# The Autism-Associated Chromatin Modifier, *Chromodomain Helicase DNA Binding Protein 8/kismet*, Affects Axon Guidance and Behavioral Phenotypes in *Drosophila melanogaster*

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## BACKGROUND

- Autism Spectrum Disorder (ASD) is a highly heritable group of neurodevelopmental disorders that afflicts 1 in 68 children in the U.S.
- Mutations in *Chromodomain Helicase DNA Binding Protein 8 (CHD8)* are among the most common *de novo* mutations associated with ASD.
- *CHD8* is a chromatin modifier that affects expression of many other ASD-risk genes.
- Mutations in *CHD8* define an ASD subtype characterized by macrocephaly and gastrointestinal (GI) problems.
- The field is currently interested in investigating the bidirectional communication between the gut microbiome and central nervous system (the gut-brain-axis) in ASD.
- Our overarching goal is to determine neural and GI phenotypes caused by loss-of-function mutations in *kismet (kis)*, the *Drosophila melanogaster* ortholog of *CHD8*, to study the role of *CHD8/kis* and the gut-brain axis in ASD.
- Here, we show that heterozygous *kis* mutants exhibit severe axon patterning defects in the adult brain, as well as severe defects in courtship behaviors.
- These assays will ultimately be used to study connections between gut microbiota and neural phenotypes.

## MATERIALS AND METHODS

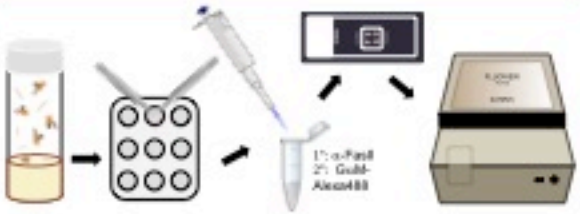


**Figure 1.** Immunohistochemistry and confocal microscopy workflow used to study axon guidance patterns in adult fly brains


**Figure 2.** Courtship assay setup
**(A)** 12 individual courtship chambers **(B)** Following **(C)** Attempted copulation **(D)** Successful copulation

## RESULTS

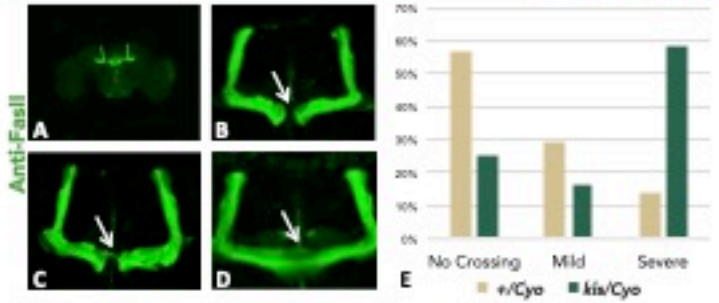


**Figure 3. *kis* is required for axon guidance.** Anti-FasII & AlexaFluor488 mark axons in the mushroom body, a neural structure in the adult brain critical for higher order sensory integration, including learning and memory. Axon guidance defects are a common cellular phenotype of ASD (white arrows are pointing at the midline of the brain).
(A) Representative image of an adult fly brain highlighting the mushroom body
(B) Representative image of a mushroom body showing **no β-lobe midline crossing.**
(C) Representative image of a mushroom body showing **mild β-lobe midline crossing.**
(D) Representative image of a mushroom body showing **severe β-lobe midline crossing.**
(E) Quantification of +/Cyo (a non-*kis* mutant carrying the same Cyo balancer chromosome; the + chromosome is marked by Sculoid) versus *kis*/Cyo β-lobe midline crossing frequencies. ($n_{+/Cyo}$ = 7, $n_{kis/Cyo}$ = 12).

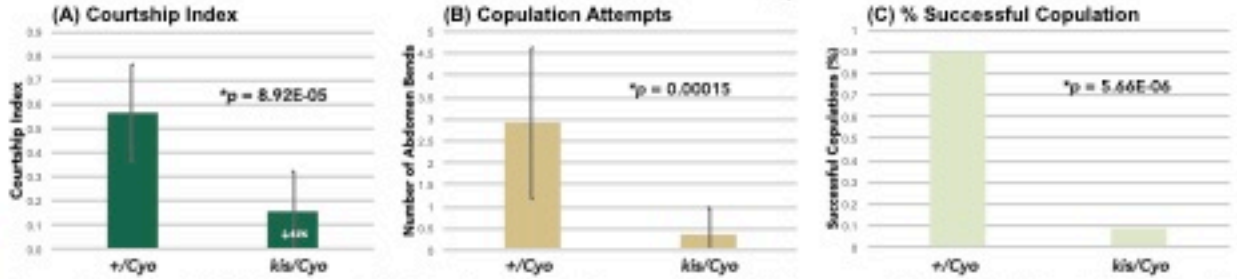


**Figure 4. *kis* is required for innate courtship behaviors.** The courtship assay is a quantitative measure of an innate behavior. This data shows that heterozygous *kis* mutants have deficits in courtship behaviors. Most critically, the courtship index (CI) is affected, which reflects the percent time spent participating in courtship behaviors for the duration of the assay (10 minutes). **(A)** The CI is significantly lower for *kis*/Cyo ($n_{+/Cyo}$ = 10, $n_{kis/Cyo}$ = 12). **(B)** Copulation attempts, measured by number of abdomen bends, is significantly lower for *kis*/Cyo ($n_{+/Cyo}$ = 10, $n_{kis/Cyo}$ = 12). **(C)** % Successful Copulation is significantly lower for *kis*/Cyo ($n_{+/Cyo}$ = 10, $n_{kis/Cyo}$ = 12). *P*-values determined by two-tailed Student's *t*-tests.

## FUTURE DIRECTIONS

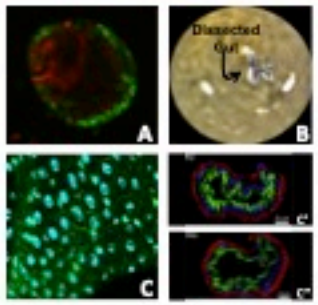


**Figure 5. Other neural phenotype and gut phenotype assays**
**(A)** Investigating neural stem cell/neuroblast (NB) proliferation in the larval brain. Green = anti-Deadpan (Type I & II NBs), Red = anti-Prospero (Type I NBs). **(B)** Measuring rates of digestion. **(C-C'')** Investigating gut phenotypes (Green = anti-Armadillo, anti-Prospero, Blue = DAPI). **(C'-C'')** Microtome sections of anterior midgut (C') and posterior midgut (C'') (Buchon *et al.*, 2013).

**Investigating the Gut-Brain Axis:** We plan to study how variations in gut microbiota influence neural phenotypes shown here.

## ACKNOWLEDGEMENTS

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