

The mechanosensitive ion channel Piezo and the interactions of a regenerating axon

Jackson Powell^{1,2} and Dr. Yuanquan Song²¹University of Pennsylvania, School of Arts and Sciences, Biochemistry, C'24²Song Lab, Children's Hospital of Philadelphia, Center for Cellular and Molecular Therapeutics

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.

Injured neurons maintain glial contact

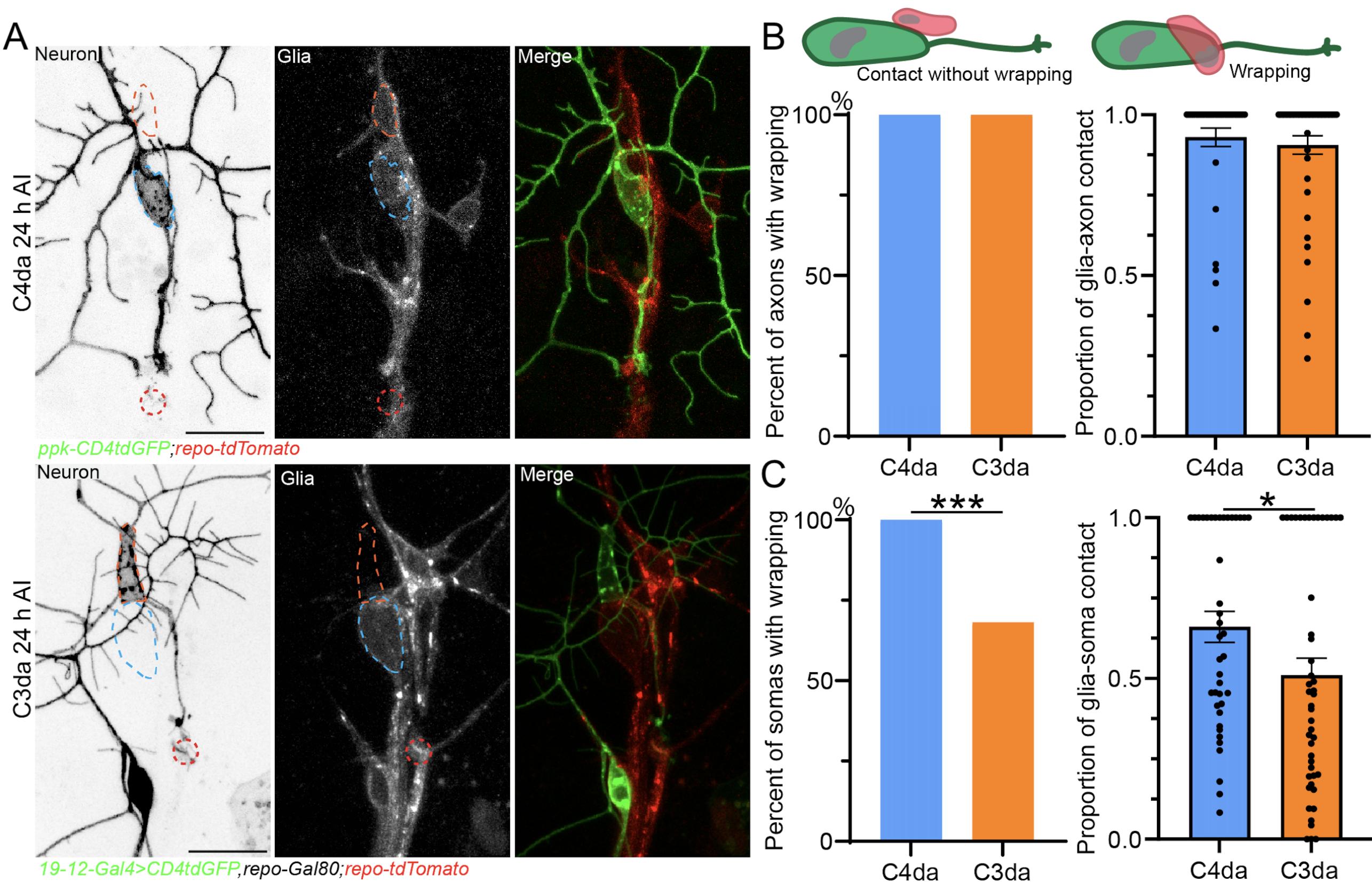


Fig. 1. (A) Representative images of C4da (top) and C3da (bottom) 24h after injury. Cell bodies of C4da are outlined in cyan dashed lines, and C3da in orange dashed lines. The injury site is demarcated by the red dashed circle. Glia are shown in red, and neurons in green. In both cases, glia wrapping is maintained around both C4da and C3da. Scale bar = 20 μ m. (B, C) Quantification is shown comparing C4da and C3da wrapping, comparing both the number of neurons showing wrapping after injury, and the proportion of neuronal areas showing wrapping, for both axons and somas.

