

Musculoskeletal Effects of Tirasemtiv and *Urtica dioica* on *Dstac* Gene Knockdown in *Drosophila melanogaster*: Applications Towards STAC3 Disorder (Native American Myopathy)

The *stac3* gene mutation, known as STAC3 disorder, is an autosomally inherited mutation first identified within the Lumbee Tribe of North Carolina. The disease affects 1 in 5000 within the Lumbee Tribe, causing congenital myopathy, musculoskeletal anomalies, and symptoms of hypotonia.

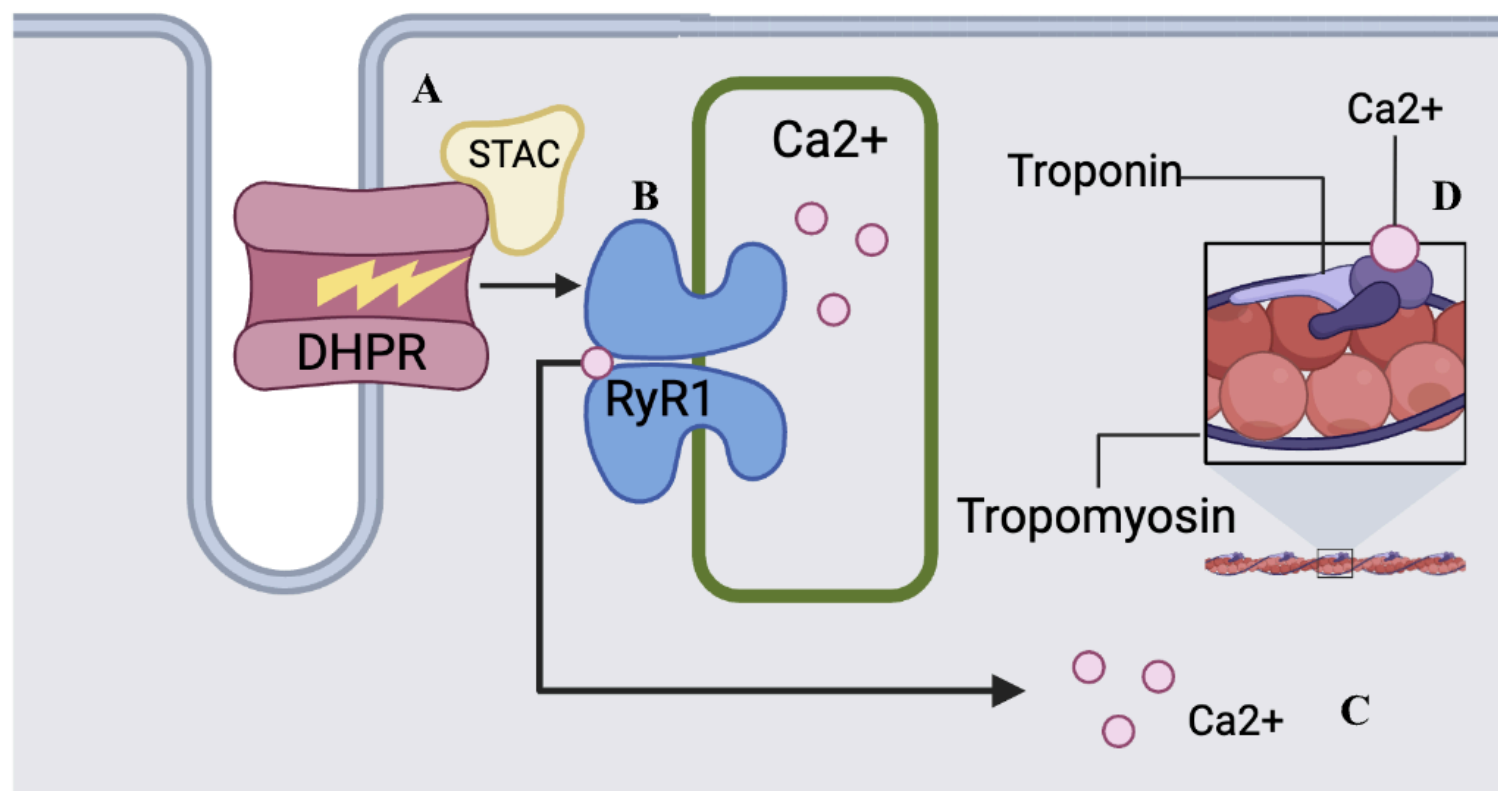


Figure 1. Excitation-Contraction Coupling Pathway. A) Stac protein activates DHPR. B) DHPR activates RyR1 to release calcium ions. C) Calcium ions enter cytosol. D) Calcium binds to troponin. (Figure made by author in BioRender)

Excitation Contraction Coupling Pathway:

- Proteins produced by the *stac3* gene function in the sarcoplasmic reticulum, initiating the activation DHPR and RyR1 receptors
- RyR1 regulates of Calcium ions within muscle cells
- Calcium ions bind to troponin proteins to remove tropomyosin from actin fibers and facilitate myosin-actin interactions.
- STAC3 Disorder prevents the activation of this pathway through a mutated *stac3* gene.

