Loss of Zdhhc8 induced apoptosis in Drosophila

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Abstract: The zinc-finger DHHC-containing 8 (Zdhhc8) gene, encoding a putative palmitoyltransferase, plays a critical role in protein palmitoylation, a post-translational modification essential for regulating protein stability, localization, and function. In this study, we investigated the effects of Zdhhc8 downregulation on Drosophila development using RNA interference (RNAi). By driving Zdhhc8-RNAi expression in specific tissues, we observed significant developmental defects in both wing and eye discs. Downregulation of Zdhhc8 led to the loss of apical constriction and vesicle formation in wing discs, accompanied by increased apoptosis. Similarly, Zdhhc8 RNAi resulted in smaller eyes and elevated apoptosis in eye discs. These findings demonstrate that Zdhhc8 is crucial for maintaining cell survival and proper tissue development in Drosophila. Our results highlight the importance of Zdhhc8 in regulating cellular processes and suggest its potential role in developmental disorders and diseases.

1 Introduction

1.1 ZDHHC8

Zinc-finger DHHC-containing 8 (ZDHHC8) is a putative palmitoyl transferase, zinc-finger DHHC-containing 8 (ZDHHC8). Contains a characteristic catalytic (DHHC) domain of stirred cysteine, highly expressed in the brain [1].Post-translational modifications (PTMs) alter the protein conformation, localization and activity and regulate protein-protein interactions to enable participation in a variety of cancer processes [2]. S-palmitoylation, in which the 16-carbon fatty acid palmitate is attached to cysteine residues via a reversible thioester linkage [3], is one of the most common PTM, which serves as a mechanism to regulate protein stability, subcellular localization, and membrane trafficking, driving cancer initiation and progression [4].

1.2 Palmitoylation

Palmitoylation is catalyzed by a member of the DHHC family of protein palmitoyl transferases (PATs), which includes 23 enzymes with the names ZDHHC1 to ZDHHC24 (excluding ZDHHC10) and contains the conserved zinc-finger-Asp-His-His-Cys (ZDHHC) motif inside a cysteine-rich domain (CRD) [5]. Indeed, proteins can cycle between palmitoylated and de-palmitoylated forms on timescales that range from seconds to hours [6]. Dynamic palmitoylation can impact protein localization, accumulation, secretion, stability, and function by altering membrane affinity [1, 4, 7]. Understanding how protein palmitoylation influences the function of individual proteins in normal and cancer cells is an important driver of current research in this area [8].

1.3 Acridine orange (AO) fluorochrome staining

Acridine orange (AO) fluorochrome staining has been in application in histology, cytology and genotoxicology for decades. It is a cell-permeable dye that shows green fluorescence when bound to double-stranded DNA and yellow/red (depending upon intensity of fragmentation) when bound to single-stranded DNA or RNA.[9]Staining with AO has been shown to be valuable in pathohistology and clinics for detection of circulating tumour cells, apoptosis or lysosomal membrane stability.[10]

1.4 Model Animal

The use of fruit flies for genetic development research has a long history, and the understanding of their chromosome composition, gene coding, location and phenotype is unmatched by other organisms. First, fruit flies are small in size, easy to operate, low in cost, simple to raise, have a short life cycle (about 10 days), are easy to reproduce, and have a large number of offspring, which is convenient for laboratory operation and phenotypic analysis. Secondly, a series of effective technologies have been developed for genetic manipulation of fruit flies, including chimera analysis technology, two-component ectopic gene expression system, site-directed homologous recombination technology, enhancer trap technology and fruit fly gene site-directed knockout technology, etc. [11] These technologies have greatly enriched the research methods using fruit flies as model organisms. [12]Next, fruit flies

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also carry many molecular markers, phenotypic markers and other chromosomes with characteristics that are easy to genetically manipulate. These tools can be used for large-scale genetic screening to isolate some lethal or visible phenotypes, and even isolate those phenotypes that are only expressed in the second or third generation of mutant individuals.[13] Finally, more than 60% of *Drosophila* genes have homology with human disease genes, which may include disease genes such as neurological diseases, tumors, metabolic abnormalities, kidney diseases and malformation syndromes. Therefore, *Drosophila* can be used as a model for the study of these human diseases. The completion of whole genome sequencing makes reverse genetics operations easier and highlights the importance and advantages of *Drosophila* as a model organism in the post-genomic era.[14]

