

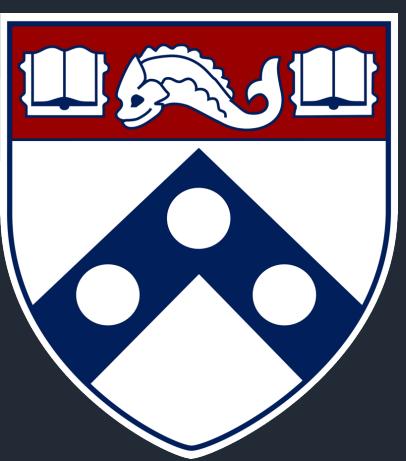
Investigating the ESCRT-III complex as an executor of Piezo's inhibition of axon regeneration in *Drosophila melanogaster* larva and human neuromuscular junction organoids

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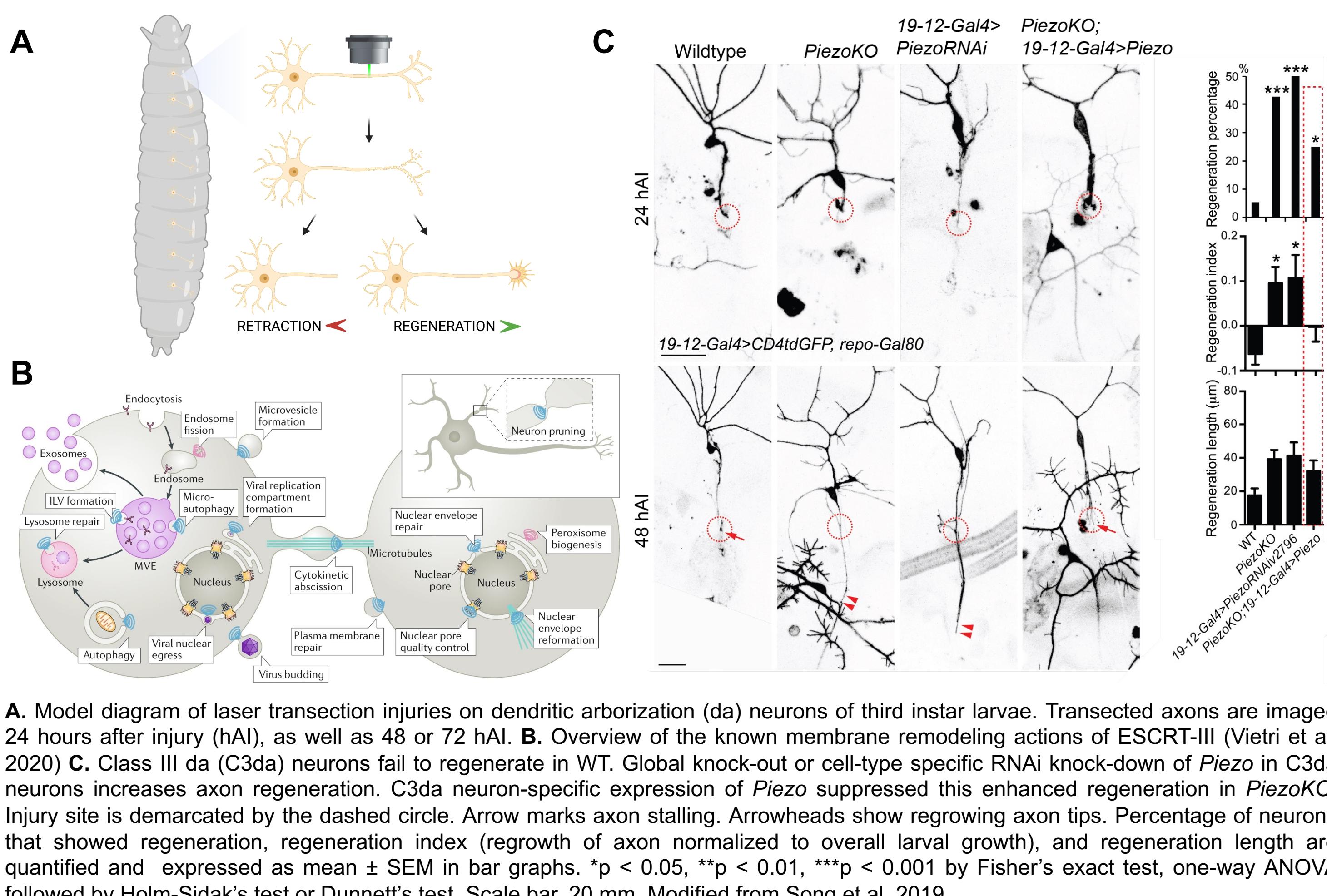
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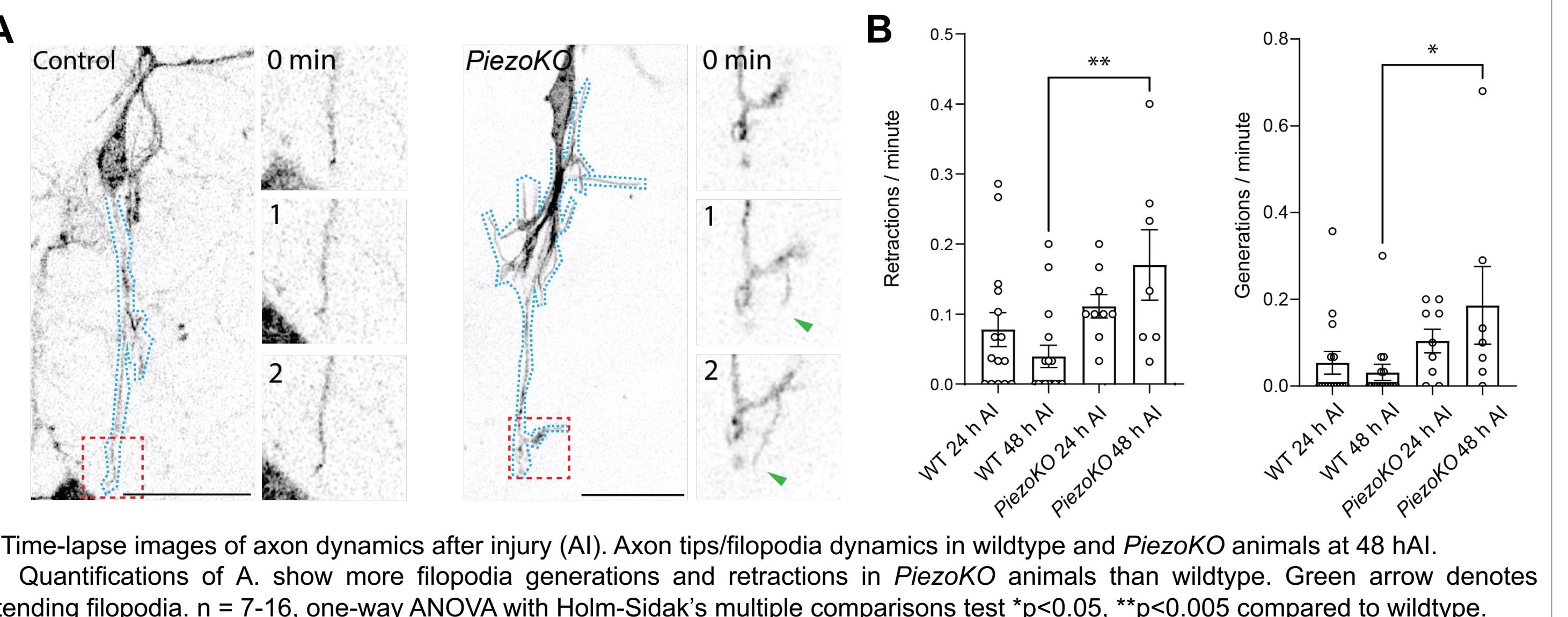
Summary

