

## Background

- Ubiquitination regulates cell growth and proliferation by targeting molecules for degradation through the ubiquitin-proteasome system (UPS). HECT E3 ligases facilitate this process, mediating the action of inflammatory responses (Park, Cho and Song, 2020).

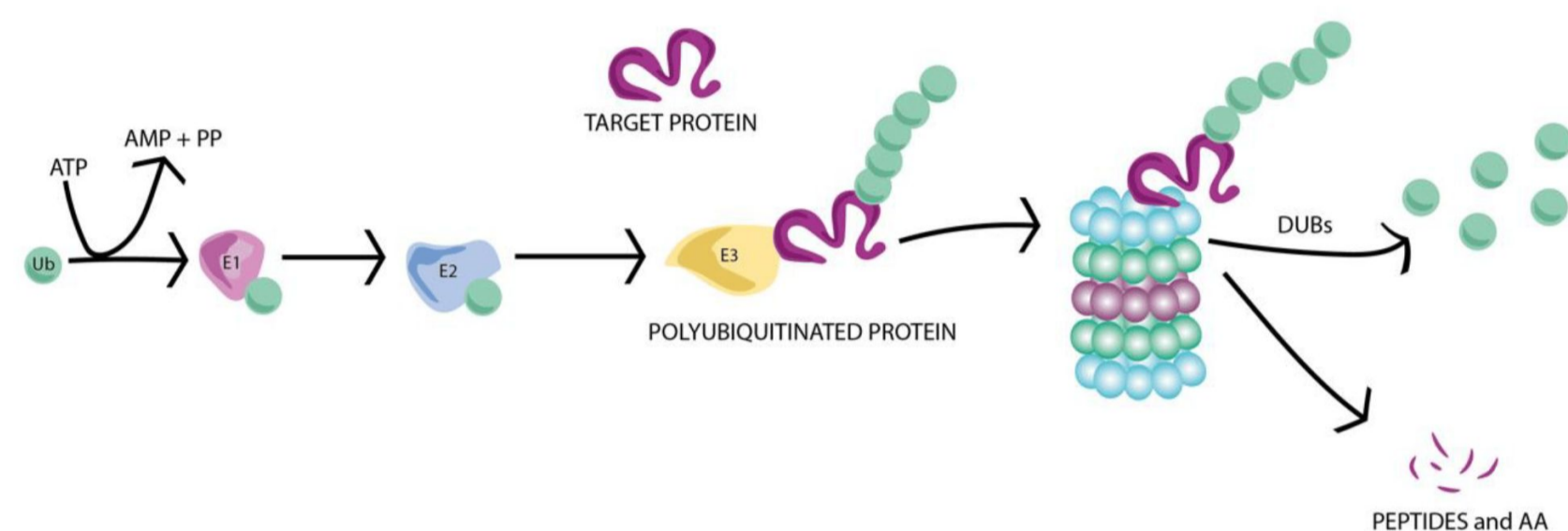


Figure 1: Process of Ubiquitination by E3 ligases, adapted from (Bachiller et al., 2020)

- Primary work in Ismail Lab suggests chronic inflammatory syndromes such as osteoarthritis are linked to dysfunction in the UPS pathway. Particularly, HECT E3 ligases mediate the NF- $\kappa$ B pathway. To investigate this we targeted the inflammatory response at an acute injury site.

## Zebrafish inflammation model

*Danio rerio* (zebrafish) is a useful model in measuring chronic inflammation, as neutrophils can be observed *in vivo* through the use of transgenic fish with GFP-tagged neutrophils.

- Acute inflammation in a zebrafish can be induced by wounding the tail fin of zebrafish embryos. Amputating the tail fin is done before 5df stage.
- After amputation, an inflammatory response is observed at site of injury with GFP tagged neutrophils which can be observed through a microscope.