In vivo timelapse protocol development

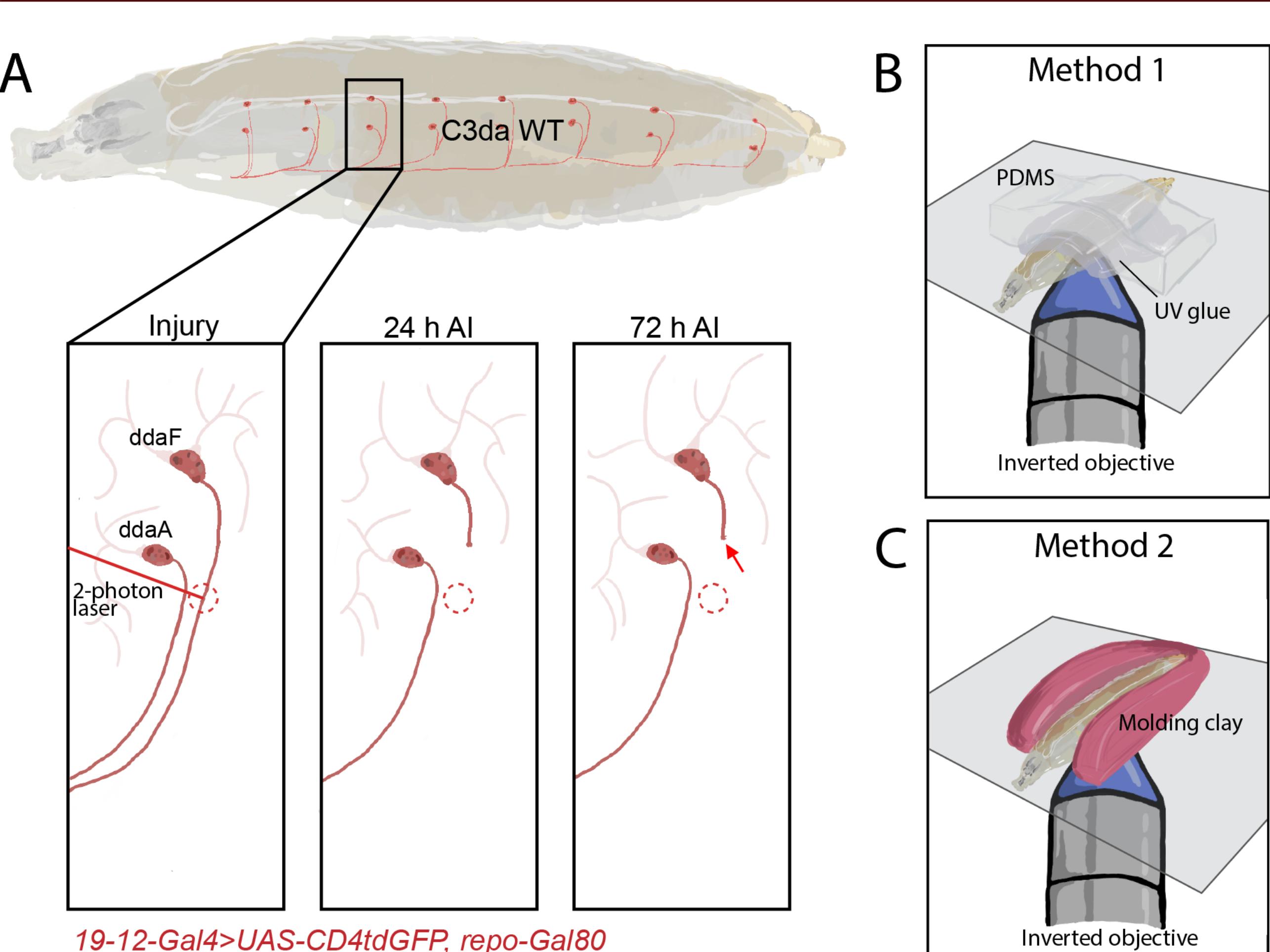


Fig. 2. (A) Axon injury protocol in *Drosophila* larva. Axons are injured via a 2-photon laser and imaged at 24 and 72 hours after injury to track their regeneration. (B) Timelapsing setup 1, using UV glue. (C) Timelapsing setup 2, using molding clay.

