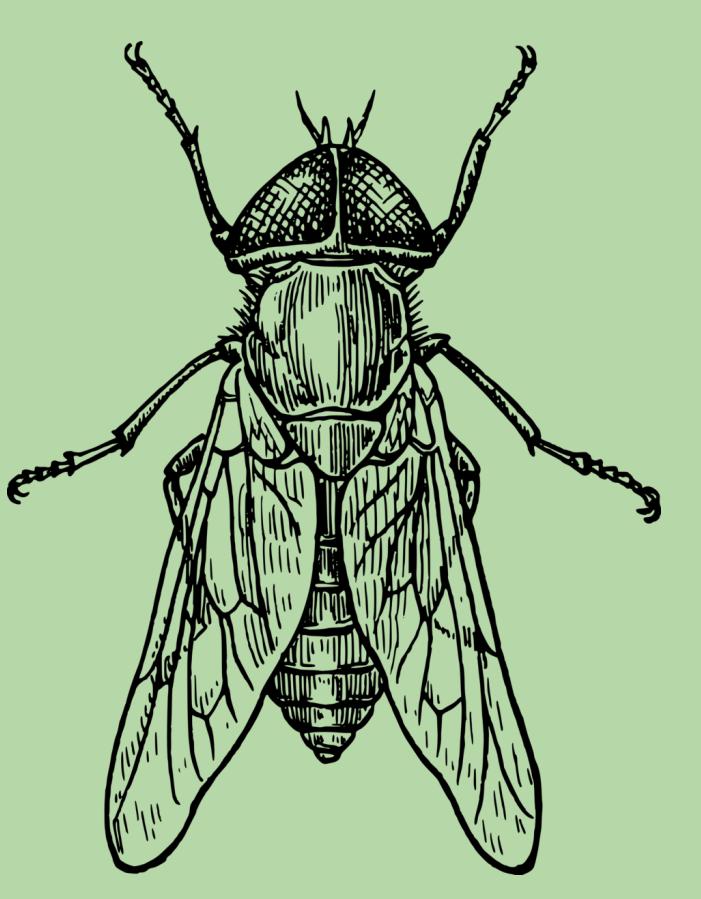




# Characterizing Alzheimer's Disease in a *Drosophila* Model Carrying Synthetic Mutations of Amyloid Beta (A $\beta$ )<sub>42</sub>



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## Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder afflicting over 55 million people globally. A hallmark of AD is the accumulation of extracellular amyloid beta (A $\beta$ ) plaques. Specifically, the A $\beta$ <sub>42</sub> peptide is known to be significantly neurotoxic. AD is characterized by disruption of neuronal communication and eventual neurodegeneration, driving disease progression. Research in our lab using *Drosophila* models expressing wild-type (WT) A $\beta$ <sub>42</sub> pan-neuronally demonstrated shortened life spans, behavioral deficits, reduced locomotor capabilities, and changes in gene expression, mirroring aspects of AD pathology. Dominant inheritance of specific A $\beta$ <sub>42</sub> amino acid substitutions in early-onset AD suggests that single-point mutations can affect A $\beta$ <sub>42</sub> aggregation and neurotoxicity.

Work in Dr. Jevgenij Raskatov's lab identified two A $\beta$ <sub>42</sub> side chain modifications that each reduces its aggregation kinetics and cytotoxicity *in vitro*. We incorporated these modifications in A $\beta$ <sub>42</sub> as missense mutations in transgenic *Drosophila*, allowing for *in vivo* characterization of the effects of these single amino acid changes on A $\beta$ <sub>42</sub>'s deleterious effects. The GAL4-UAS system is used to conduct assays to compare the phenotypes of flies expressing WT A $\beta$ <sub>42</sub> and flies expressing these mutant forms of A $\beta$ <sub>42</sub>.

