^{LM27} homozygous mutant MARCM clones display abonormal positioning of soma cluster compared to commissural axon bundles (left arrow), soma that have developed outside of the normal cluster (arrowhead), and disrupted commissural axon migration (right arrow). (C) Wild type MARCM clones (FRT 40A) in adult DC neurons displaying normal dendritic morphology on ipsilateral brain hemisphere. (D) *kis*^{LM27} homozygous mutant MARCM clones in adult brains. Overall morphology of dendritic field appears normal. (E) Wild type MARCM clones (FRT 40A) in adult DC neurons displaying normal axonal morphology on contralateral brain hemisphere. (F) *kis*^{LM27} homozygous mutant MARCM clones in adult brains. Arrow displays abonormal and reduced number of axonal extensions from lobulla into the medulla.

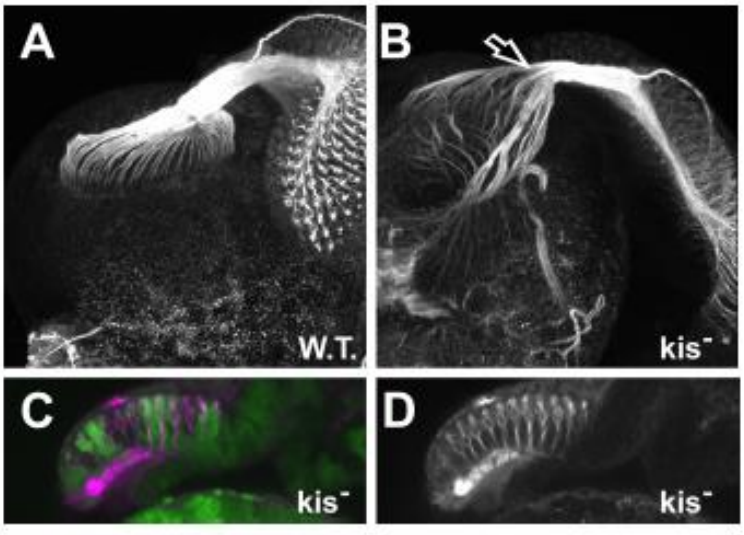

Figure 8. Kismet is required for proper photoreceptor axon migration into the developing brain. (A-D) Late third instar larval retinas with attached brains. (A) Wild type retina with attached larval brain stained for the Futsch protein (white) display normal axonal bundles in the optic stalk that innervate the developing optic lobes. (B) *kis*^{LM27} homozygous mutant clones in the developing retina stained for Futsch (white) display abnormal axonal migration into the developing brain. Arrow indicates defasciculation of axon bundles in the optic stalk. (C) Cross section of *kis*^{LM27} homozygous mutant clones in the developing retina posterior to the morphogenetic furrow. Heterozygous (control) tissue is denoted by presence of GFP (green). Homozygous clones are denoted by lack of GFP. Retinas are stained with Futsch (magenta). Note normal positioning of ommatidia clusters near the apical (top) portion of the retina. (D) Futsch stain (white) from panel (C).

TABLE 1. Gal4 lines tested for Climbing Behavior

Name	BL#	Organ/Tissue	Climbing affected
ELAV-Gal4	458	Pan-neuronal	No
Ple-Gal4	8845	Dopaminergic neurons	No
Cha-Gal4	6798/6793	Cholinergic neurons	No
OK371-Gal4	26160	Glutamatergic neurons	No
OK107-Gal4	854	entire MB	No
1471-Gal4	9465	Gamma lobes of MB	No
D42-Gal4	8816	Motor neurons	No
Pdf-Gal4	6900	Ventrolateral neurons	No
Repo-Gal4	7415	Glial Cells	No
C309-Gal4	6906	MB, Thoracic Ganglion, Eye	Yes
OK307-Gal4	6488	Giant Fiber Interneuron	Yes
A51-Gal4	8764	Muscles and Motor neurons	Yes
DJ757-Gal4	8184	Muscles	Yes

The effect of different Gal4 lines tested for climbing behavior with *UAS:kis RNAi* constructs. BL indicates Bloomington Stock number. MB indicates Mushroom Body. Climbing is affected compared to Wild type flies, as well as Gal4 and UAS controls outcrossed to w^-.