Tracking growth cone activity

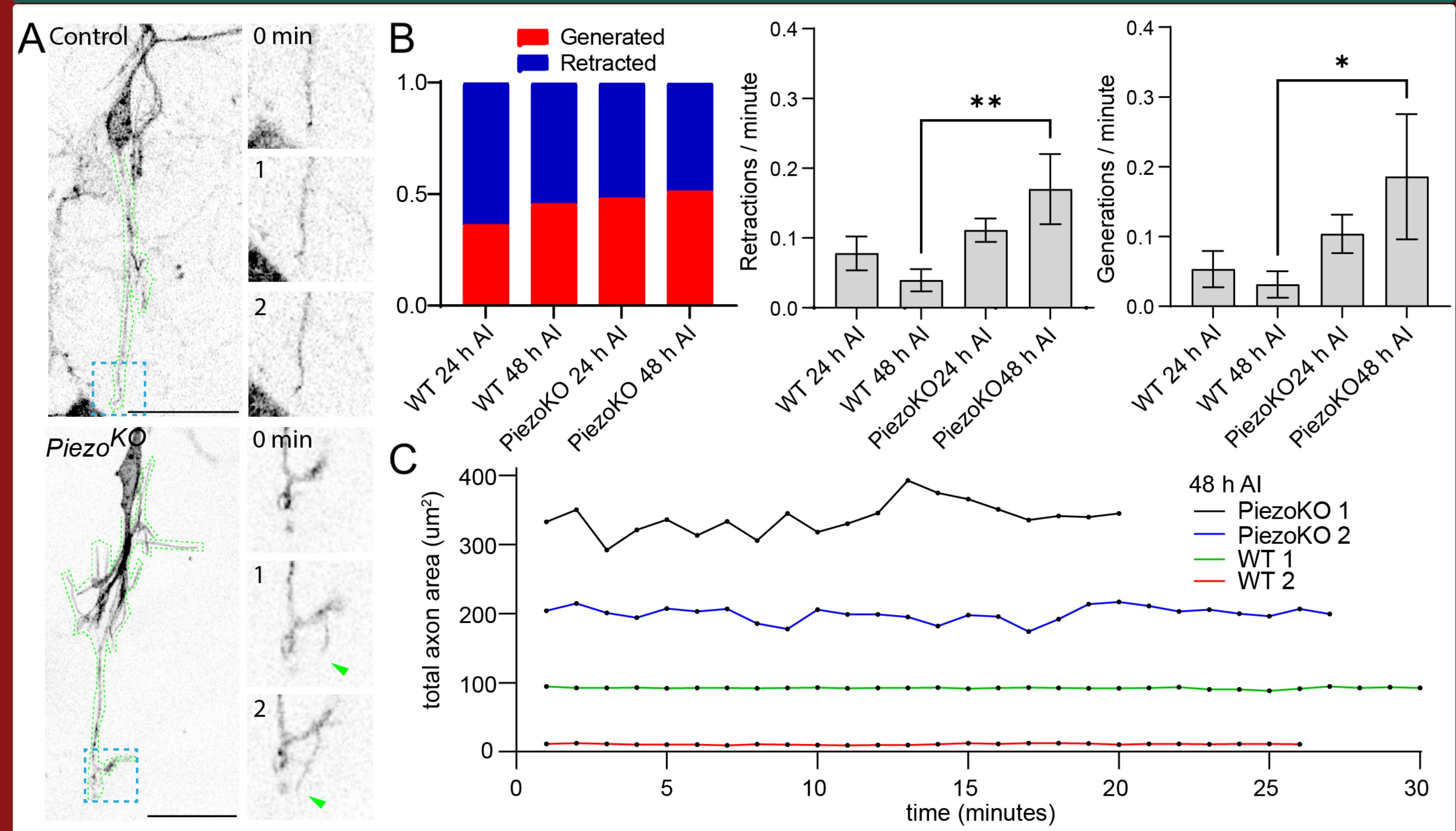


Fig. 3. (A) Representative images of injured neurons. A dotted red circle marks the injury site. An arrow marks a stalled axon, while a double arrowhead marks a regenerating axon. Scale bar=20 μ m. Quantification by (B) percentage of total axons that showed regeneration and (C) normalized regeneration index.