Drosophila are small flies in the order Diptera and family Drosophilidae. Commonly known as fruit or vinegar flies, they are often found on rotting fruit or other decaying matter [15]. *Drosophila* melanogaster, in the subgenus Sophophora, was first made famous at the beginning of the 20th century when the Morgan lab at Columbia University confirmed the chromosome theory of inheritance [16]. Now studied by >1800 labs around the world, D. melanogaster is a powerful model organism. Flies are easily cultured in the lab and have many offspring and short generation times; in addition, they have a compact genome, are easy to manipulate genetically, and have many orthologous genes associated with human disease.[15]

1.5 Gal4-*UAS* system

The Gal4-*UAS* system is one of the commonly used gene expression tools in *Drosophila* genetics research. The Gal4 (galactose-regulated upstream promoter element, Gal) protein is a type of transcription factor in yeast involved in galactose transport and glucose-6-phosphate conversion. This protein can bind to the upstream DNA sequence (upstream activating sequence, *UAS*) and activate the expression of downstream genes. [17] The Gal4 protein consists of 881 amino acids, including functional domains such as an N-terminal DNA-binding domain, a Gal80 protein-binding site, a transcriptional activation region, and a nuclear localization signal. [18] The *UAS* sequence is another yeast sequence similar to the enhancer sequences of higher eukaryotes, capable of binding to the Gal4 protein. Each *UAS* sequence has binding sequences of varying lengths, containing the sequence CGGA(GC)GACATCAGGCAGGC. [19] In the presence of the Gal4 protein, the *UAS* sequence acts as a modular element that activates the transcriptional activity of downstream genes. The interaction between the two is independent of the orientation and distance of the *UAS* sequence, functioning as an enhancer sequence. [20] The key to the Gal4-*UAS* system lies in the fact that the Gal4 gene and the *UAS*-target gene are located in two separate transgenic lines, and neither can activate target gene expression on its own. [21] Only by crossing the two transgenic lines can the specific expression of the target gene be controlled. [22]

Certainly, the Gal4-*UAS* system offers numerous advantages. Firstly, by utilizing specific *UAS*-target gene transgenic lines, it is possible to study the same target gene across different tissues or developmental stages. Conversely, by employing specific Gal4 transgenic lines, the roles of various target genes can be investigated within specific tissues or at particular developmental stages. Secondly, transgenic lines can be constructed with *UAS* sequences linked to lethal or toxic genes. In these lines, the lethal or toxic genes remain unexpressed when the transgenic line exists alone; it is only upon crossing with a Gal4 transgenic line that the target gene's expression is activated. Lastly, this system is capable of exponentially increasing the expression of sequences downstream of the *UAS* sequence [23].

1.6 RNA interference (RNAi)

RNA interference (RNAi), also known as post-transcriptional gene silencing (PTGS), is a phenomenon where RNA effectively blocks the expression of homologous genes. In eukaryotic organisms, certain RNAs inhibit the expression of homologous genes by preventing mRNA translation, degrading mRNA, or controlling the promoters of mRNA expression and inducing post-transcriptional silencing. Among these, miRNA and dsRNA are widely expressed across different species. Firstly, in animal and plant cells, non-protein-coding genes are transcribed into miRNA precursors. These transcripts, 70-90 nucleotides long, form hairpin structures containing double-stranded regions, which then enter the RNA interference system to produce active miRNAs. These small RNAs, approximately 22 nucleotides in length, form double strands with the target gene's mRNA through base pairing, directly blocking further translation or inducing conformational changes in the mRNA. Secondly, early studies showed that double-stranded RNA (dsRNA) is more effective at suppressing homologous gene expression than antisense or sense strands. It was later demonstrated that dsRNA is processed by the RNA interference system to produce small RNAs that induce silencing. These small double-stranded RNAs, 21-23 nucleotides long, often have two free nucleotides at the 3' end. Because they can complementarily pair with homologous mRNA and subsequently degrade the paired mRNA, they are termed small interfering RNAs (siRNAs) [24, 25].

