

Introduction and Abstract

We previously established the mechanosensitive ion channel Piezo as being an inhibitor of axon regeneration. After axon injury, Piezo's localization in the growth cone increases, and its activation leads to decreased regeneration. However, it is not well understood what leads to Piezo's activation. Therefore, this project has **two aims**:

1. Develop a protocol to investigate the activity of the growth cone over longer time scales.
2. Determine the physical interactions leading to Piezo activation.

Timelapsing *Drosophila* Neurons *in vivo*

To investigate growth cone dynamics over time, we first use our established axon injury paradigm (Li et al, 2018) (Fig. 1A). We then developed two methods to timelapse neurons *in vivo*. The first (Fig. 1B) works better for longer time scales and is based off of (Ji & Han, 2020). The second (Fig. 1C) has higher throughput, and works best for shorter timescales.

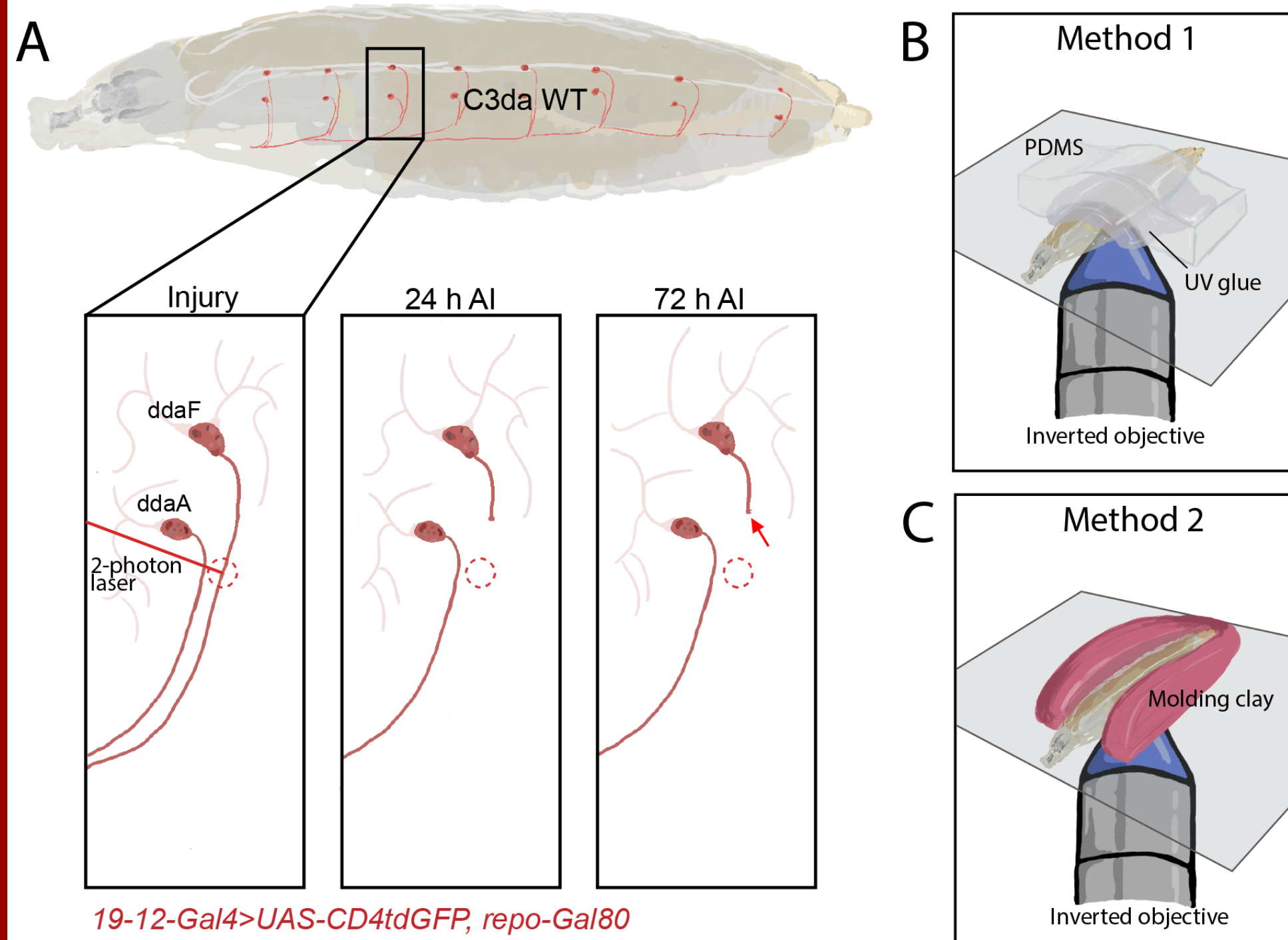


Fig. 1. (A) Axon injury protocol in *Drosophila* larva. Axons are injured via a 2-photon laser and imaged at 24 and 72 hours after injury to track their regeneration. (B) Timelapsing setup 1, using UV glue. (C) Timelapsing setup 2, using molding clay.