Treatments

- *Urtica dioica* (UD), an herb traditionally used by the Lumbee Tribe, is high in calcium.
- Tirasemtiv (TRV) is a Fast Skeletal Muscle Troponin Activator that increases troponin sensitivity.

Modeling STAC3 Ortholog in *D. melanogaster*

- Male Gal4/Gal4 flies were crossed with *Dstac-RNAi* virgins to yield larvae with *stac* gene knockdown (Mef2:GAL4 > UAS:*DstacRNAi*)
- Male Gal4/Gal4 were crossed with *RNAi-Luciferase* virgins larvae with Luciferase knockdown (Mef2:GAL4 > UAS:*LuciferaseRNAi*).
- Shown in Figure 2.

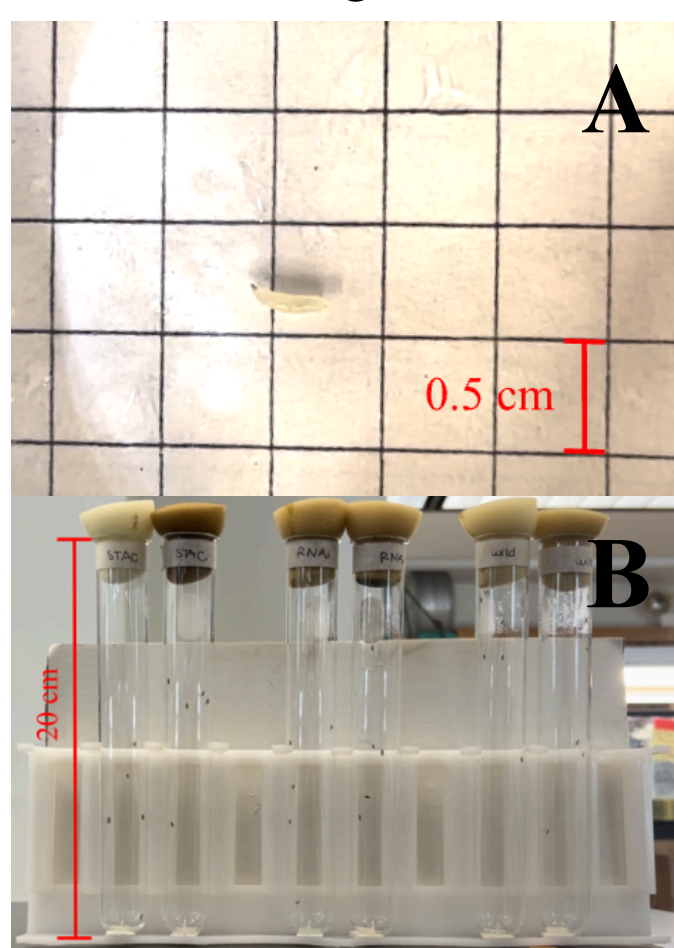


Figure 3. A) *Drosophila* Larvae Motility Assay B) *Drosophila* Adult RING Assay. (Photographs taken by author, 2025)

Larval Motility Assay

- Larvae exposed to 500 μ L of control (H₂O), Tirasemtiv, *Urtica dioica*, or combined treatment for 30 minutes.
- Larvae placed onto glass petri dish back with grid paper and recorded for a 10 second periods displaying constant motion in the same direction (shown in Figure 3).

Rapid Iterative Negative Geotaxis Assay

- Adult flies were exposed to control (H₂O), Tirasemtiv, *Urtica dioica*, or combined treatment through food source for 24 hours.
- Vials were tapped on table until all flies fell to the bottom and heights climbed in an 8 second period were recorded.