The RNA interference system in vivo is associated with many related proteins, protein complexes, and reaction intermediates. [26]The general process is as follows: The enzyme Dicer cleaves the double-stranded RNA entering the system into fragments of about 23 base pairs, a process that also requires the participation of the receptor-destroying enzyme (RDE-4). Once assembled, the nuclease in the complex catalyzes the deformation and displacement of dsRNA with the help of ATP. Due to the RNA displacement reaction, the antisense strand of siRNA can complementarily pair with homologous mRNA, either degrading the mRNA or inhibiting its degradation [24] [27]. In *Drosophila*, dsRNA also suppresses gene expression. Injecting dsRNA into *Drosophila* embryos can strongly inhibit the expression of homologous genes. [28]By combining the Gal4-*UAS* system, linking RNA inhibitory gene sequences downstream of the *UAS* sequence, and crossing with specific Gal4 transgenic lines, the expression of target proteins can be specifically downregulated at certain developmental stages or in specific tissues, avoiding manipulation at the gene level. [29]Using this technology, researchers have successfully established multiple genome-wide RNAi libraries in *Drosophila*, significantly advancing the study of gene functions in vivo.[30]

1.7 *Drosophila* P Element

Since the 1910s, a small number of abnormal phenomena have been observed in the offspring of crosses between certain strains of *Drosophila* melanogaster*, such as abnormal segregation ratios, ovarian dysgenesis, high mutation rates during meiosis in males, recombination, and chromosomal aberrations. In-depth studies have shown that the cause of hybrid dysgenesis in these strains is the presence of P elements in these so-called P strains of fruit flies. P elements share many similarities with transposons in bacteria: the full-length P element is 2907 base pairs (bp) long, with 31 bp inverted repeat sequences at both ends. Its coding region includes 4 exons and 3 introns. [31]In somatic cells, introns 1 and 2 are excised, and the transcript is translated into a 66 kDa transposase repressor protein, which inhibits downstream gene expression. In germ cells, intron 3 is spliced out, and the transcript is translated into an 87 kDa transposase, which facilitates P element transposition. [32]After insertion, the P element creates an 8 bp direct repeat in the target gene. If part of the sequence is deleted but the corresponding exons providing the transposase remain, these incomplete P elements can still transpose.

P elements can excise from their original locations, a process known as excision. There are two types of excision: precise excision can revert the mutation caused by P element insertion, and the P element completely disappears from the insertion site; imprecise excision can lead to gene mutations and chromosomal aberrations, with the P element either completely disappearing and taking away part of the sequence or leaving behind a partial remnant in the chromosome. The number and location of P elements are analyzed using isotope-labeled P element probes. Results show that the position and number of P elements vary among the genomes of different *Drosophila* strains. In addition to P elements, other transposons in *Drosophila* include *copia*, *412*, *279*, *Tip*, and *FB*.[33]

2 Materials and Methods

2.1 *Drosophila* hybridization experiment

The experiment involves crossing male and female fruit flies of different genotypes as parents, thus the sorting of male and female fruit flies plays a crucial role in the experiment. The larvae of fruit flies are quite similar in morphology, with male larvae being more slender. Under light observation, one can notice symmetrical vesicular structures at their tails, which eventually develop into male reproductive organs. The adult forms of fruit flies, however, have distinct differences and are easily distinguishable (see Table 1).

