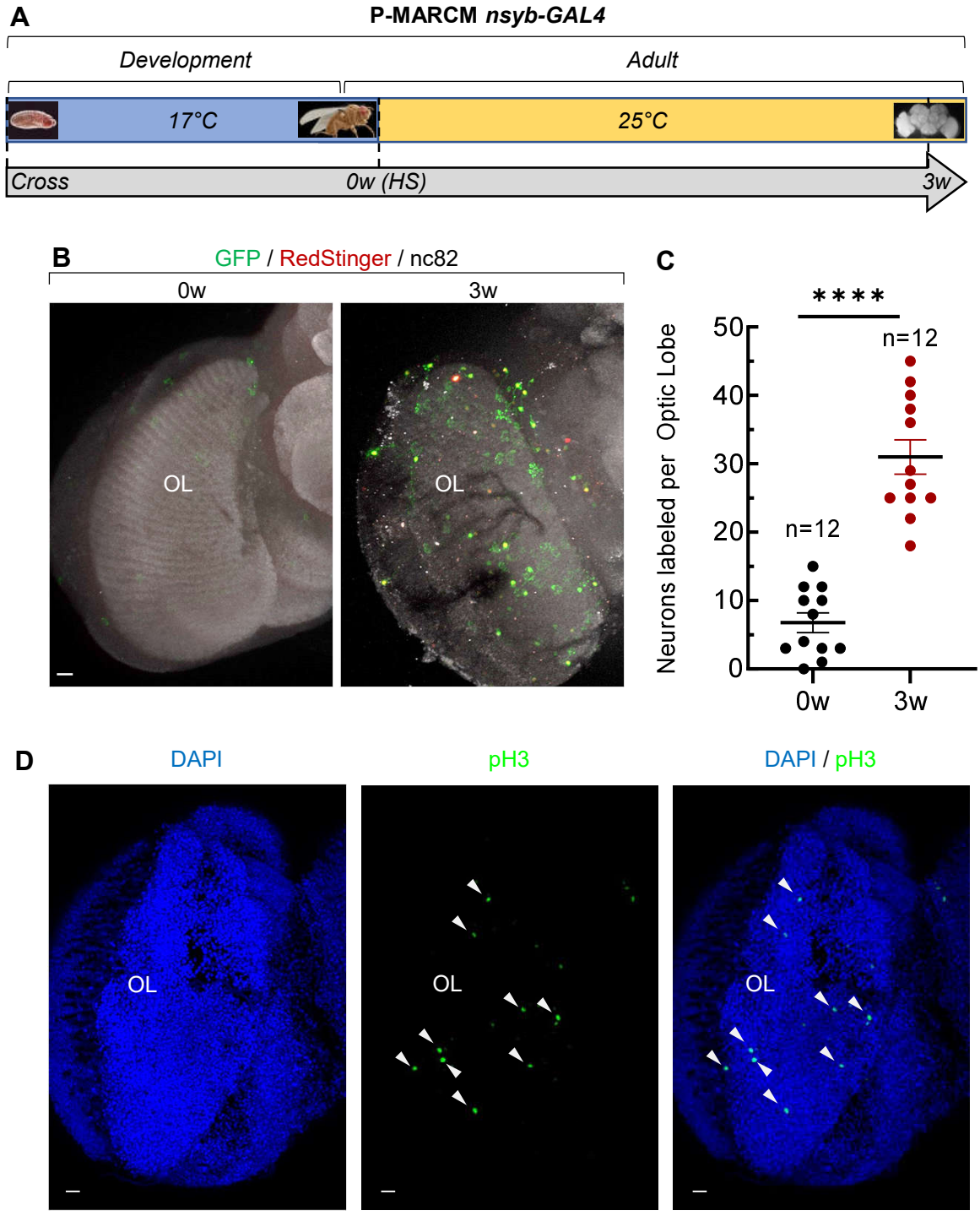


Fig. S1. P-MARCM captures adult neurogenesis in the *Drosophila* optic lobes (OL)

(A) Experimental strategy to reveal adult neurogenesis with P-MARCM *nsyb-GAL4*. Flies 2-5 days–old were Heat-Shocked (HS) to activate the P-MARCM system and brains were dissected 3 weeks (3w) after.

(B) Adult-born neurons in the optic lobes (OL) are labeled by P-MARCM with *nsyb-GAL4* line 3 weeks after HS.

(C) Amount of adult-born neurons in OL at 3 weeks (n=12 OL) is significantly higher than background levels (n=12 OL) (*p=0.0000002,* Student's t-test). Error bars represent s.e.m.

(D) Cell proliferation is also detected by anti-pH3 antibody in the OL (9.2+/-1,0 s.e.m. pH3+ cells/OL; n=26 OL). Scale bars for all panels: 10μm.

