The *Drosophila* intellectual disability-associated histone demethylase, KDM5, is required during early neurodevelopment for proper neuronal morphology

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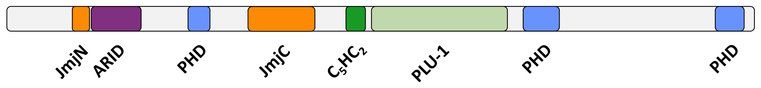
Abstract

Intellectual disability (ID) disorders affect 2% of the population and are characterized by an IQ score lower than 70 with deficits in adaptive functioning. Mutations in over 400 genes contribute to the pathogenesis of ID disorders, with patients presenting with learning and memory impairments and often syndromic features such as epilepsy, anxiety, short stature, and aggressive tendencies. Our research focusses on the KDM5 family of transcriptional regulators, mutations in which account for 1-3% of inherited ID ranging from mild to severe. The molecular mechanisms by which KDM5 proteins impact neuronal function remain largely unknown, leaving patients without effective treatment strategies. Thus, the overarching goal of this project is to understand how KDM5 contributes to neuronal and transcriptional outputs that influence cognition, and how these processes are altered by *kdm5* mutations associated with ID.

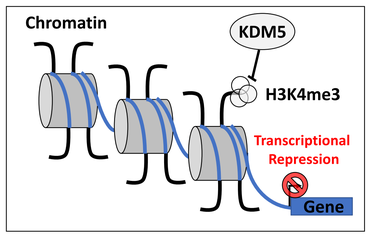
Here, we demonstrate that *Drosophila*, which possesses a single *kdm5* ortholog, serves as a suitable and genetically tractable model to investigate the molecular and cellular defects associated with ID disorders. Combining analyses of a *kdm5* null mutant with powerful genetic tools and behavioral paradigms, we are able to probe the neuronal requirements of KDM5 *in vivo*. Here, we present a role for KDM5 during early neurodevelopment in regulating neuronal morphology. We (1) demonstrate that KDM5 is required within immature neurons of the mushroom body, a brain structure critical for cognition, but not in mature cells, for proper mushroom body development, and (2) utilize Targeted DamID (TaDa) to identify differentially expressed KDM5 target genes within immature neurons of *kdm5*^{140} null mutants, allowing us to elucidate KDM5 transcriptional regulatory networks critical for neuronal development and cognitive function.

Background

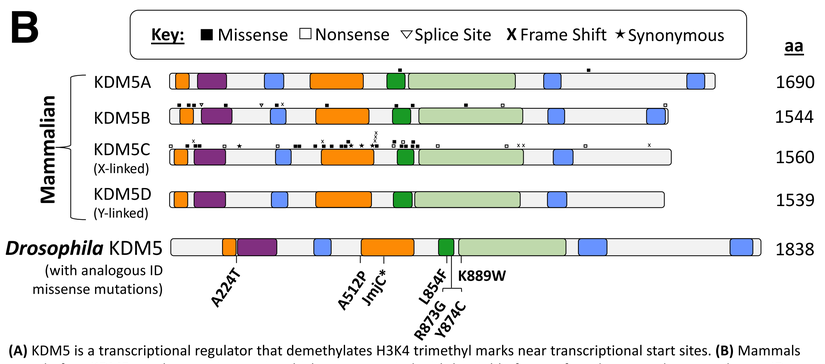
A ***Drosophila* KDM5**



KDM5 Domain	Protein Function
JmjN	Required for JmjC demethylase activity
ARID	DNA binding
PHD	Reader of H3K4 methylation status
JmjC	Demethylation of H3K4me3
C_5HC_2 / PLU-1	Unknown