In the experiment, we typically select virgin flies, i.e., unmated fruit flies, as the parental stock for hybridization. Since they are less likely to reject mating with male fruit flies and have a stronger reproductive capacity than mated flies, it is easier to obtain offspring of specific genotypes, thereby enhancing the reliability of the experiment and eliminating the influence of other genotypes in the progeny. After removing all the fruit flies from the culture bottles to be harvested, we collect newly emerged flies within 8 hours under a 25°C culture condition. If the culture temperature is below 25°C, the collection time is extended accordingly. The newly emerged flies are transparent and swollen, with black spots on their abdomen known as meconium, which serves as an indicator to identify virgin flies. If uncertain, the fly can be cultured separately to observe whether larvae hatch; if not, it is considered a virgin fly.[34]

Table 1. Sexual Dimorphism in *Drosophila* melanogaster

	Female	male
Size	Larger body size	Smaller body size
Sex Comb	No sex comb	Black bristle-like sex comb located on the outer side of the base of the first pair of tarsal segments
Dorsal-Abdominal Stripes	5 distinct black stripes	3 distinct black stripes: the first two are thin, and the last one is wide, extending to the ventral side
Abdominal Segments	6 abdominal segments	4 abdominal segments
Tail	Sharper tail with an ovipositor at the base, cylindrical and protruding	Rounded end with a copulatory organ at the base, appearing as a black circular shape

2.2 Rearing of *Drosophila*

In our laboratory, we use a brown sugar-cornmeal-yeast medium, with the following formulation (for 1 liter):

- Brown sugar: 135 g
- Cornmeal: 85 g
- Agar: 7 g
- Water: 1 L
- Yeast: 8 g
- Propionic acid: 4 mL

2.3 Preparation process

1. Insert clean glass tubes upside down into a stainless steel rack to facilitate the drainage of water from the tubes.

2. Place the rack in an electric thermostatic blast drying oven and sterilize at 100 °C for at least 1 hour.

3. Weigh brown sugar, cornmeal, agar, and yeast using an electronic balance; measure water and propionic acid using a measuring cylinder.

4. Mix the weighed brown sugar and agar in an electric rice cooker, then add an appropriate amount of deionized water.

5. Heat while stirring until the agar powder is completely dissolved and the brown sugar is free of lumps.

6. Mix the cornmeal with cold water to form a paste, then add the paste to the pot. Heat and stir until the mixture boils.

7. Turn off the heat and allow the mixture to cool to around 70 °C. Add the yeast paste and propionic acid, then stir thoroughly.

8. Dispense the medium into small tubes, plug with cotton, and let them dry in a cool place before use.

2.4 *Drosophila* Hybridization

The fruit flies required for the experiment are anesthetized using carbon dioxide (CO_2) and sorted on a microporous ventilation operating board. Selected parental virgin flies are reared in glass tubes containing food. Once a sufficient number of flies are obtained, hybridization is performed. Each cross is completed using 4-5 virgin females of a specific genotype and corresponding males. After hybridization, the flies are transferred to new tubes every 2-3 days based on egg-laying conditions. Approximately 10 days later, offspring flies emerge, and the corresponding progeny are selected based on genotype for phenotypic observation.

2.5 Observation and Recording of Adult *Drosophila* Phenotypes

The phenotypes of adult *Drosophila* are highly diverse, providing great convenience for in vivo functional studies. Using an optical microscope, we can observe different phenotypes of *Drosophila*, and capture images and record phenotypes using a connected CCD camera. Different phenotypes can be photographed after various treatments. For subtle phenotypic changes, we can increase the sample size and use software to perform statistical analysis on the captured images to obtain experimental results.

2.6 Photographing and Recording Eye Phenotypes of Adult *Drosophila*

1) Sample preparation: Collect a certain number of offspring flies in a 1.5 mL EP tube and place them in a -80 °C freezer for about 5 minutes for later use.

2) Material preparation: Prepare a 1% transparent agar plate and allow it to cool and solidify for later use.

3) Photographing and recording: Pour the frozen flies onto the agar plate and place it under the field of view of an upright microscope. Use tweezers to place a single fly in the center of the field of view, adjust the orientation, magnification, and fine-tune the microscope's coarse and fine focus knobs until the image is completely clear. Focus and take a photo, then save the image after renaming it according to the genotype.

4) Repeat sampling: Repeat the above steps until the number of images meets the experimental requirements.

5) Statistical analysis: Use Photoshop software to measure the size of the *Drosophila* compound eyes and use graphing software to analyze the differences in this phenotype among different genotypes.