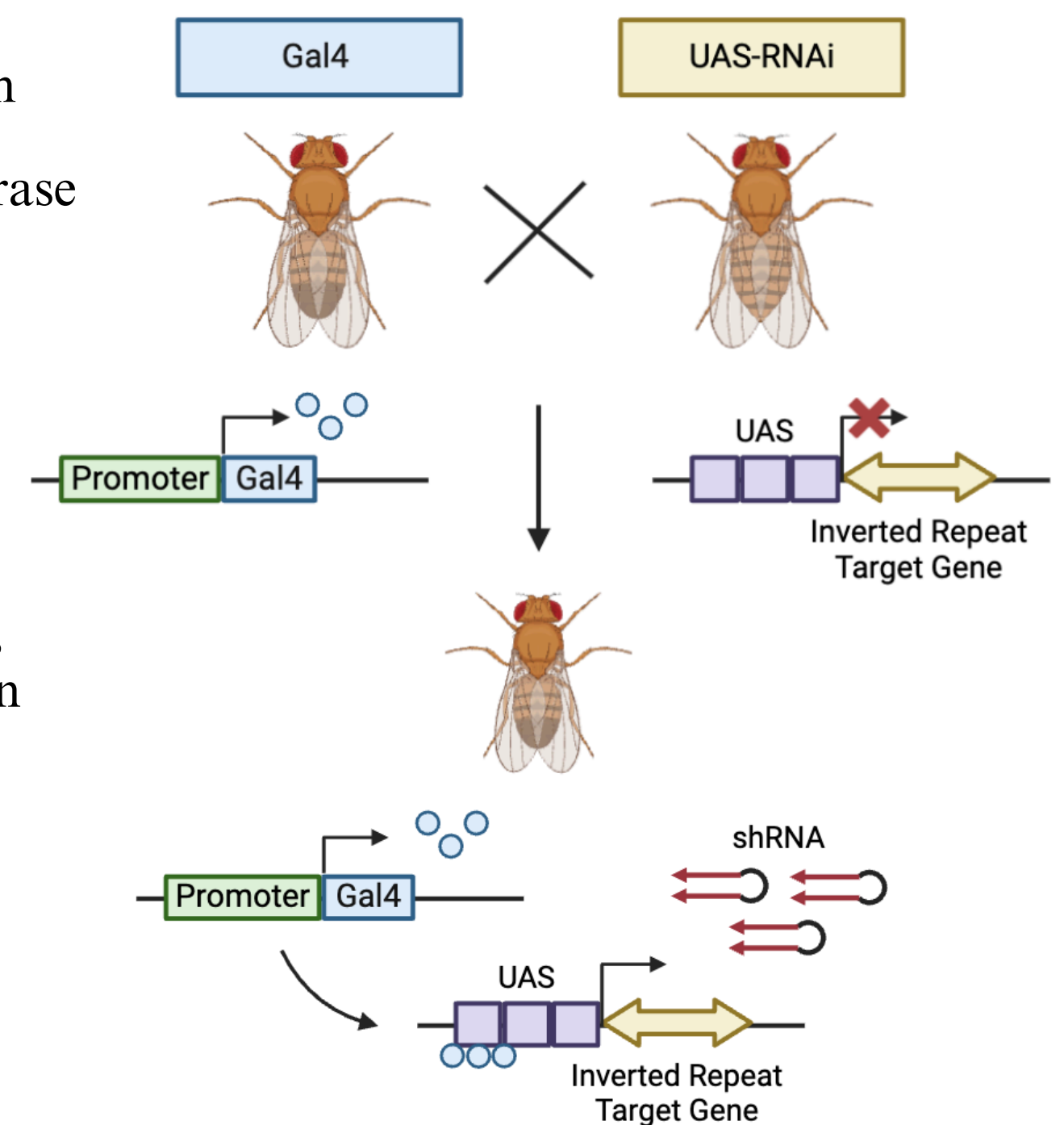


Figure 2. *Drosophila* UAS-*RNAi* cross system. (Figure made by author in BioRender)