Flot1 Inhibits Regeneration

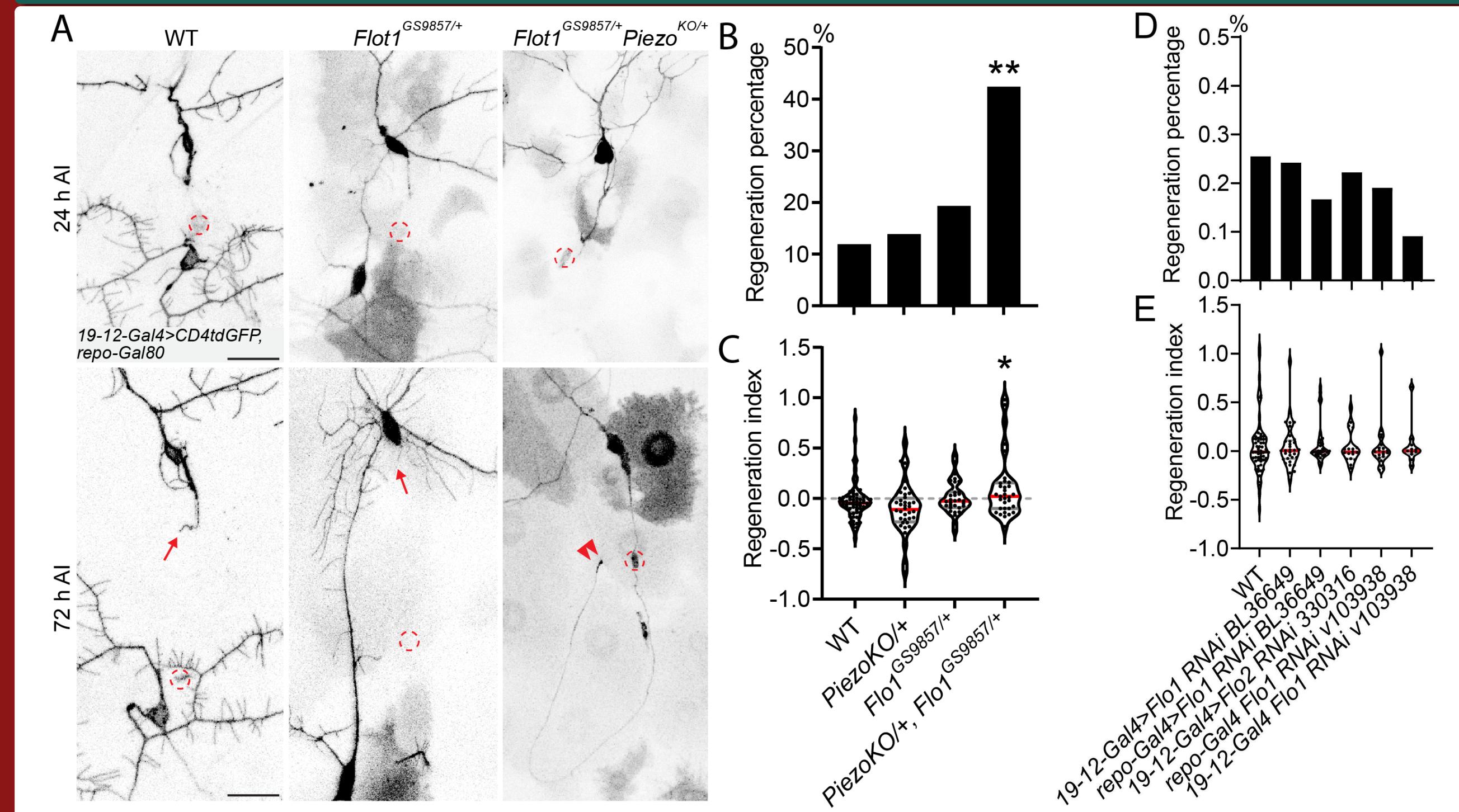


Fig. 5. (A) Representative images of injured neurons. A dotted red circle marks the injury site. An arrow marks a stalled axon, while a double arrowhead marks a regenerating axon. Scale bar=20 μ m. Quantification by (B) percentage of total axons that showed regeneration and (C) normalized regeneration index.