2.7 Photographing and Recording Wing Phenotypes of Adult *Drosophila*

1) Sample preparation: Select the required offspring flies on a CO_2 operating board, and use tweezers to remove the wings of the flies and place them on a glass slide for later use.

2) Material preparation: Prepare a 1:1 mixture of alcohol and glycerol. To avoid bubble formation, the mixture can be centrifuged at low speed. Additionally, prepare a small amount of pure alcohol for later use.

3) Photographing and recording: Add a drop of alcohol to the glass slide with the wings to allow the wing tissue to spread flat on the slide. Then, add the alcohol-glycerol mixture and cover with a coverslip to prevent bubble formation, ensuring the wing tissue lies flat for easier imaging. Place the prepared slide under the microscope, adjust the magnification, and fine-tune the coarse and fine focus knobs until the image is completely clear. Focus and take a photo, then save the image after renaming it according to the genotype.

4) Repeat sampling: Repeat the above steps until the number of images meets the experimental requirements.

5) Statistical analysis: Count the wing vein loss phenotype in the wing tissue and use graphing software to analyze the differences in this phenotype among different genotypes.

2.8 *Drosophila* Strains

GMR-Gal4, *ptc*-Gal4, *Zdhhc8-RNAi*, and *UAS-GFP-RNAi* were obtained from the Bloomington Stock Center.

3 Results

3.1 Downregulation of *Zdhhc8* Leads to Loss of presence of apical constriction and vesicle in *Drosophila* Wing Disc

By driving *Zdhhc8*-RNAi expression with *ptc*-Gal4, we found that downregulation of *Zdhhc8* significantly reduced the presence of apical constriction and vesicle in the *Drosophila* wing disc (Figure 1). Compared to the wild-type and GFP-RNAi control groups, the *Zdhhc8*-RNAi group showed a significant decrease in ACV presence.

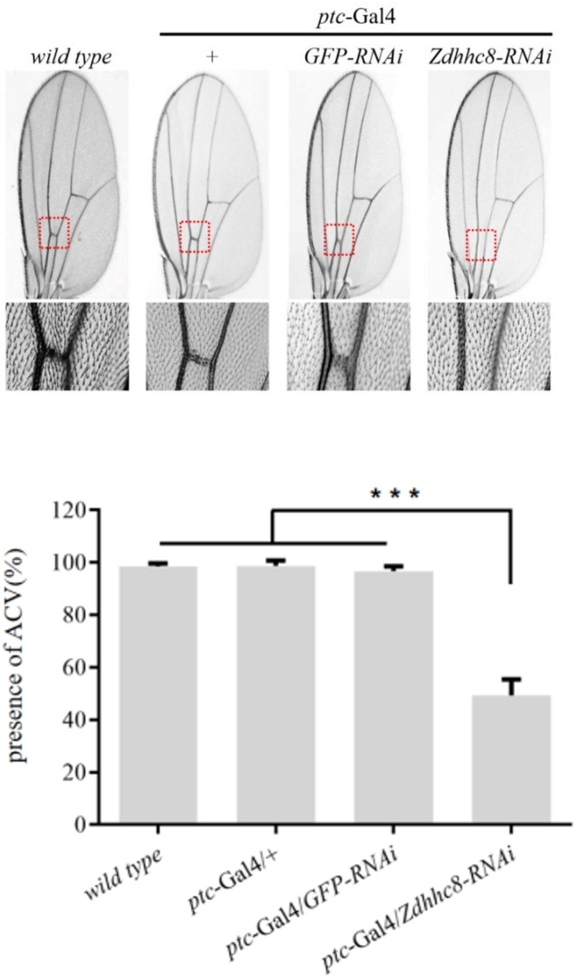

Figure 1. Down-regulation of *Zdhhc8* by RNAi leads to ACV lost in *Drosophila.*

3.2 Downregulation of *Zdhhc8* Induces Apoptosis in *Drosophila* Wing Disc

Further studies revealed that downregulation of *Zdhhc8* significantly increased the number of apoptotic cells in the *Drosophila* wing disc (Figure 2). Compared to the control groups, the *Zdhhc8*-RNAi group exhibited a significant increase in the number of apoptotic cells, indicating that *Zdhhc8* plays a crucial role in maintaining cell survival.