Tracking Growth Cone Activity

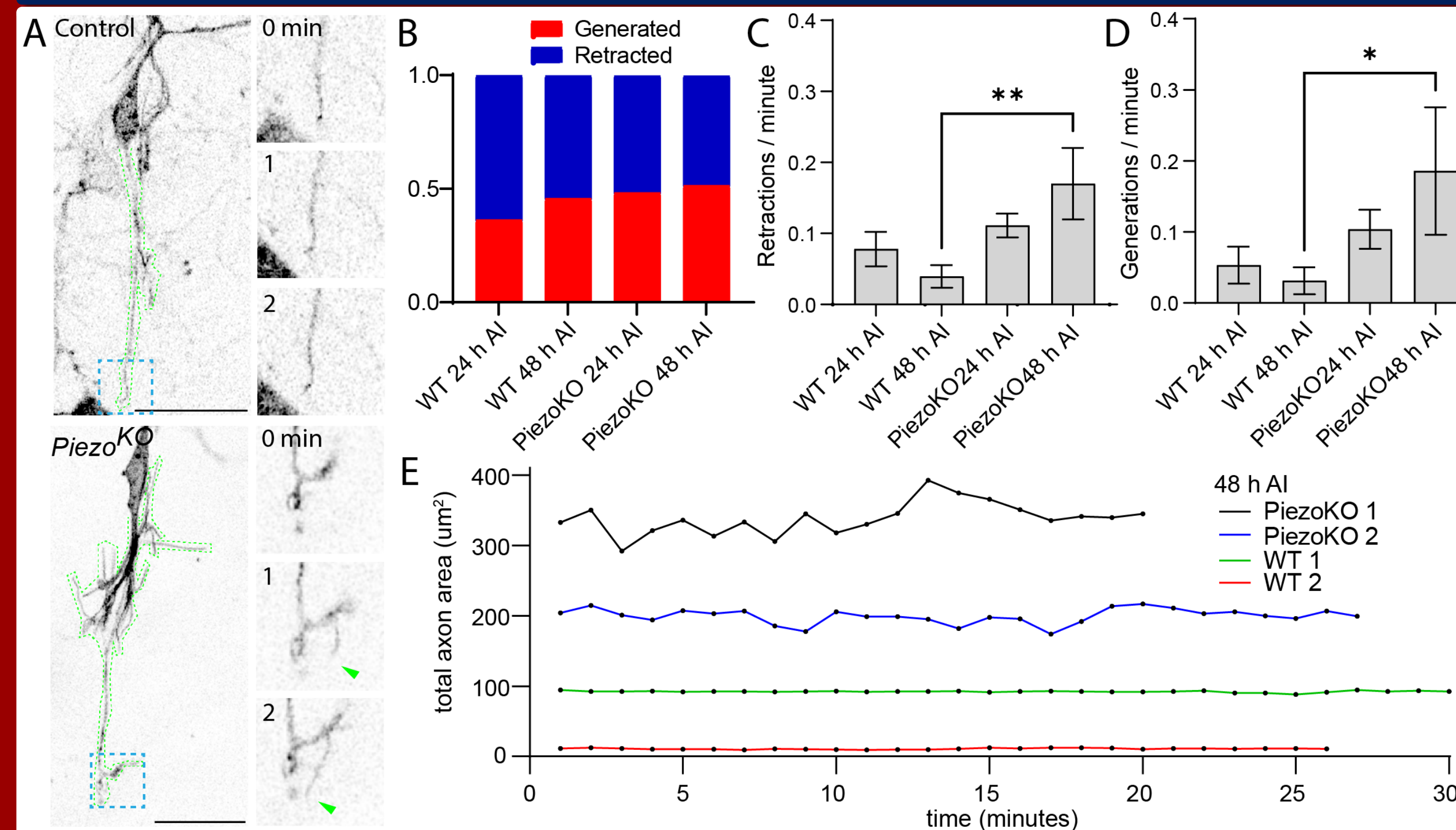


Fig. 2. (A) Representative images of wildtype (WT) vs. Piezo knockout *PiezoKO*. Scale bar=20µm. (B) Proportion of outgrowths generated vs. retracted. (C) Outgrowths retracted per minute and (D) generated per minute. (E) Examples of whole axon area over time.