The failure of damaged axons to functionally regenerate can cause the debilitating pain and loss of important sensory and motor functions seen in patients with peripheral and central nervous system injuries. Due to the dearth of effective treatments for neural injury, there is an urgent need to research the mechanisms that control axon regeneration. Dynamic remodeling of the cytoskeleton into a specialized motile structure called a growth cone is necessary for axon regeneration. The Song lab has shown an evolutionarily conserved role of the mechanosensitive channel Piezo in the inhibition of axon regeneration (Song et al. 2019). However, how Piezo impacts growth cone and cytoskeletal dynamics locally remains a gap in knowledge. A published study, Carrillo-Garcia et al. 2021, established the ability for Piezo to recruit the ESCRT-III complex, a well-known membrane and cytoskeletal remodeling complex, in human cell systems. To uncover whether the ESCRT-III complex functions downstream of Piezo in the inhibition of axon regeneration, we are employing an established *Drosophila melanogaster* larval injury model in which we perform laser transections of single, trackable axons of peripheral dendrite arborization sensory neurons that display varying capacities to regenerate. Preliminary data pairing this injury assay and live timelapse imaging shows increased extension and retraction events of growth cones in Piezo knock-out animals suggesting a role of Piezo in modulating cytoskeletal dynamics. Additionally, utilizing this model as well as available *Drosophila melanogaster* mutant lines, we have identified the ESCRT-III complex component Shrub as a genetic interactor with Piezo in the inhibition of axon regeneration. In line with the Piezo-dependent Rab11 trafficking of the ESCRT-III targeting factor ALIX described by Carrillo-Garcia et al., genetic interactions between Piezo, Rab-11, and ALIX were also found in the inhibition of axon regeneration. This preliminary data suggests a novel role of the ESCRT-III complex in Piezo's inhibition of axon regeneration that we will explore further through genetic and live imaging analyses in the *Drosophila melanogaster* model system. Additionally, we will employ a neuromuscular junction organoid model to test whether these genetic modulators of regeneration are conserved in human systems. By studying the genetic, cellular, and molecular mechanisms underlying axon regeneration, our work is helping to advance the field of regenerative medicine and identifying novel targets for therapeutic treatment of nervous system injury.