Ppk may work with Piezo to inhibit regeneration

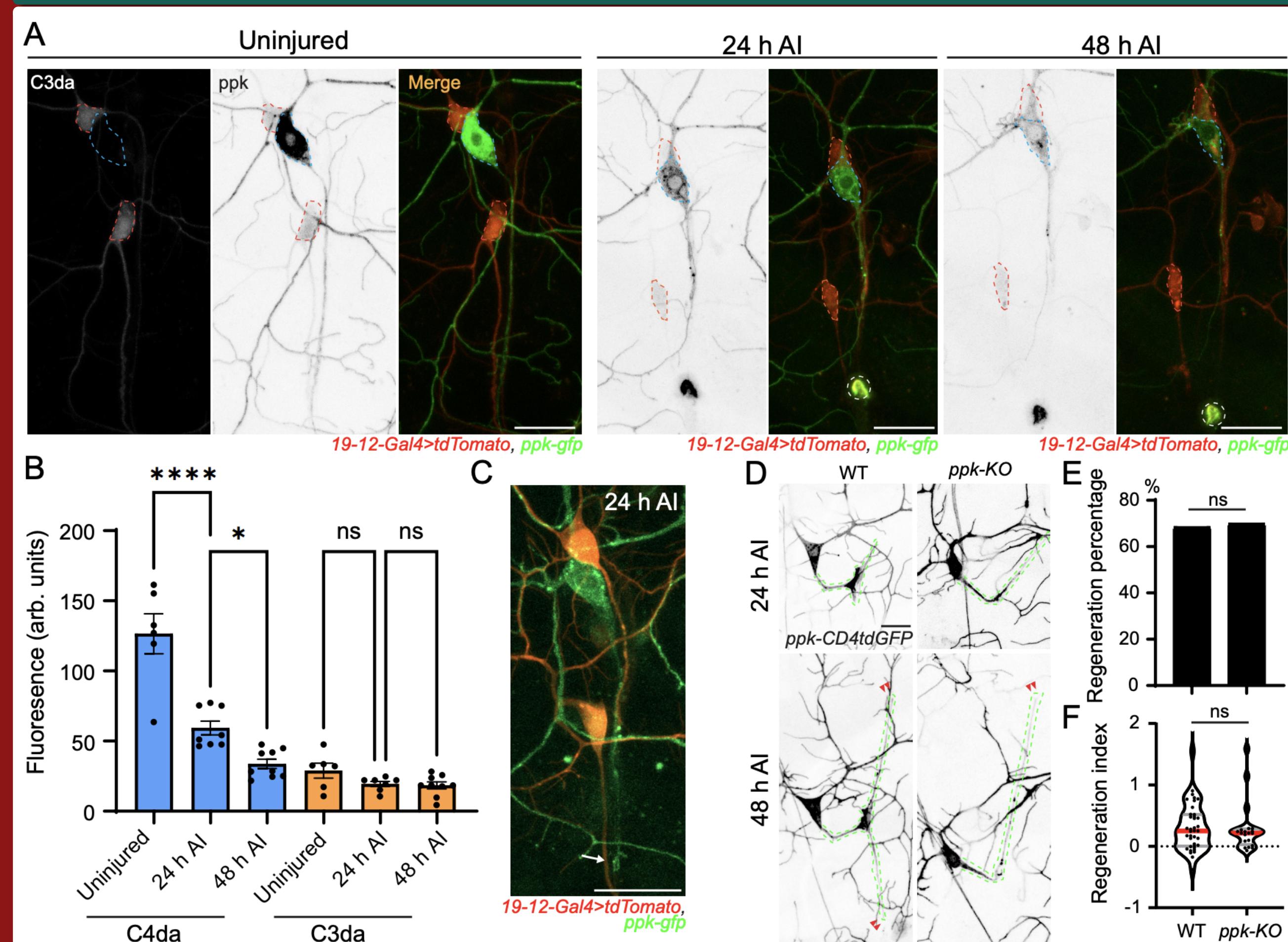


Fig. 6. (A) Representative images of uninjured and injured WT neurons labeled with *ppk-gfp*. C3da cell bodies are traced with an orange dashed line, and C4da with a blue dashed line. The injury site is marked by the white dotted circle. Scale bar = 20 μ m. (B) Quantification of *ppk-gfp* fluorescence in the somas of C4da and C3da. (C) Representative image of injured C4da showing *ppk* protein enrichment at the growth cone (marked by the white arrow). Scale bar = 20 μ m. (D) Representative images of WT and *ppk*-KO regeneration. Axons are traced with a green dashed line, and regenerating axon tips are marked by the red double arrowhead. Scale bar = 20 μ m. (E) Quantifications of regeneration indexes and percentages.

While *ppk*-KO did not show an affect on regeneration in C4da, initial tests of Piezo and *ppk* knockout transhets (*ppk*^{KO/+}; *Piezo*^{KO/+}) have yielded promising results in C3da regeneration. With an $N = 29$, the regeneration index mean was 0.23, and percentage was 48% (data not shown). Both of these quantifications were statistically significant (**) against wildtype. Altogether, this work provides both a method and a biological framework for further investigation. Further investigation will be needed to fully uncover the pathways initiated here.

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