TABLE 2. Neuronal defects observed in *kismet* MARCM mutants

Neuron	Gal4-line	n	Defects observed	%	P
γ neurons, M.B.	201Y				
- control (pupal)		5	unpruned axons	0%	-
- *kis* mutant (pupal)		10	unpruned axons	40%	<.05
α,β,γ neurons, M.B.	OK107				
- control (adult)		9	lobe structure	0%	-
			cell migration	0%	-
			axon migration	11%	-
- *kis* mutant (adult)		11	lobe structure	55%	<.05
			cell migration	36%	<.05
			axon migration	55%	<.05
D.C. Neurons			Atonal		
- control (larval)		9	cell migration	0%	-
			axon migration	0%	-
			cells out of cluster	0%	-
- control (adult)		4	axon extension	0%	-
		14.25	axons in medulla		
- *kis* mutant (larval)		19	cell migration	53%	<.001
			axon migration	32%	<.05
			cells out of cluster	32%	<.05
- *kis* mutant (adult)		3	axon extension	100%	<.05
		6	axons in medulla		

Quantification of defects observed in different *kismet* mutant neurons. M.B. refers to the Mushroom Body. D.C. refers to the Dorsal Cluster. Unpruned axons refers to the number of clones with unpruned γ axons extending into the α lobe at 18 hours APF. Lobe structure indicates missing or altered lobe morphology in α, β, or γ lobes. Cell migration indicates number of soma that failed to migrate to their appropriate locations by the developmental time indicated. Axon migration indicates either axons crossing the midline for adult Kenyon cells, or commissural axons failing to cross the midline or failing to migrate to their proper contralateral targets in larval DC neurons. Axon extension indicates presence of axons failing to extend from lobulla into the medulla in adult brains. P values are indicated from a 2 tailed student's t-test comparing *kis* mutant to control.

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18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____X_No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal

abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Kismet/CHD7 regulates axon morphology, memory, and locomotion in a Drosophila model of Charge Syndrome.	David J. Melicharek, Laura C. Ramirez, Sukhdeep Singh, Rhea Thompson, and Daniel R. Marenda	Human Molecular Genetics	May 2010	☐ Submitted ☐ Accepted ■ Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____X____ No_____

If yes, please describe your plans:

Once the targets are identified that regulate the phenotypes we see in our *kismet* mutants, we will publish those results separately.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:

Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME Daniel R. Marenda	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME dmarenda	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Loyola University Chicago	BS	1996	Biology
Syracuse University	PhD	2003	Biology/Genetics
Emory University School of Medicine	Post-doc	2003-2006	Developmental Biology

Positions and Honors

Positions:

2008-present	Assistant Professor, Dept. of Biology, Drexel University
2008-present	Assistant Professor, Dept. of Neurobiology and Anatomy, Drexel University School of Medicine
2006-2008	Assistant Professor, Dept of Biological Sciences, University of the Sciences in Philadelphia
2005-2006	NRSA Research Fellow, Dept. of Cell Biology, Emory University School of Medicine
2003-2005	Post-doctoral Fellow, Dept. of Cell Biology, Emory University School of Medicine
1998-2003	Graduate Student, Dept. of Biology, Syracuse University
1996-1998	Research Assistant, Dept. of Ophthalmology, University of Iowa School of Medicine

Honors:

GSA 52nd Annual Drosophila Research Conference Platform presentation	2011
Cover Article in *Human Molecular Genetics*	2010
Drexel University Human Cognition Enhancement Award (HCEP)	2010
Pennsylvania Department of Health CURE Award	2009
Article featured in "This Issue" section of *Development*	2006
NIH Ruth L. Kirschstein National Research Service Award (NRSA)	2005-2006
GSA 44th Annual Drosophila Research Conference Platform presentation	2003
Syracuse University Research and Creative Project Grant	2003
Certificate of College Science Teaching, Syracuse University	2003
Syracuse University Fellowship	2003
Syracuse University Travel Grant	2003
Marilyn Albelow Summer Scholarship	1999-2003
Syracuse University Teaching Associate	1999-2002
Syracuse University Outstanding Teaching Assistant Award Nomination	2001

C. Selected peer-reviewed publications (undergraduate authors are underlined)

Most relevant to the current application (in reverse chronological order)

1. *Melicharek D, Ramirez LC, Singh S, Thompson R, and **Marenda DR.** (2010) Kismet/CHD7 regulates axon morphology, memory, and locomotion in a *Drosophila* model of CHARGE Syndrome. *Hum. Mol. Genet.* **19**(21): 4253-4262 *Cover Article
2. Majumdar N, Paez G, D'Rozario M, Inamadar S, and **Marenda DR.** (2010) MAP Kinase phosphorylation is dispensable for cell division, but required for cell growth in Drosophila. *Fly.* **4:** 204-212
3. Melicharek D, Shah A, DiStefano G, Gangemi AJ, Orapallo A, Vrailas AD, and **Marenda DR.** (2008) Identification of novel regulators of *atonal* expression in the developing *Drosophila* retina. *Genetics*. **180:** 2095-2110
4. Vrailas AD, Majumdar N, Middleton G, Cooke EM, and **Marenda DR**. (2007) Delta and Egfr expression are regulated by Importin-7/Moleskin in *Drosophila* wing development. *Dev. Biol.* **308**: 534-546
5. Vrailas AD, **Marenda DR**, Cook S, Powers MA, Lorenzen JA, Perkins LA, and Moses K. (2006) *smoothened* and *thickveins* regulate Moleskin/Importin-7 mediated MAP Kinase signaling in the developing *Drosophila* eye. *Development* **133**: 1485-1494

Additional recent publications of importance to the field (in reverse chronological order)

1. Curtis BJ, Zraly CB, **Marenda DR,** Dingwall AK. (2010). Histone lysine demethylases function as co-repressors of SWI/SNF remodeling activities during Drosophila wing development. *Developmental Biology* (In Press)
2. **Marenda DR**, Vrailas AD, Rodrigues AB, Cook S, Powers MA, Lorenzen JA, Perkins LA, and Moses K. (2006) MAP Kinase subcellular localization controls both pattern and proliferation in the developing *Drosophila* wing. *Development* **133:** 43-51
3. **Marenda DR**, Zraly CB, and Dingwall AK (2004) The *Drosophila* Brahma (SWI/SNF) chromatin remodeling complex exhibits cell-type specific activation and repression functions. *Dev. Biol.* **267**: 279-293
4. Zraly CB, **Marenda DR,** and Dingwall AK (2004). SNR1 (INI1/SNF5) mediates important cell growth functions of the *Drosophila* Brahma (SWI/SNF) chromatin remodeling complex. *Genetics* **168**: 199-214
5. **Marenda DR**, Zraly CB, Feng Y, Egan S, and Dingwall AK (2003) **The *Drosophila* SNR1 (SNF5/INI1) subunit directs essential developmental functions of the Brahma chromatin remodeling complex**. *Mol. Cell. Biol.* **23**: 289-305
6. Zraly CB, **Marenda DR**, Nanchal R, Cavalli G, Muchardt C, and Dingwall AK (2003) SNR1 is an essential component in a subset of *Drosophila* Brm complexes, targeting specific functions during development. *Dev. Biol.* **253**: 291-308
7. Howell N, Bogolin C, Jamieson R, **Marenda DR**, and Mackey DA (1998) mtDNA mutations that cause Optic Neuropathy: How do we know? *Am J Hum Genet* **62**: 196-202