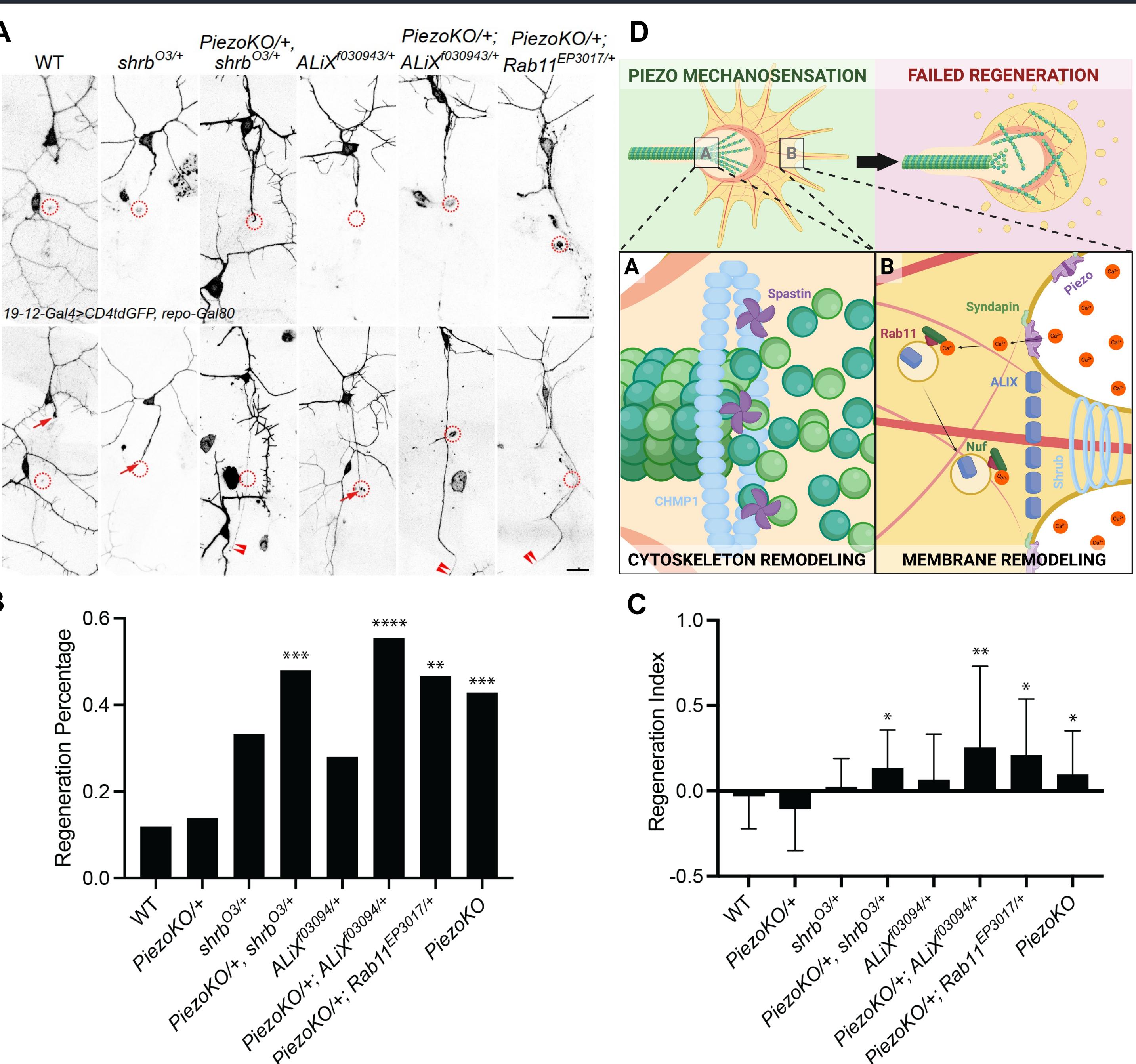
Background & Methodology



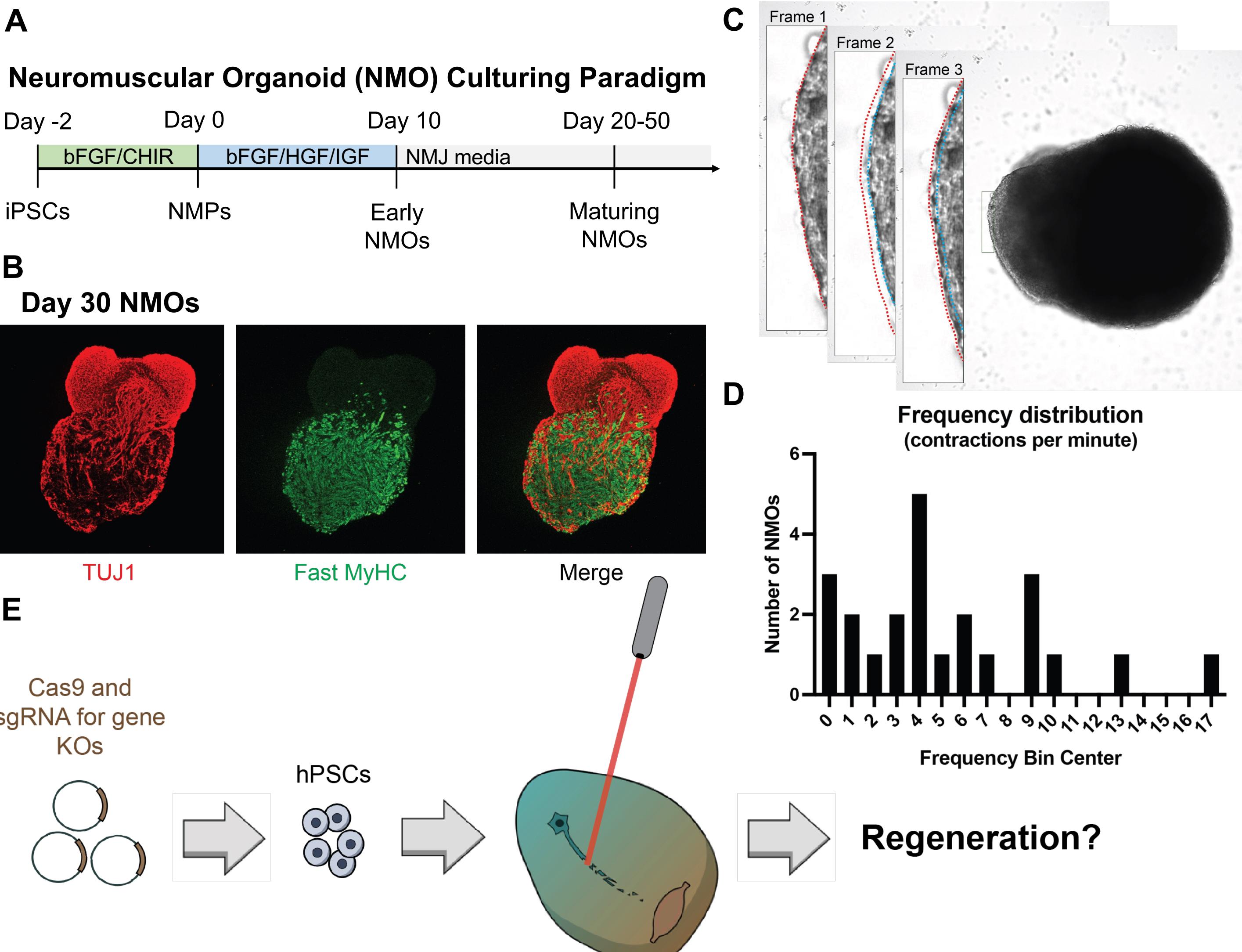
Piezo inhibits regenerative cytoskeletal dynamics after injury



ESCRT-III functions with Piezo to inhibit axon regeneration



Establishing an axon regeneration model in neuromuscular junction organoids



Conclusions & Future Directions

- Our work suggests that the ESCRT-III complex functions together with Piezo to inhibit axon regeneration.
- Ongoing work is focused on investigating genetic interactions between Piezo and the ESCRT-III complex component Chmp1, as well as validating Shrub as a downstream effector of Piezo.
- In future studies, we hope to further implicate Piezo and the ESCRT-III pathway in the disruption of cytoskeletal dynamics at the regenerating axon tip through live imaging of transgenic animals.
- We also plan to implement our NMO model to test whether these results are conserved in a human model system.

References

- Diagrams created with Biorender.com
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