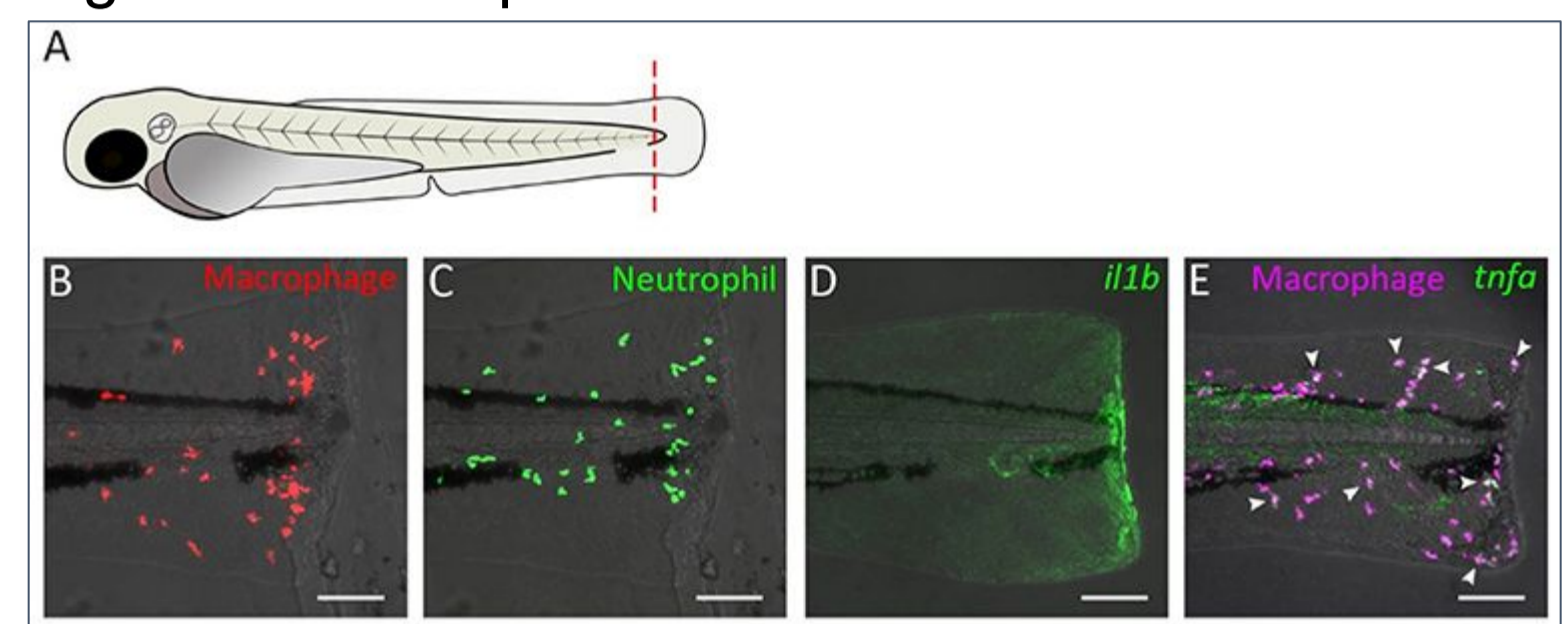
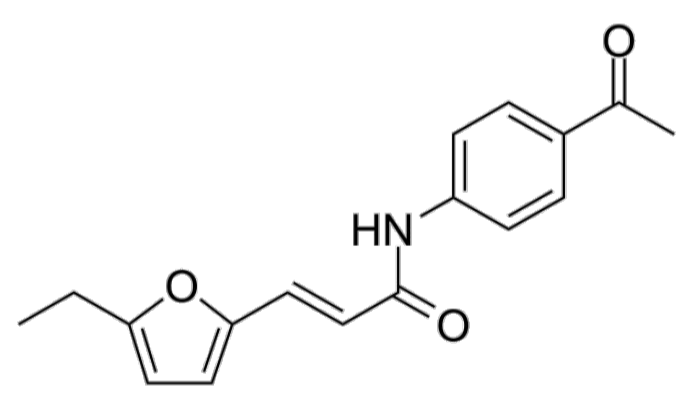


Figure 2: Zebrafish Inflammation model taken from (Xie, Meijer and Schaaf, 2021), outlining recruitment of different inflammatory responses. Note the neutrophils at site of injury in Image C.