To visualize morphological differences in neuronal development, we use Scanning Electron Microscopy (SEM) to examine eye morphology in mutant flies and quantify the results by comparing them to flies expressing WT A $\beta$ <sub>42</sub> and non-expressing controls. Preliminary results indicate that 2 $\Delta$  (Mutant A and B) expressing flies have fewer morphological abnormalities than Mutant A or Mutant B, which had fewer than WT A $\beta$ <sub>42</sub> expressing flies. PCR confirmed the presence of our mutant inserts. RT-PCR will confirm mutation expression. A longevity assay assessed the survivorship of flies expressing the mutant A $\beta$ <sub>42</sub> pan-neuronally wherein Mutant B brought a near complete rescue effect in lifespan when compared to non-expressing controls.

This research aims to advance the understanding of AD by identifying how specific A $\beta$ <sub>42</sub> side chain modifications affect aggregation and toxicity, potentially increasing our understanding of the mechanism(s) by which A $\beta$ <sub>42</sub> negatively affects neuron function and inform future therapeutic strategies.

## A $\beta$ <sub>42</sub>- Mutant A Shows Reduced Neurotoxicity *In Vitro* Compared to WT A $\beta$ <sub>42</sub>

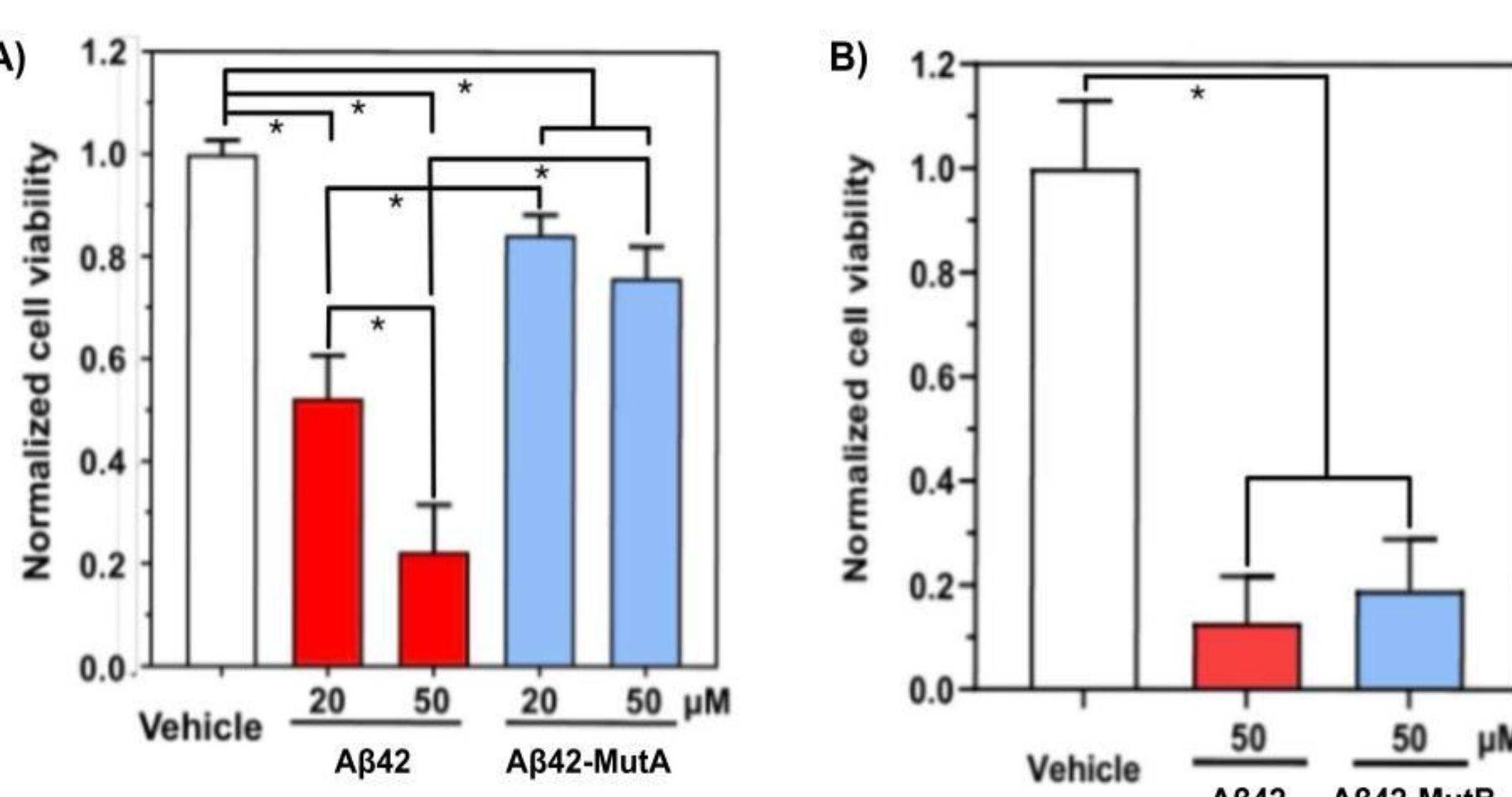


Figure 1. A) Neurotoxicity of A $\beta$ <sub>42</sub> Mutant A against SH-SY5Y neuroblastoma cells at 20 & 50  $\mu$ M, p < 0.05. B) Neurotoxicity of A $\beta$ <sub>42</sub> and Mutant B against SH-SY5Y neuroblastoma cells at 50  $\mu$ M, p < 0.05. Data from Raskatov Lab.

## PCR Confirmation

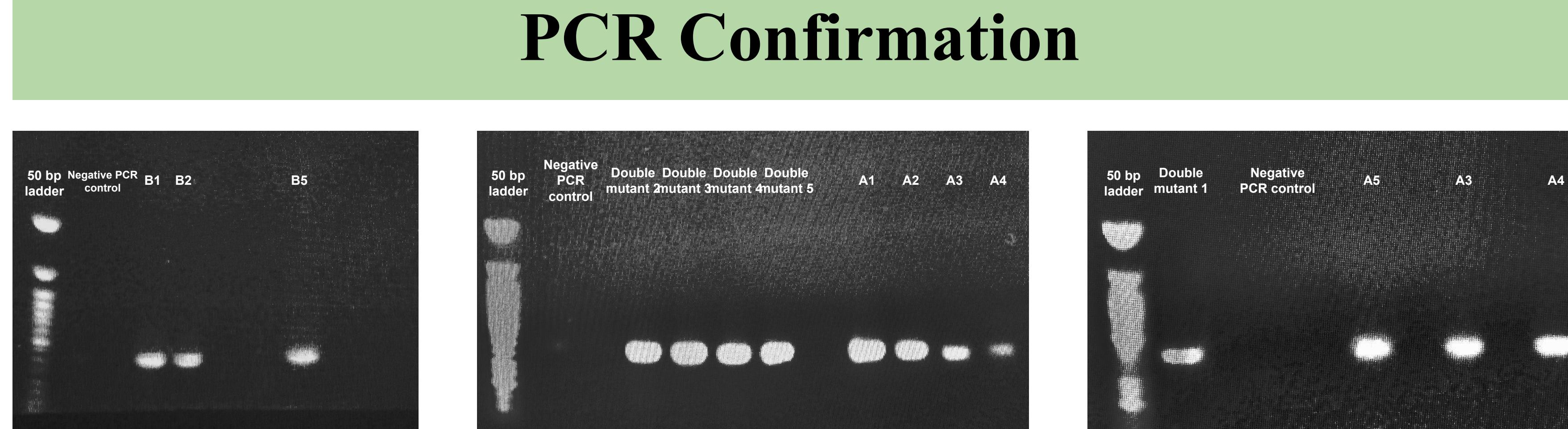


Figure 2. PCR results for 5 lines of each UAS transgenic fly (15 lines total) confirming the insertions of the transgenes. A1-5 correspond to 5 different *Drosophila* lines of Mutant A. B1-5 correspond to 5 different *Drosophila* lines of Mutant B.