Neuronal Outgrowths Interact with Glial Membrane

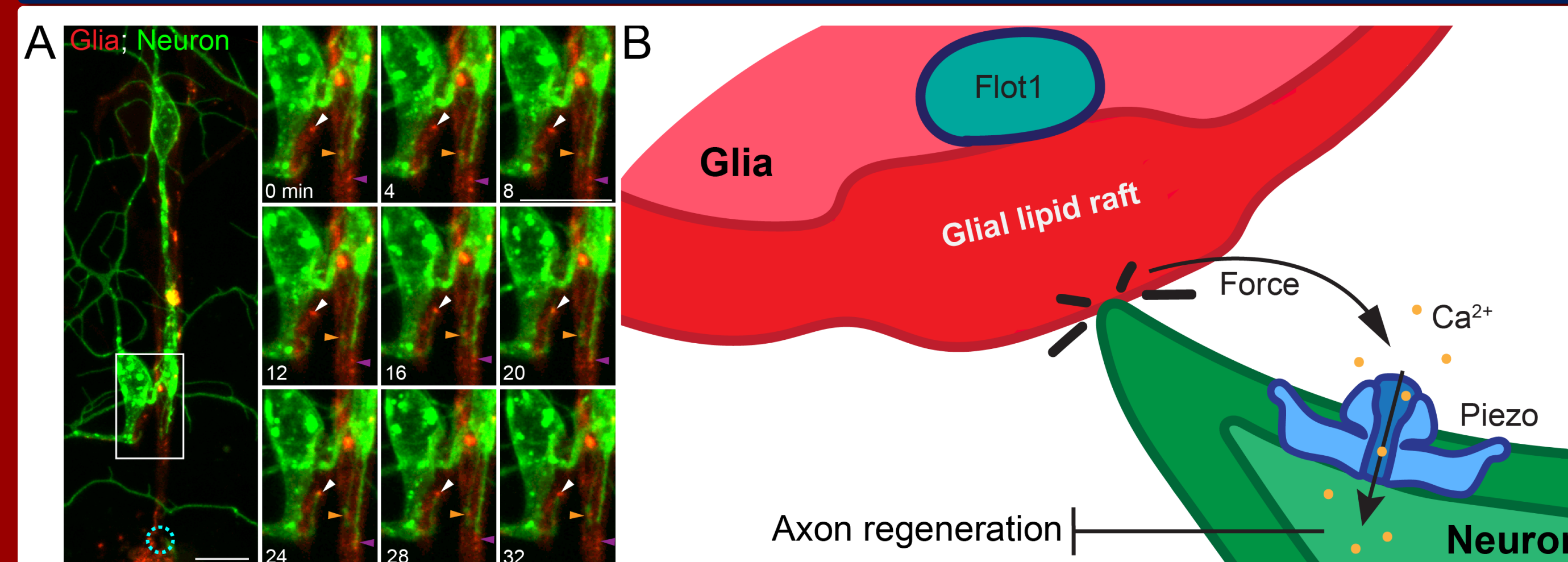


Fig. 3. (A) Neuronal outgrowths are seen interacting with glia at areas of high glial-membrane density (arrowheads). Scale bar=20µm. (B) We hypothesize that these areas may mark unique membrane features, like lipid rafts, in the glia mediated by Flotilin1 (Flot1).