3.3 Downregulation of *Zdhhc8* Results in Smaller Eyes in *Drosophila*

By driving *Zdhhc8*-RNAi expression with GMR-Gal4, we found that downregulation of *Zdhhc8* significantly reduced the size of adult *Drosophila* eyes (Figure 3). Compared to the wild-type and GFP-RNAi control groups, the *Zdhhc8*-RNAi group showed a significant reduction in eye size.

3.4 Downregulation of *Zdhhc8* Induces Apoptosis in *Drosophila* Eye Disc

Finally, we observed that downregulation of *Zdhhc8* significantly increased the number of apoptotic cells in the *Drosophila* eye disc (Figure 4). Compared to the control groups, the *Zdhhc8*-RNAi group exhibited a significant increase in the number of apoptotic cells, further confirming the critical role of *Zdhhc8* in cell survival.

In summary, downregulation of *Zdhhc8* not only affected the development of the *Drosophila* wing and eye discs but also significantly induced apoptosis, indicating that *Zdhhc8* plays a crucial regulatory role in *Drosophila* development.

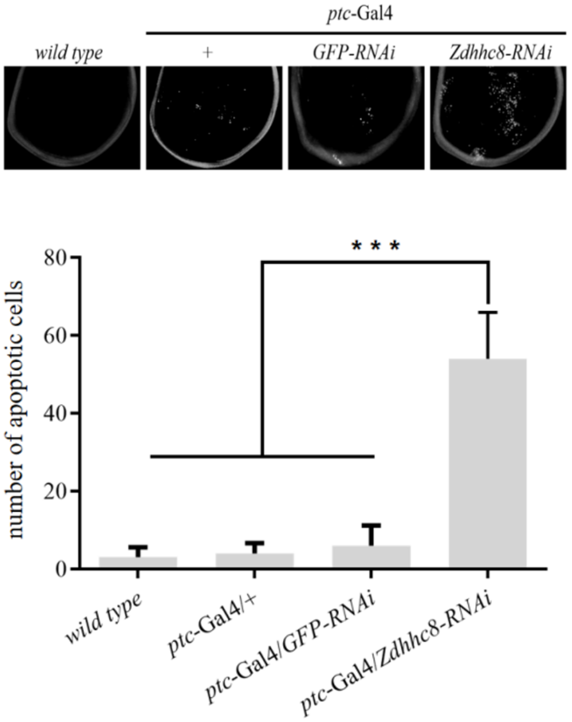

Figure 2. Downregulation of *Zdhhc8* induced apoptosis in *Drosophila* wing disc

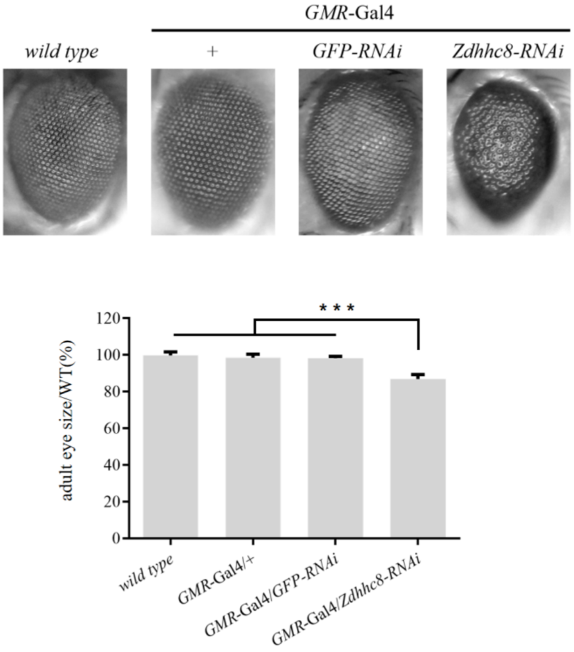

Figure 3. Down-regulation of *Zdhhc8* by RNAi leads to smaller eyes in *Drosophila.*