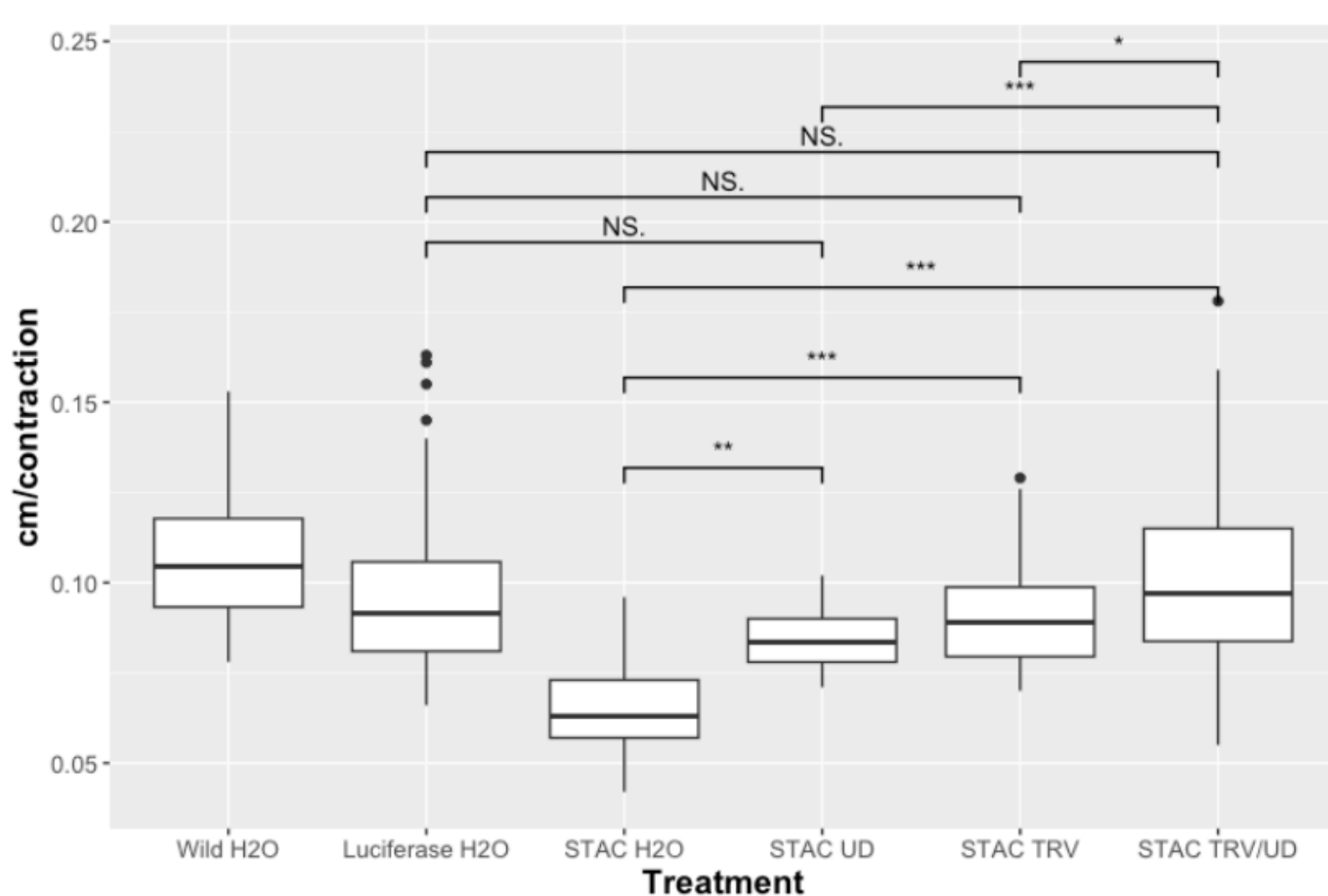


Figure 4. Comparison of distance traveled per contraction by *Dstac-RNAi* strains post-individual and combined treatment and preliminary control strains. (Kruskal-Wallis, Dunn Test, $df = 5$, $n=50$ each group)