Flot1 Inhibits Regeneration

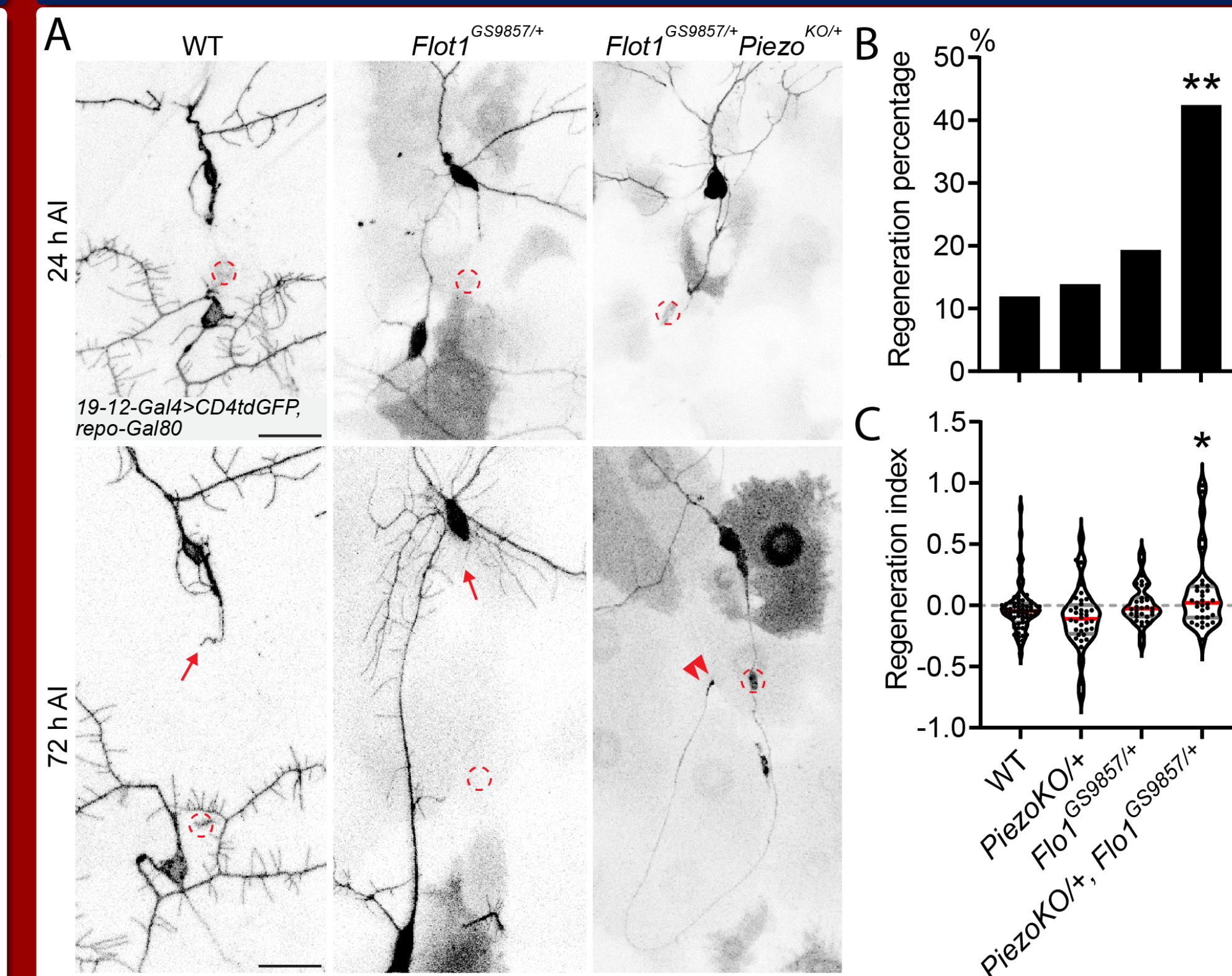


Fig. 4. (A) Representative images of injured neurons. A dotted red circle marks the injury site. An arrow marks a stalled axon, while a double arrowhead marks a regenerating axon. Scale bar=20µm. Quantification by (B) percentage of total axons that showed regeneration and (C) normalized regeneration index.

Discussion

This work provides both a method and a biological framework for further investigation. We have established a protocol for tracking growth cone dynamics *in vivo*. We used this method to identify how *PiezoKO* alters activity and a possible glia-neuron interaction. We believe this glia-neuron interaction may be related to lipid rafts formed using Flot1. Further investigation will be needed to fully uncover this pathway.

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