# Characterization of a predicted H3.3K36 trimethylation reader in *Drosophila*

**Christopher Abdullah[1,4], Jackson M. Moorefield[3], Julia Goncalves[3], Tiffany Riascos[3], and Robert J. Duronio[2,3,4,5]**

[1]SPIRE Postdoctoral Fellowship Program; [2]Department of Genetics; [3]Department of Biology;
[4]Integrative Program for Biological and Genome Sciences; [5]Lineberger Comprehensive Cancer Center
University of North Carolina at Chapel Hill

## BACKGROUND

### ZMYND11 is an H3.3 specific K36 trimethylation reader protein

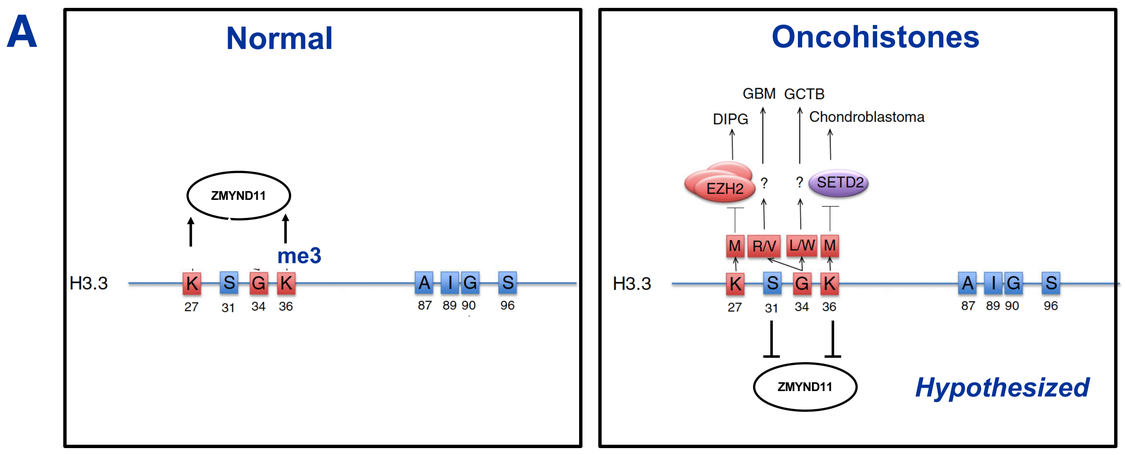






**Figure 1**: **(A)** ZMYND11 is a protein in mammals that has been demonstrated to be a specific variant histone H3.3 lysine 36 trimethylation (H3.3K36me3) reader (Wen 2014). **(B)** Crystal structures have demonstrated that the serine residue at position 31 of the H3.3 protein likely conveys specificity towards H3.3 rather than the canonical, replication-dependent histone H3 (Wen 2014). The reader has been shown to repress transcription via regulation of RNA polymerase II elongation in a H3K36me$^3$-dependent manner. Additionally, data suggest that the reader also regulates RNA processing, specifically by promoting intron retention. H3.3 interactions with ZMYND11. Predicted interactions with the "A29-G33" segment of H3.3 (left) and H3.1(right) and Bromo-PWWP domains of ZMYND11 (Wen 2014)