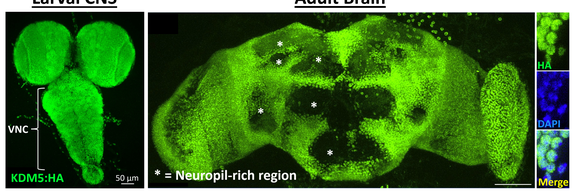
B



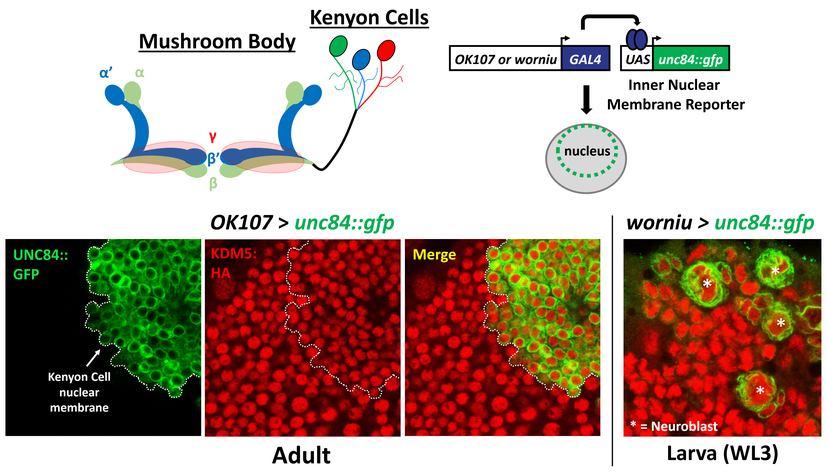
(A) KDM5 is a transcriptional regulator that demethylates H3K4 trimethyl marks near transcriptional start sites. **(B)** Mammals encode four KDM5 paralogs, mutations in which are associated with heritable forms of syndromic and non-syndromic ID in humans. We have generated six fly strains, each bearing a missense mutations in an analogous ID-associated KDM5 residue, as well as a synthetic, demethylase-dead strain (JmjC*).

Results

A **KDM5 is ubiquitously expressed within nuclei of the *Drosophila* CNS**

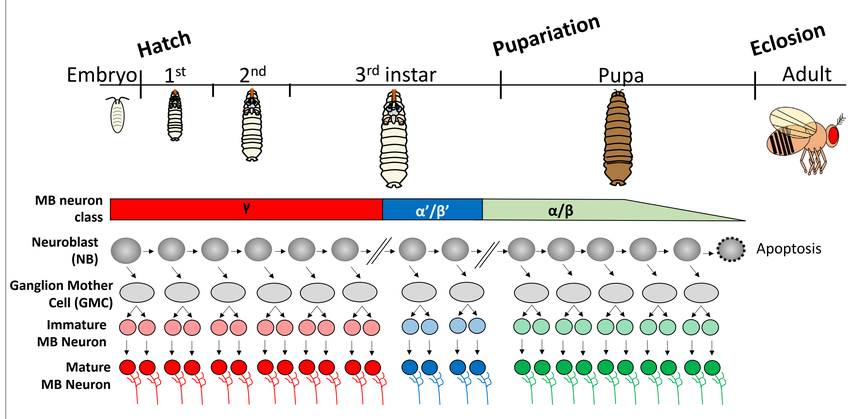


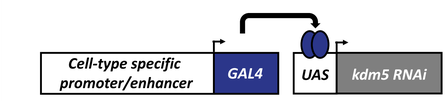
B **KDM5 is expressed within nuclei of *Drosophila* mushroom body neurons and neural progenitor cells**

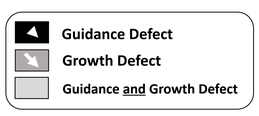


(A) Endogenous HA-tagged KDM5 is ubiquitously expressed in nuclei of the *Drosophila* adult brain and larval CNS. Adult brain KDM5:HA expression is from Zamurrad *et al.,* 2018. **(B)** An inner nuclear membrane-tethered GFP reporter, UNC84::GFP, reveals KDM5:HA expression within Kenyon Cell nuclei of the *Drosophila* adult, as well as within neuroblast nuclei of the late 3rd instar larval brain. Neuroblasts are labeled by asterisks, with UNC84::GFP perduring for 1-3 cell divisions.

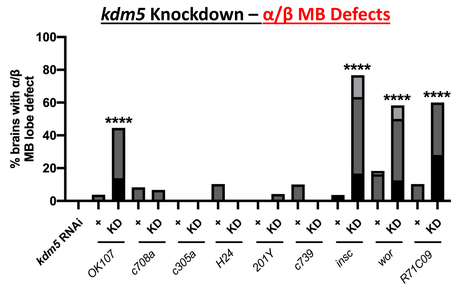
C **KDM5 is required during early neurodevelopment for proper mushroom body formation**