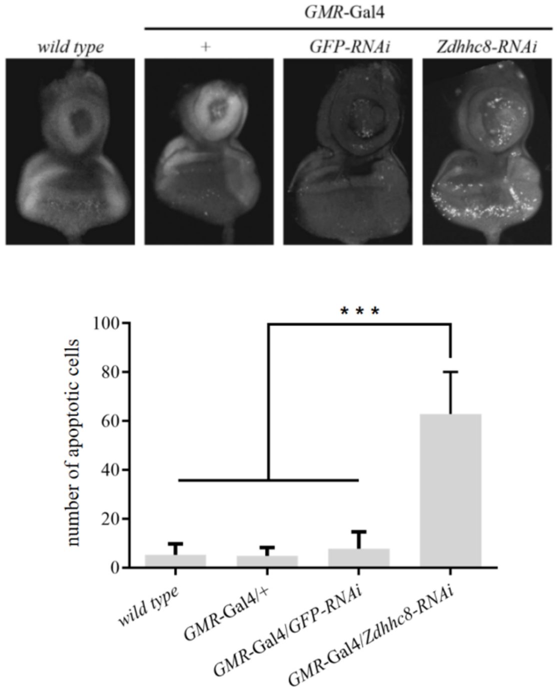

Figure 4. Downregulation of *Zdhhc8* induced apoptosis in *Drosophila* eye disc.

4 Conclusion

In conclusion, this study provides compelling evidence that *Zdhhc8* plays a vital role in *Drosophila* development. Downregulation of *Zdhhc8* through RNAi led to significant morphological and cellular defects in both wing and eye discs, including the loss of apical constriction, reduced vesicle formation, and increased apoptosis. These findings underscore the importance of *Zdhhc8* in maintaining cell survival and tissue integrity during development. The observed phenotypes suggest that *Zdhhc8* is involved in critical cellular processes, such as protein palmitoylation, which regulates protein function and localization. Future studies should explore the molecular mechanisms by which *Zdhhc8* influences these processes and investigate its potential implications in human developmental disorders and diseases. Overall, this work contributes to a deeper understanding of the role of *Zdhhc8* in development and highlights its potential as a target for further research in developmental biology and disease pathology.

References

1. Villanueva, C.E. and B. Hagenbuch, Palmitoylation of solute carriers. Biochem Pharmacol, 2023. 215: p. 115695.
2. Han, Z., et al., The post-translational modification, SUMOylation, and cancer (Review). Int J Oncol, 2018. 52(4): p. 1081-1094.
3. Jin, J., et al., Protein palmitoylation and its pathophysiological relevance. J Cell Physiol, 2021. 236(5): p. 3220-3233.
4. Tabata, K., et al., Palmitoylation of ULK1 by ZDHHC13 plays a crucial role in autophagy. Nat Commun, 2024. 15(1): p. 7194.
5. Jin, J., et al., Protein palmitoylation and its pathophysiological relevance. J Cell Physiol, 2021. 236(5): p. 3220-3233.
6. Hansen, A.L., et al., STING palmitoylation as a therapeutic target. Cell Mol Immunol, 2019. 16(3): p. 236-241.
7. Shi, C., et al., ZDHHC18 negatively regulates cGAS-mediated innate immunity through palmitoylation. EMBO J, 2022. 41(11): p. e109272.
8. Zhou, B., et al., Protein palmitoylation in cancer: molecular functions and therapeutic potential. Mol Oncol, 2023. 17(1): p. 3-26.
9. Albertini, D.F., Journal of Assisted Reproduction and Genetics. From the editor. J Assist Reprod Genet, 2010. 27(1): p. 1-2.
10. Liu, M., et al., New applications of the acridine orange fluorescence staining method: Screening for circulating tumor cells. Oncol Lett, 2017. 13(4): p. 2221-2229.
11. Cohen, S. and G. Jurgens, Drosophila headlines. Trends Genet, 1991. 7(8): p. 267-72.
12. Kravitz, E.A. and M.D.L.P. Fernandez, Aggression in Drosophila. Behav Neurosci, 2015. 129(5): p. 549-63.
13. Bastock, R. and D. St Johnston, Drosophila oogenesis. Curr Biol, 2008. 18(23): p. R1082-7.
14. Asjad, H.M.M., et al., Pharmacochaperoning in a Drosophila model system rescues human dopamine transporter variants associated with infantile/juvenile parkinsonism. J Biol Chem, 2017. 292(47): p. 19250-19265.
15. Trotter, M.B., et al., The Drosophila model system to study tau action. Methods Cell Biol, 2017. 141: p. 259-286.
16. Hales, K.G., et al., Genetics on the Fly: A Primer on the Drosophila Model System. Genetics, 2015. 201(3): p. 815-42.
17. Kogenaru, V., M. Isalan and M. Kogenaru, A drug stabilizable GAL80(ds) for conditional control of gene expression via GAL4-UAS and CRISPR-Cas9 systems in Drosophila. Sci Rep, 2024. 14(1): p. 5893.
18. Iacopino, S., F. Licausi and B. Giuntoli, Exploiting the Gal4/UAS System as Plant Orthogonal Molecular Toolbox to Control Reporter Expression in Arabidopsis Protoplasts. Methods Mol Biol, 2022. 2379: p. 99-111.
19. Busson, D. and A. Pret, GAL4/UAS targeted gene expression for studying Drosophila Hedgehog signaling. Methods Mol Biol, 2007. 397: p. 161-201.
20. Poulton, B.C., et al., Using the GAL4-UAS System for Functional Genetics in Anopheles gambiae. J Vis Exp, 2021(170).