### ZMYND11 and the *Drosophila* CG8569 protein are well-conserved

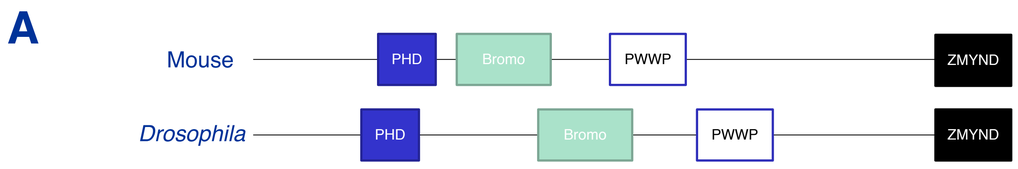
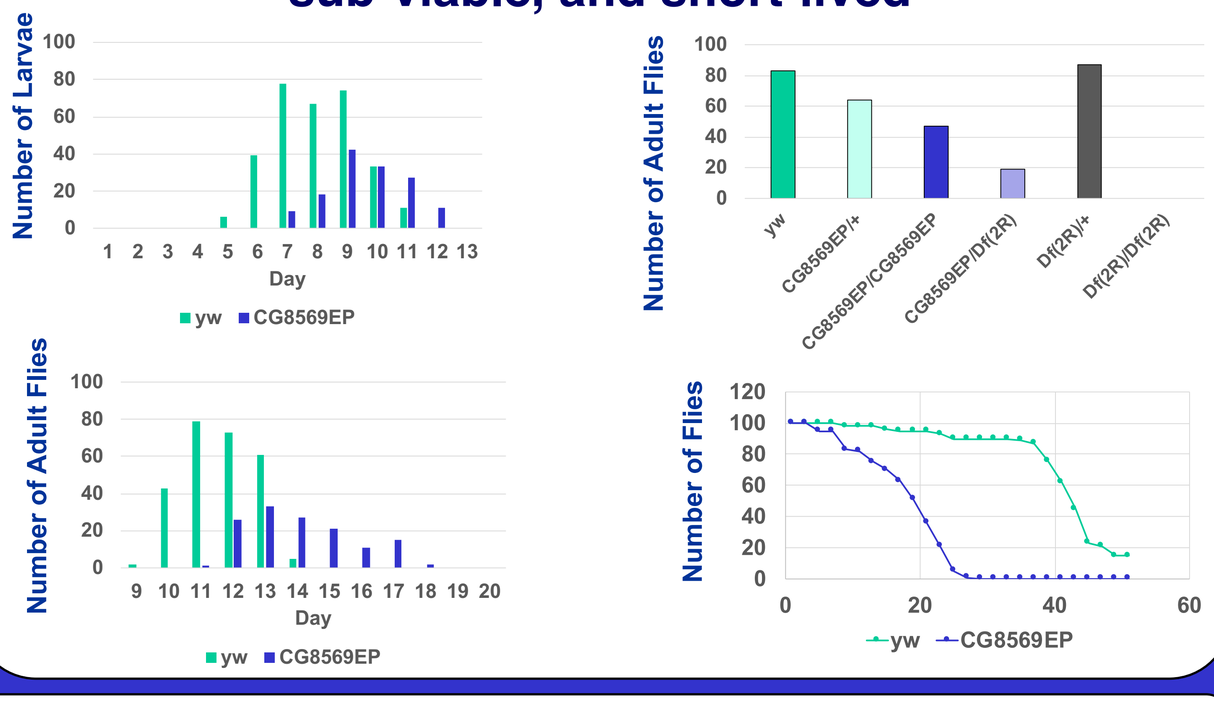


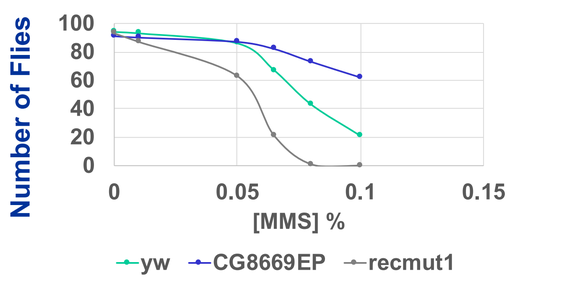

**Figure 2**: CG8569 protein domain conservation and existing truncation allele, CG8569$^{EP}$. (A) Schematic of functional domains of the mouse ZMYND11 protein (602 aa) and the *Drosophila* CG8569 protein (608 aa). Functional domains include the Bromo and PWWP which have been shown to bind K36me$^3$, the PHD domain which also binds histone marks. Additionally the ZMYND domain is highly conserved and a Zinc finger-like domain. (B) Schematic of the function domans of a truncation mutant of the CG8569 protein.

## RESULTS

### CG8569$^{EP}$ mutants are developmentally delayed, sub-viable, and short-lived




### CG8569$^{EP}$ mutants are less sensitive to DNA damaging agents




## RESULTS

### RNAi-mediated knockdown of CG8569 decreases viability

**A**

$\frac{yw}{yw} \times \frac{yw}{Y};\frac{UAS-CG8569RNAi}{TM6B}$

$\frac{yw}{yw};\frac{tubGal4}{CyO} \times \frac{yw}{Y}$

$\frac{yw}{yw};\frac{tubGal4}{CyO} \times \frac{yw}{Y};UAS-CG8569RNAi$

| Genotype | # scored | Observed | Expected |
|---|---|---|---|
| $yw;\frac{+}{+};\frac{UAS-CG8569RNAi}{+}$ | 378 | 201 (53.2%) | 189 (50%) |
| $yw;\frac{tubGal4}{+};\frac{+}{+}$ | 349 | 164 (46.9%) | 175 (50%) |
| $yw;\frac{tubGal4}{+};\frac{UAS-CG8569RNAi}{+}$ | 386 | 70 (18.1%) | 193 (50%) |

$\frac{yw}{yw} \times \frac{yw}{Y};\frac{UAS-CG8569RNAi}{TM6B}$

$\frac{yw}{yw};\frac{actGal4}{TM6B} \times \frac{yw}{Y}$

$\frac{yw}{yw};\frac{actGal4}{TM6B} \times \frac{yw}{Y};UAS-CG8569RNAi$

| Genotype | # scored | Observed | Expected |
|---|---|---|---|
| $yw;\frac{+}{+};\frac{UAS-CG8569RNAi}{+}$ | 378 | 201 (53.2%) | 189 (50%) |
| $yw;\frac{actGal4}{+}$ | 413 | 223 (54.0%) | 207 (50%) |
| $yw;\frac{actGal4}{UAS-CG8569RNAi}$ | 434 | 83 (19.1%) | 217 (50%) |