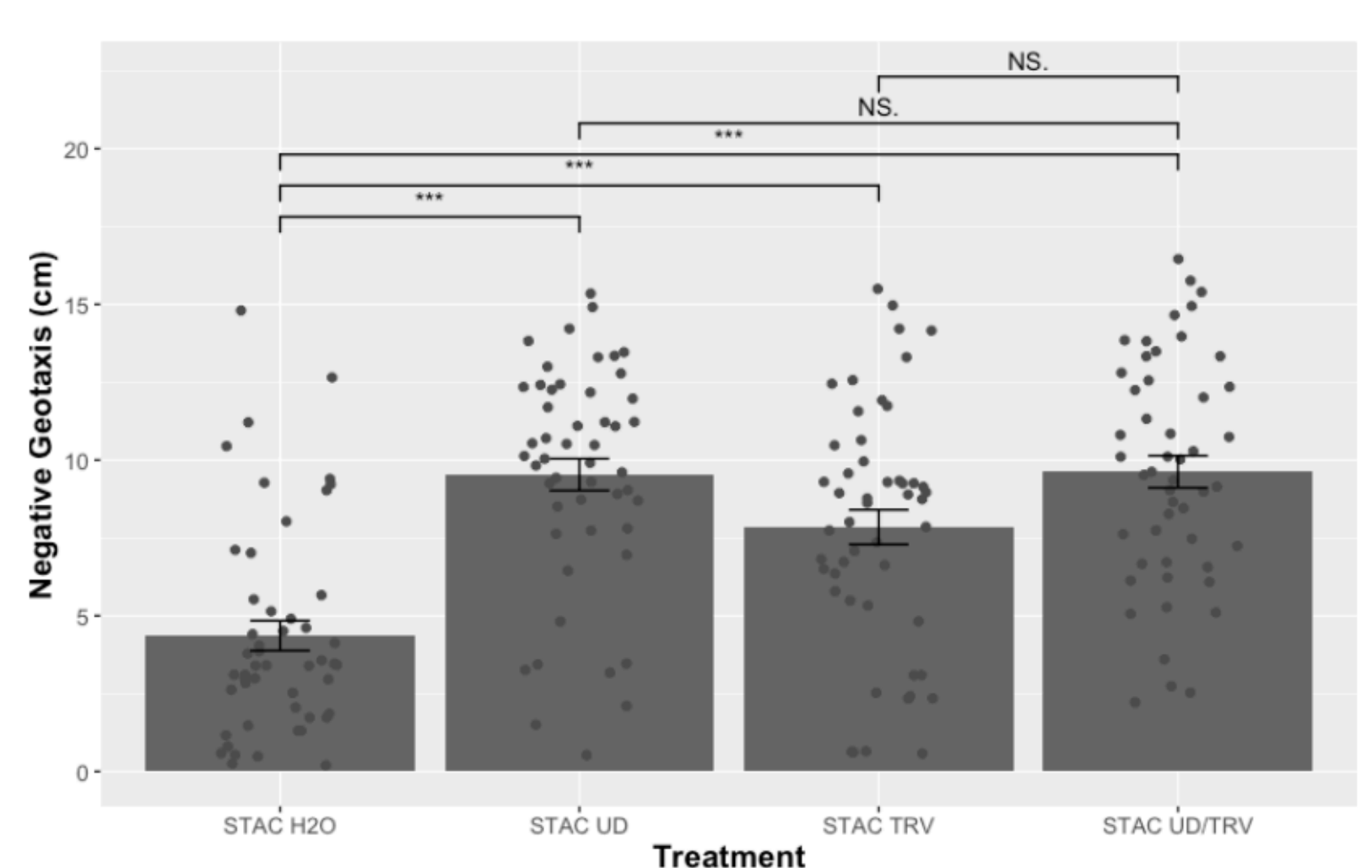


Figure 5. Comparison of *Dstac-RNAi* with treatments and without treatment. (Kruskal-Wallis, Dunn Test, $df = 3$, $n=50$ each group)

Conclusions

- *Dstac-RNAi* larvae has similar rate of contraction to wild-type and positive control *RNAi-Luciferase*.
- *Urtica dioica* extract displayed highest increase in distance and contraction rate of *Dstac-RNAi* larvae, but least increase in distance per contraction.
- Combined Tirasemtiv and *Urtica dioica* extract has the highest increase of locomotion per contraction in *Dstac-RNAi* larvae.
- *Urtica dioica*, Tirasemtiv, and combined treatment all increase adult motility.
- *Urtica dioica* and combined treatment have greatest increase on adult motility.

As the largest tribe east of the Mississippi River and the ninth largest tribe in the United States with continual growth, the treatments in this study have the potential to improve the quality of life for individuals within the Lumbee tribe affected by STAC3, while also bringing awareness to population-specific diseases.

References

- Boughman, Arvis Locklear, and Loretta O. Oxendine. *Herbal Remedies of the Lumbee Indians*. McFarland, 2004.
- Hsu, I.-U., et. al. (2020). *Dstac* Regulates Excitation-Contraction Coupling in *Drosophila* Body Wall Muscles. *Frontiers in Physiology*, 11, 573723. <https://doi.org/10.3389/fphys.2020.573723>
- Rufenach, B., & Petegem, F. V. (2021). Structure and function of STAC proteins: Calcium channel modulators and critical components of muscle excitation-contraction coupling. *The Journal of Biological Chemistry*, 297(1). <https://doi.org/10.1016/j.jbc.2021.100874>