21. Qian, Y., et al., A Single-Component Optogenetic Gal4-UAS System Allows Stringent Control of Gene Expression in Zebrafish and Drosophila. ACS Synth Biol, 2023. 12(3): p. 664-671.
22. Busson, D. and A. Pret, GAL4/UAS targeted gene expression for studying Drosophila Hedgehog signaling. Methods Mol Biol, 2007. 397: p. 161-201.
23. Elliott, D.A. and A.H. Brand, The GAL4 system: a versatile system for the expression of genes. Methods Mol Biol, 2008. 420: p. 79-95.
24. Grishok, A., RNAi mechanisms in Caenorhabditis elegans. FEBS Lett, 2005. 579(26): p. 5932-9.
25. Kedmi, R., et al., A modular platform for targeted RNAi therapeutics. Nat Nanotechnol, 2018. 13(3): p. 214-219.
26. Fletcher, S.J., et al., A Perspective on RNAi-Based Biopesticides. Front Plant Sci, 2020. 11: p. 51.
27. Fitzgerald, K., et al., A Highly Durable RNAi Therapeutic Inhibitor of PCSK9. N Engl J Med, 2017. 376(1): p. 41-51.
28. Lundstrom, K., Are Viral Vectors Any Good for RNAi Antiviral Therapy? Viruses, 2020. 12(10).
29. Chen, Y. and K. De Schutter, Biosafety aspects of RNAi-based pests control. Pest Manag Sci, 2024. 80(8): p. 3697-3706.
30. DeMell, A. and S.P. Dinesh-Kumar, Ca(2+) kickstarts antiviral RNAi. Cell Host Microbe, 2021. 29(9): p. 1339-1341.
31. Wierzbicki, F., et al., On the origin of the P-element invasion in Drosophila simulans. Mob DNA, 2025. 16(1): p. 7.
32. Paulouskaya, O., et al., Levels of P-element-induced hybrid dysgenesis in Drosophila simulans are uncorrelated with levels of P-element piRNAs. G3 (Bethesda), 2023. 13(2).
33. Sohn, E.J. and S. Oh, P-Element-Induced Wimpy Testis Proteins and P-Element-Induced Wimpy Testis-Interacting RNAs Expression in Ovarian Cancer Stem Cells. Genet Test Mol Biomarkers, 2023. 27(2): p. 56-64.
34. Donas, K.P., The myth of Daedalus and Icarus: fly between the extremes - pushing the limits of endovascular therapy in complex aortic aneurysms carries risks. J Cardiovasc Surg (Torino), 2024. 65(2): p. 83-84.