## E3 ligases inhibition by Heclin slows Neutrophils migration to injury site

### Heclin

Heclin is a HECT E3 ligase inhibitor selective for Nedd4, Smurf2 and WWP1. This research aimed to investigate the inhibitory action of Heclin on neutrophil recruitment.



Molecular structure of Heclin

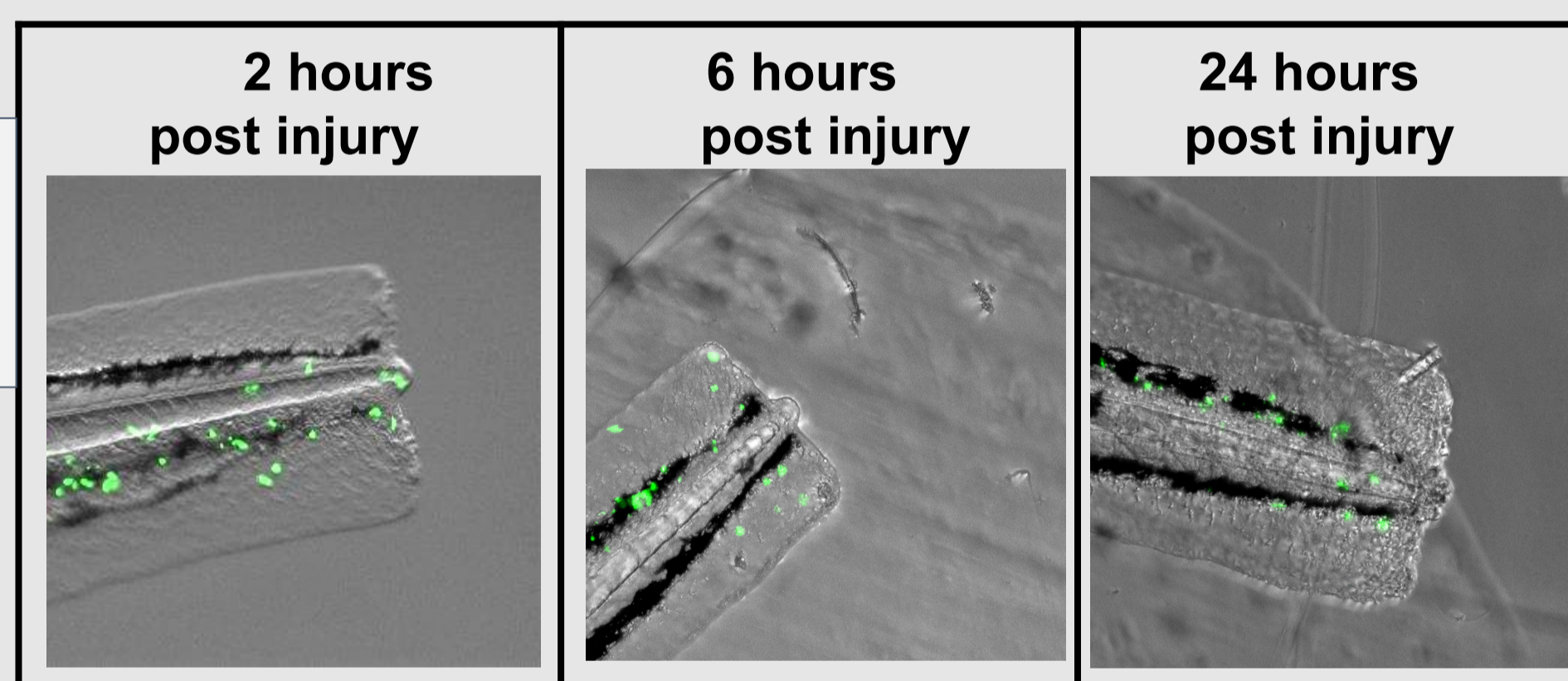


Figure 3: These images show the tail fin injury site at 2 hours, 6 hours and 24 hours post injury. A clean scalpel was used to make the incision. The zebrafish used were at stage 2dpf. GFP tagged neutrophils can be observed at site of injury.