**B**

$\frac{yw}{yw} \times \frac{yw}{Y};\frac{UAS-CG8569RNAi}{TM6B}$

$\frac{yw}{yw};\frac{ElavGal4}{CyO} \times \frac{yw}{Y}$

$\frac{yw}{yw};\frac{ElavGal4}{CyO} \times \frac{yw}{Y};UAS-CG8569RNAi$

| Genotype | # scored | Observed | Expected |
|---|---|---|---|
| $yw;\frac{+}{+};\frac{UAS-CG8569RNAi}{+}$ | 421 | 236 (56%) | 210 (50%) |
| $yw;\frac{ElavGal4}{+};\frac{+}{+}$ | 357 | 168 (47%) | 179 (50%) |
| $yw;\frac{ElavGal4}{+};\frac{UAS-CG8569RNAi}{+}$ | 386 | 205 (53%) | 193 (50%) |

**C**

$\frac{yw}{yw} \times \frac{yw}{Y};\frac{UAS-CG8569RNAi}{TM6B}$

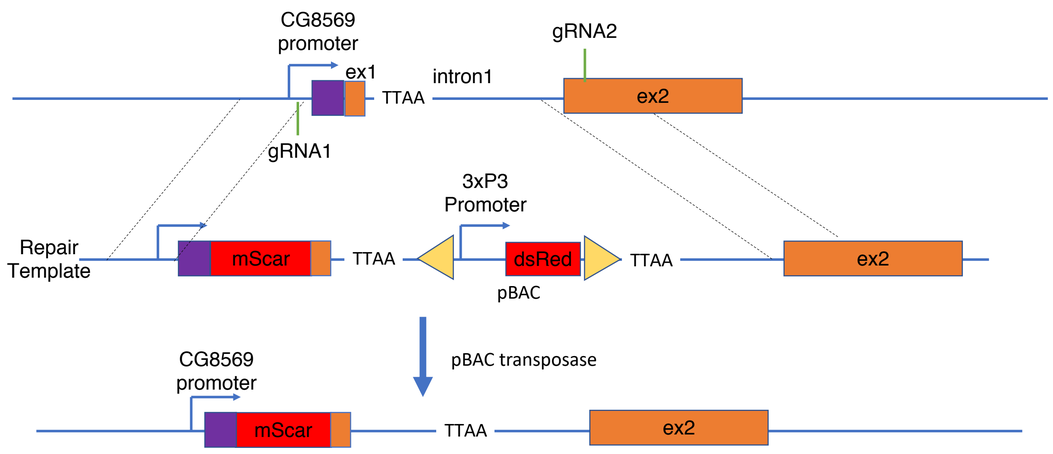
$\frac{yw}{yw};\frac{RepoGal4}{TM6B} \times \frac{yw}{Y}$

$\frac{yw}{yw};\frac{RepoGal4}{TM6B} \times \frac{yw}{Y};UAS-CG8569RNAi$

| Genotype | # scored | Observed | Expected |
|---|---|---|---|
| $yw;\frac{+}{+};\frac{UAS-CG8569RNAi}{+}$ | 364 | 193 (53%) | 182 (50%) |
| $yw;\frac{RepoGal4}{+}$ | 339 | 183 (54%) | 170 (50%) |
| $yw;\frac{RepoGal4}{UAS-CG8569RNAi}$ | 396 | 75 (19%) | 198 (50%) |

**Figure 3**: Viability assay using several different Gal4 drivers to express UAS-mediated CG8569 RNAi. Eclosion rates of *Drosophila* were assayed according to the crosses outlined above. (A) Viability assays were performed using two different ubiquitous Gal4 drivers, Tubulin (bottom) and actin (top) to drive expression in all cells. (B) The Elav-Gal4 driver was used to express CG8569-RNAi in neurons. (C) The Repo-Gal4 driver was used to express CG8569 in glial cells.

### Generating CG8569 Reagents: mScarlet-tagged allele, null allele, antibody




## FUTURE DIRECTIONS

Generating CG8569 Protein

- to screen against histone modifications using the Strahl Lab's histone peptide array

Generating CG8569 Mutants

- Cloning CG8569 binding mutants that will disrupt CG8569 and H3.3K36me$^3$ interactions
- Cloning mutants found in human patients related to cancer , mental retardation and intellectual disability

## ACKNOWLEDGEMENTS

I thank the Duronio Lab for support and assistance and the UNC Histone Replacement Group for helpful insight into this project.

Funding: SPIRE Postdoctoral Fellowship Program (K12-GM000678), R01DA036897 to RJD, ITCMS Training Grant, UNCCreativity Hub Grant

## REFERENCES

Wen H, et. al. ZMYND11 links histone H3.3K36me3 to transcription elongation and tumour suppression. *Nature*. 2014 Apr 10;508(7495):263-8.

Shi, et. al. The Histone Variant H3.3 in Transcriptional Regulation and Human Disease. Journal of Molecualr Biology. 2017.

Fang, et. al. The histone H3.3K36M mutation reprograms the epigenome of chondroblastomas. Science. 2016