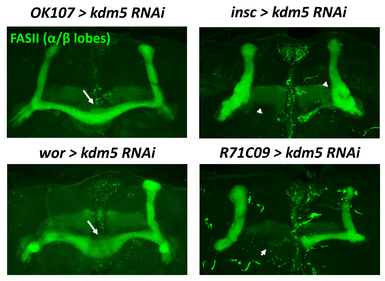




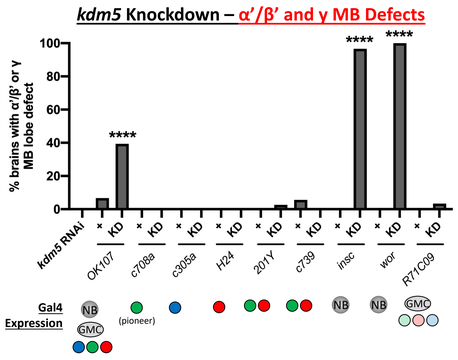


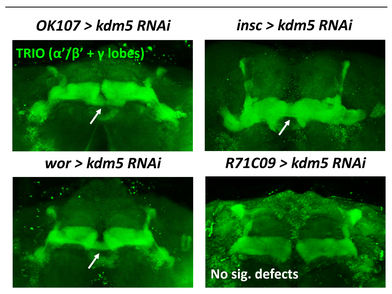
kdm5 Knockdown – α/β MB Defects

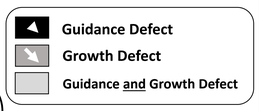




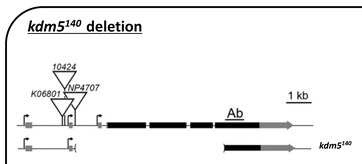
kdm5 Knockdown – α'/β' and γ MB Defects





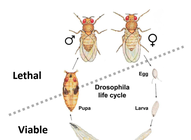


kdm5^{140} deletion

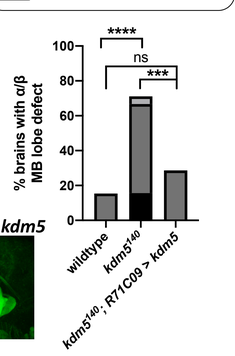


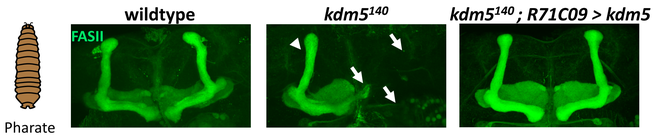
Drelon et al., 2018

kdm5^{140} viability

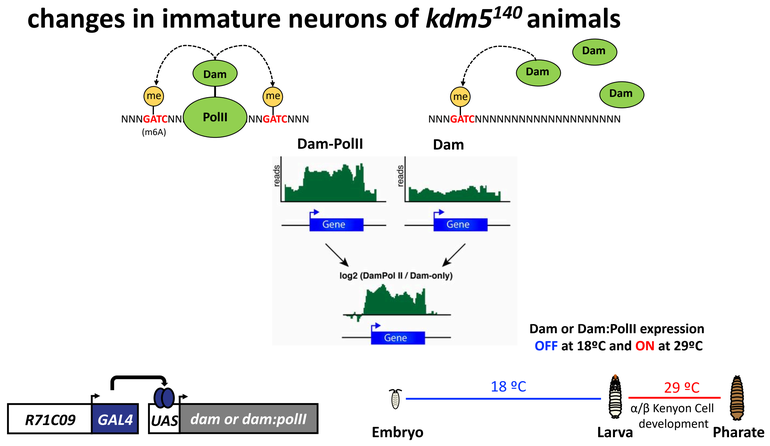


Torres et al., 2011





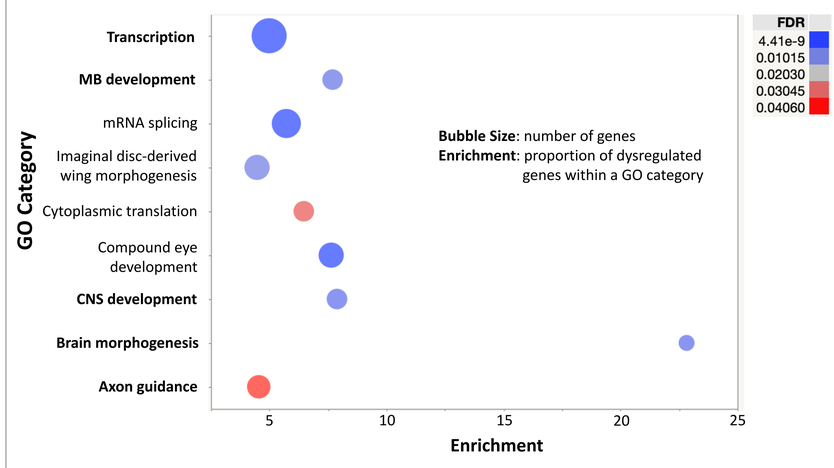
D **Utilizing Targeted DamID (TaDa) to identify gene expression changes in immature neurons of *kdm5*^{140} animals**