## *Drosophila* Expressing Mutated A $\beta$ <sub>42</sub> Live Longer Than Those Expressing WT A $\beta$ <sub>42</sub>

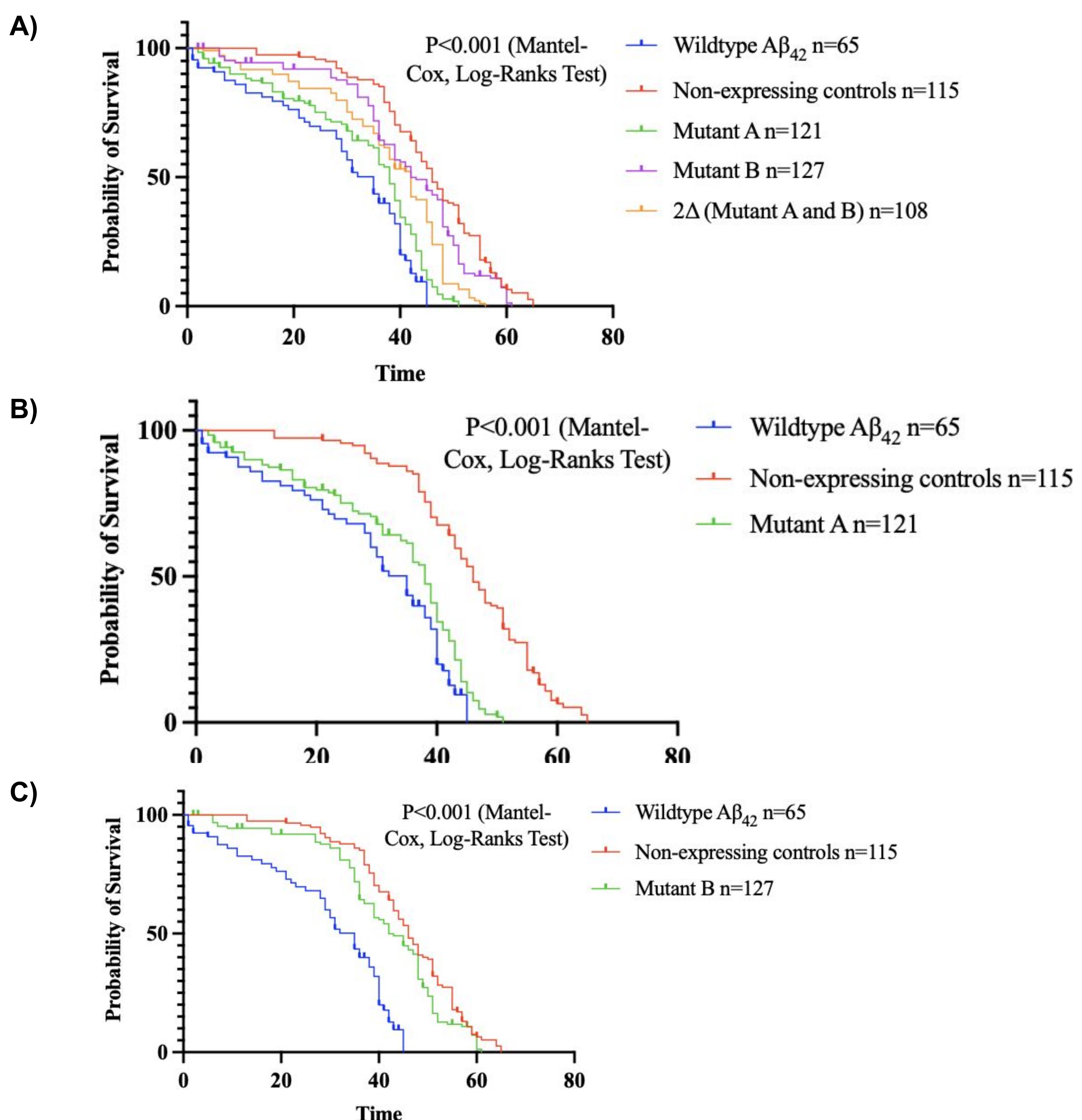


Figure 3. The APPL-GAL4 driver was utilized to achieve pan-neuronal expression. A) Comparison of all genotypes. B) Comparison of Mutant A, controls, and WT A $\beta$ <sub>42</sub>. C) Comparison of Mutant B, controls, and WT A $\beta$ <sub>42</sub>. Results from Mutant B demonstrate a stronger rescue effect compared to that of Mutant A, although neither are a complete rescue.