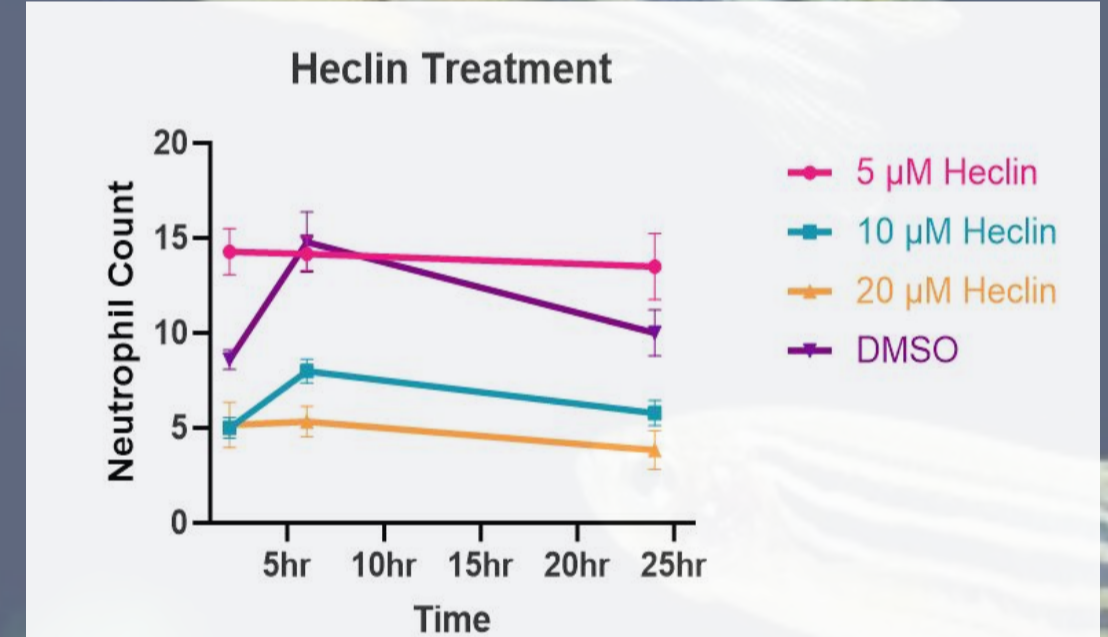


Figure 4: Heclin is a known HECT E3 ligase inhibitor. Neutrophil response graphs for differing treatments of Heclin concentrations.