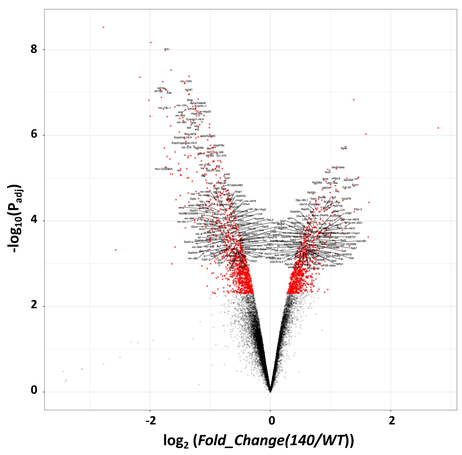
(C) Kenyon Cells are born in a temporally-controlled developmental sequence. *kdm5* knockdown using the *OK107-Gal4* driver, which drives *kdm5* RNAi expression in all MB neuroblasts (NBs), ganglion mother cells (GMCs), and mature Kenyon Cells (KCs), results in significant growth and guidance defects of the MB lobes. Additionally, *kdm5* knockdown specifically in NBs (*worniu-Gal4* and *insc-Gal4*) results in similar defects. Interestingly, knockdown in GMCs and immature KCs (*R71C09-Gal4*) results in defects that are specific to the α/β lobes. Importantly, *kdm5* knockdown in subsets of post-mitotic, mature KCs does not result in any apparent gross morphological defects of the MB. *kdm5*^{140} null mutants, which were previously generated *via* imprecise excision of the p-element *NP4707*, are pupal lethal. *kdm5*^{140} null pharate adults present with severe MB defects compared to an isogenic control. Significantly, these defects were rescued upon reintroduction of KDM5 in GMCs and immature neurons, suggesting that KDM5 is required during early neurodevelopment for proper MB formation. *** $p < 0.001$; **** $p < 0.0001$ *via* χ^2 test with Bonferroni correction, n = 24-49. **(D)** Targeted DamID (TaDa) can be used to identify gene expression changes within in a cell specific manner. *R71C09-Gal4* drives expression of a transgene encoding a Dam or Dam::PolII fusion protein within immature neurons in a *kdm5*^{140} null mutants. Dam methylates GATC motifs flanking regions bound by PolII. Pupae are then processed for NGS, allowing us to indirectly measure gene expression changes in a cell-specific, and temporally-controlled manner (by pulsing Dam or Dam-PolII expression using a *tub-Gal80[ts]* transgene. Figure is modified from Widmer et al. 2018.

E **TaDa analysis of *kdm5*^{140} animals reveals disruptions in networks critical for neurodevelopment and cell division**

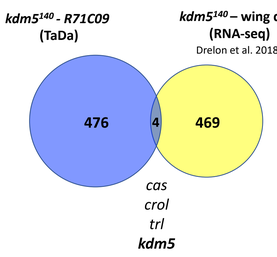
Gene Ontology Analysis of Downregulated Genes



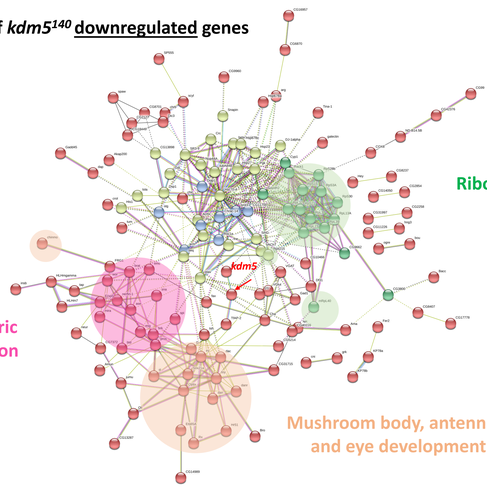
Differentially Expressed Genes



kdm5^{140} gene dysregulation is tissue-specific



STRING analysis of *kdm5*^{140} downregulated genes



(E) TaDa analysis of GMCs and immature neurons of *kdm5*^{140} mutants. Gene ontology (GO) analysis of downregulated genes shows enrichment for genes regulating neurodevelopment and cell division, among other processes. A volcano plot shows a significant number of genes that are both up- and downregulated within *kdm5*^{140} animals (FDR < 0.05). Notably, such changes appear to be tissue specific, as RNA-seq analysis of wing discs reveals a different subset of genes that are dysregulated within the same mutant. STRING analysis of downregulated genes further demonstrates major genetic networks that are disrupted by loss of *kdm5*.

Future Directions

- Perform Targeted DamID (TaDa) to assess gene expression changes within neural progenitor cells and immature MB neurons of *kdm5* knockdown and *kdm5* ID mutant backgrounds.
  - Identify candidate pathways through which KDM5 may act to regulate mushroom body development and cognitive function.
  - Perform rescue experiments with candidate genes identified through TaDa.
- Assess the cognitive and behavioral phenotypes of KDM5 ID mutants with learning and memory, seizure, and aggression assays.

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NIH National Institute of Neurological Disorders and Stroke

NIH National Institute of General Medical Sciences