## Scanning Electron Microscopy

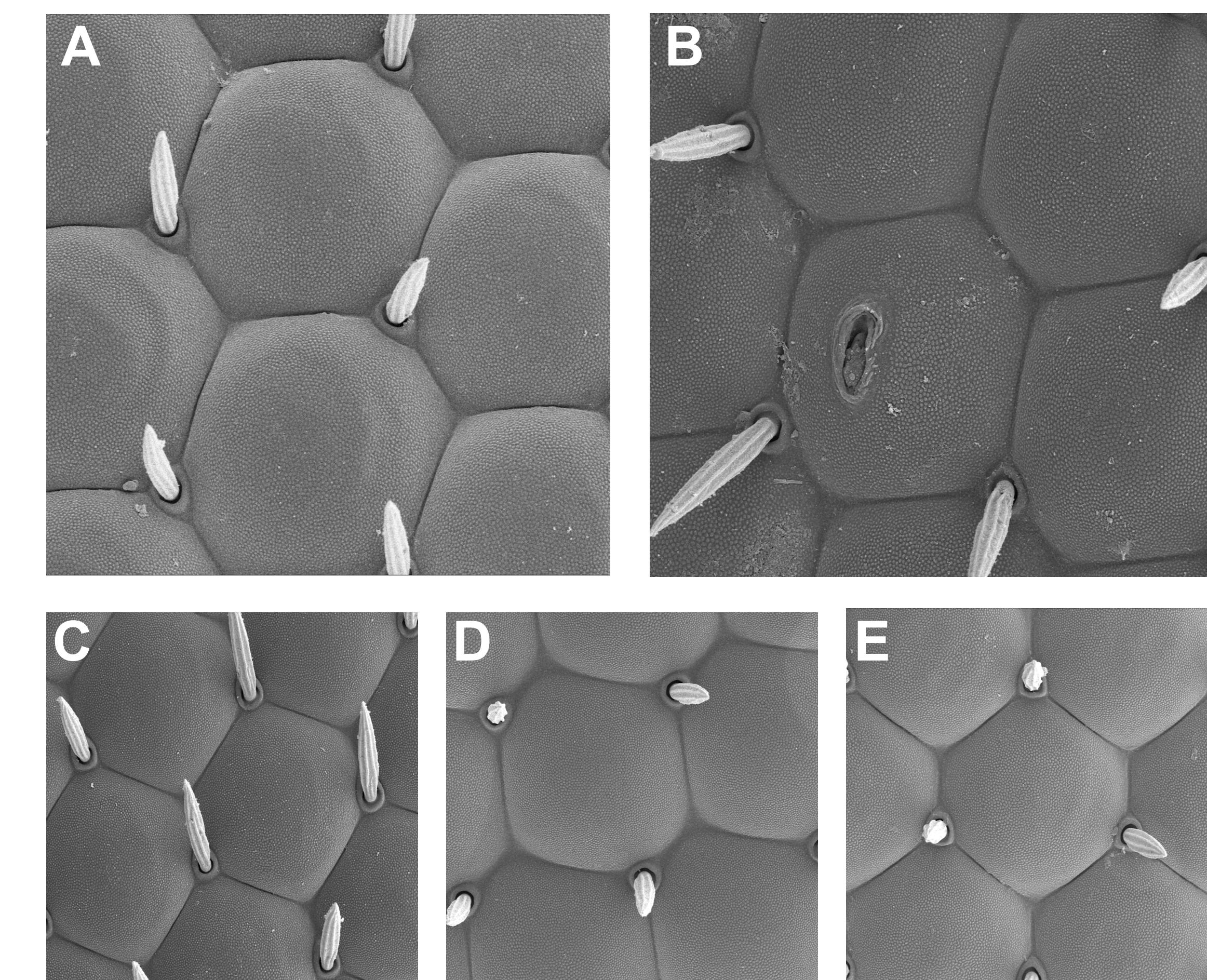
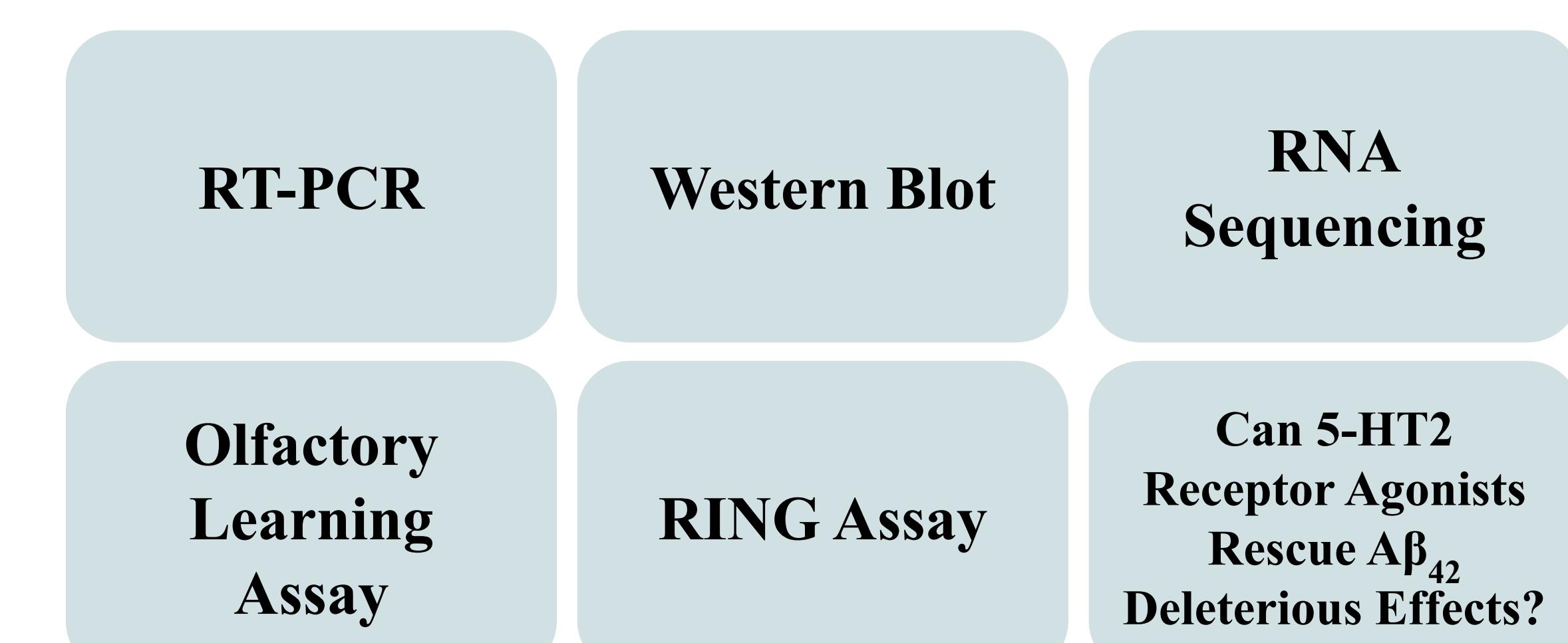


Figure 4. Scanning electron microscope (SEM) imaging was performed at 4000x to visualize degeneration of the fly compound eyes 3 days after eclosion. The GMR-GAL4 system was utilized to achieve expression in neurons of the eye. A) +/+ Control B) A $\beta$ <sub>42</sub>/+ C) Mutant A/+ D) Mutant B/+ E) 2 $\Delta$ /+.

## Future Directions



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