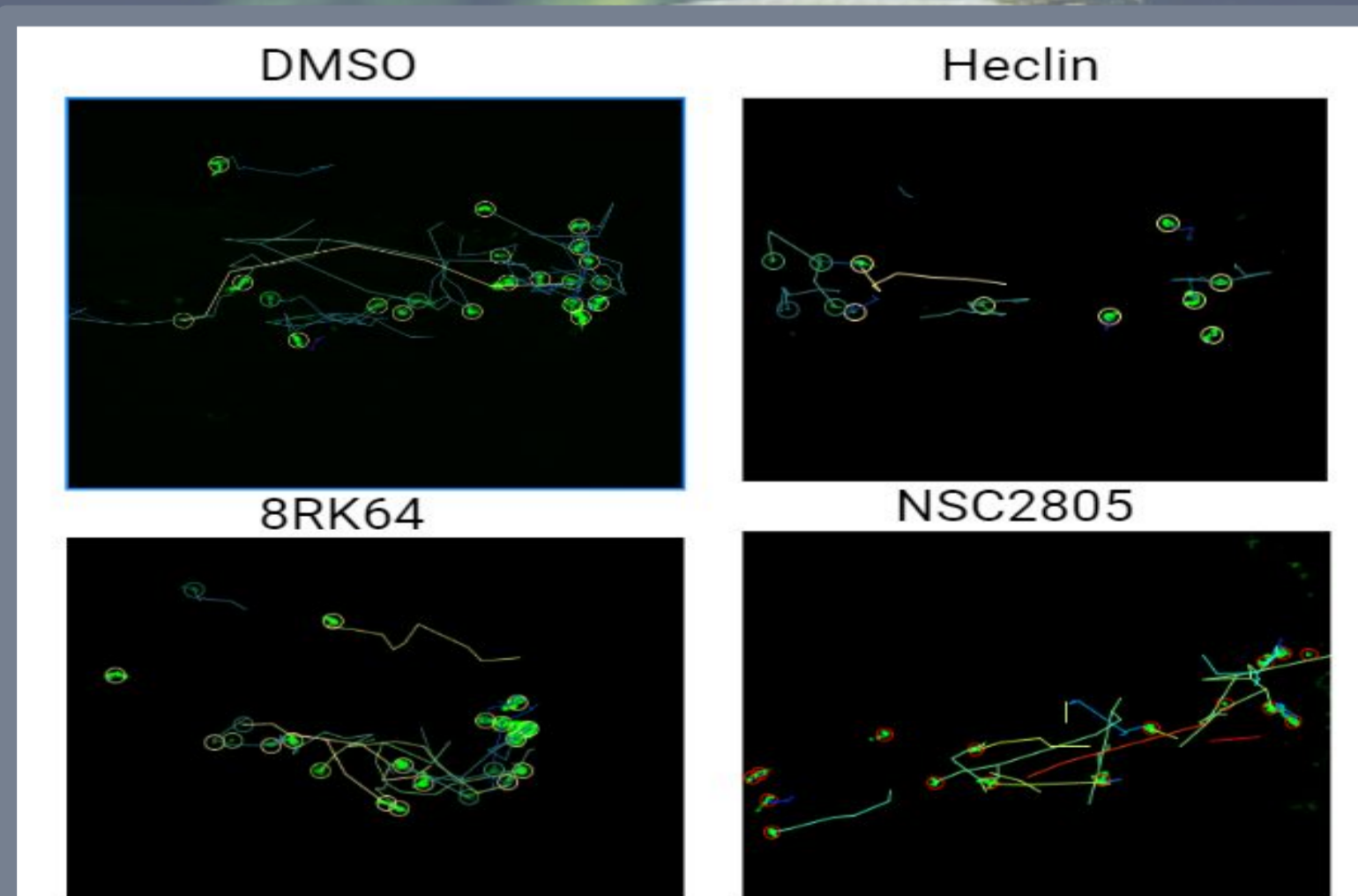


Figure 5: 5dpf zebrafish were treated with differing inhibitors. GFP tagged neutrophils were recorded with a confocal microscope. An overlay was produced using ImageJ and tracked using a Kalman tracker.

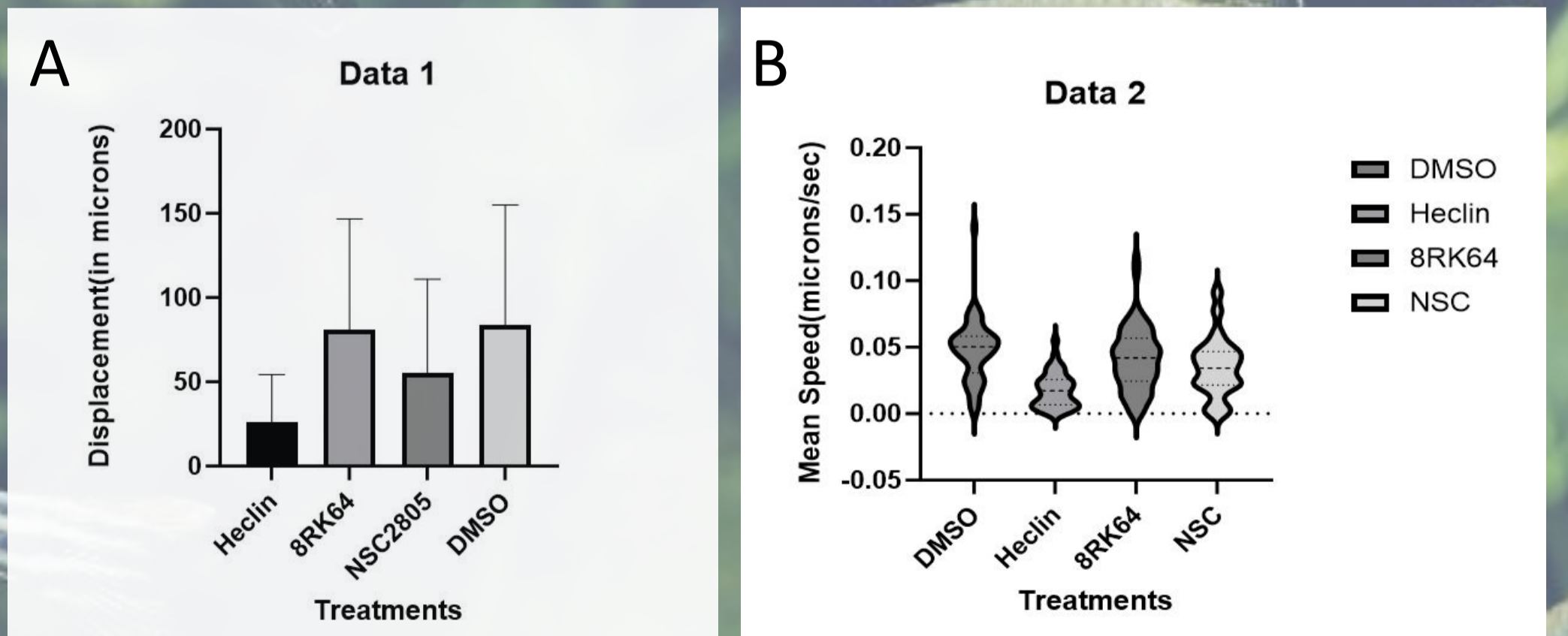


Figure 6: Displacement and speed data obtained from neutrophil tracking in Fiji. using a kalman tracker, neutrophil speed was tracked. A) mean displacement and B) mean speed over 1 hour.

## Conclusions

- Targeting HECT E3 ligases using Heclin slowed inflammatory response mediators, i.e, neutrophils. This is clearly evident at higher concentrations of compound at 10 $\mu$ M and higher.
- Most surprisingly, E3 inhibitors not only slowed neutrophils but also reduced mean displacement.
- Therefore, targeting HECT E3 ligases can be an explorable target for therapeutic drugs to treat chronic inflammation.

## References

- Bachiller, S., Alonso-Bellido, I.M., Real, L.M., Pérez-Villegas, E.M., Venero, J.L., Deierborg, T., Armengol, J.Á. and Ruiz, R. (2020). The Ubiquitin Proteasome System in Neuromuscular Disorders: Moving Beyond Movement. *International Journal of Molecular Sciences*, [online] 21(17), p.6429. doi:<https://doi.org/10.3390/ijms21176429>.
- Park, J., Cho, J. and Song, E.J. (2020). Ubiquitin–proteasome system (UPS) as a target for anticancer treatment. *Archives of Pharmacol Research*, 43(11), pp.1144–1161. doi:<https://doi.org/10.1007/s12272-020-01281-8>.
- Xie, Y., Meijer, A.H. and Schaaf, M.J.M. (2021). Modeling Inflammation in Zebrafish for the Development of Anti-inflammatory Drugs. *Frontiers in Cell and Developmental Biology*, 8. doi:<https://doi.org/10.3389/fcell.2020.620984>