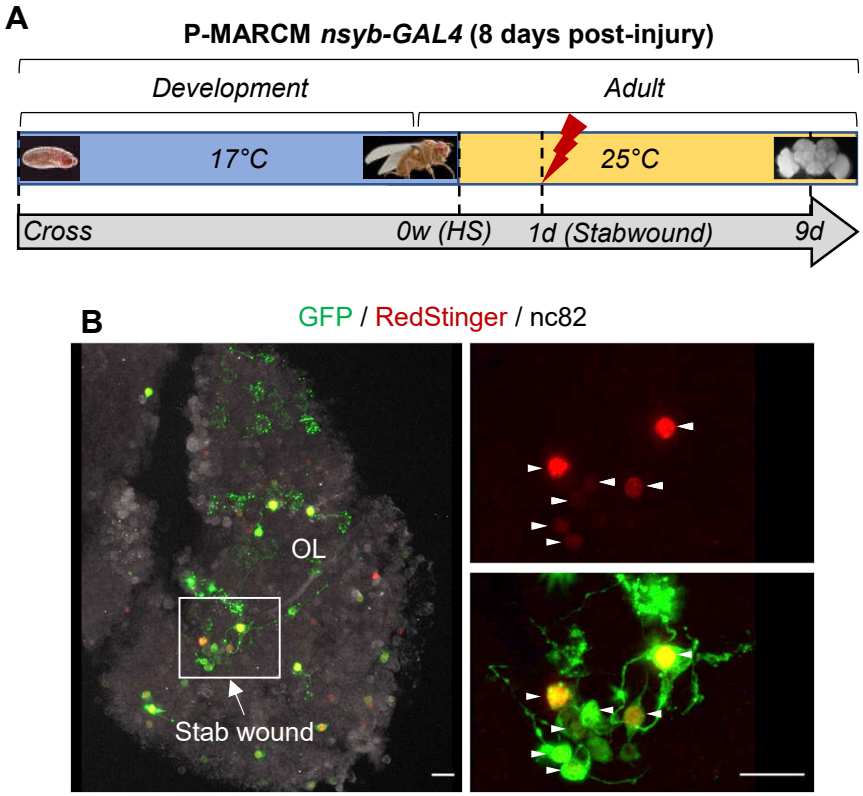


Fig. S2. P-MARCM captures injury-induced neuronal regeneration in the *Drosophila* optic lobe (OL)

(A) Experimental strategy to capture injury-induced neuronal regeneration in OL with P-MARCM *nsyb-GAL4*. Two to 5 days–old flies were Heat-Shocked (HS) to activate the P-MARCM system. Stab wound was applied to the left OL by a fine needle 1 day after HS. Flies were dissected and imaged 8 days later.

(B) Regenerated neurons in the OL (arrowheads) are labeled by P-MARCM with *nsyb-GAL4* line 8 days after stab wound. Scale bar: 10 μm.

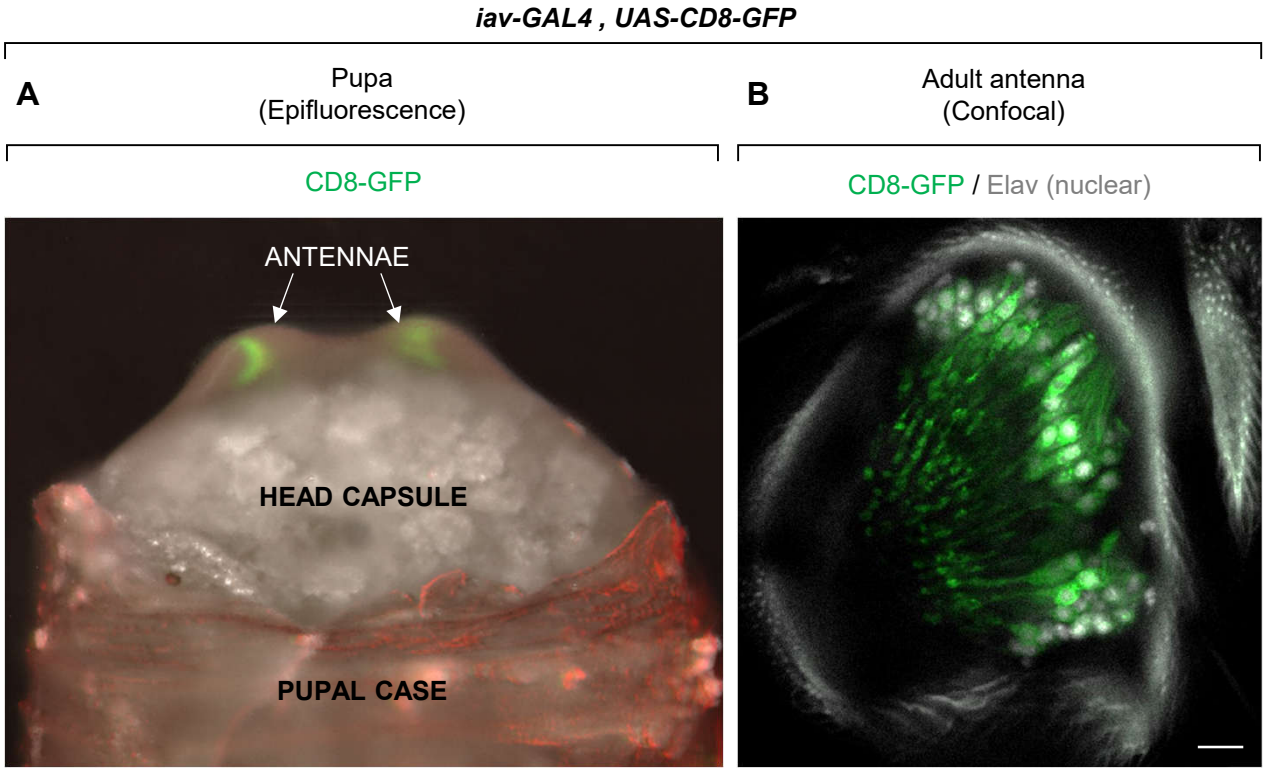


Fig. S3. Expression pattern of *iav-GAL4* line in the antennae.

(A) Expression of *iav-GAL4* in the antennae begins in pupal stage.
(B) *iav-GAL4* expression is restricted to JO neurons in adult antenna. Scale bar: 10μm.

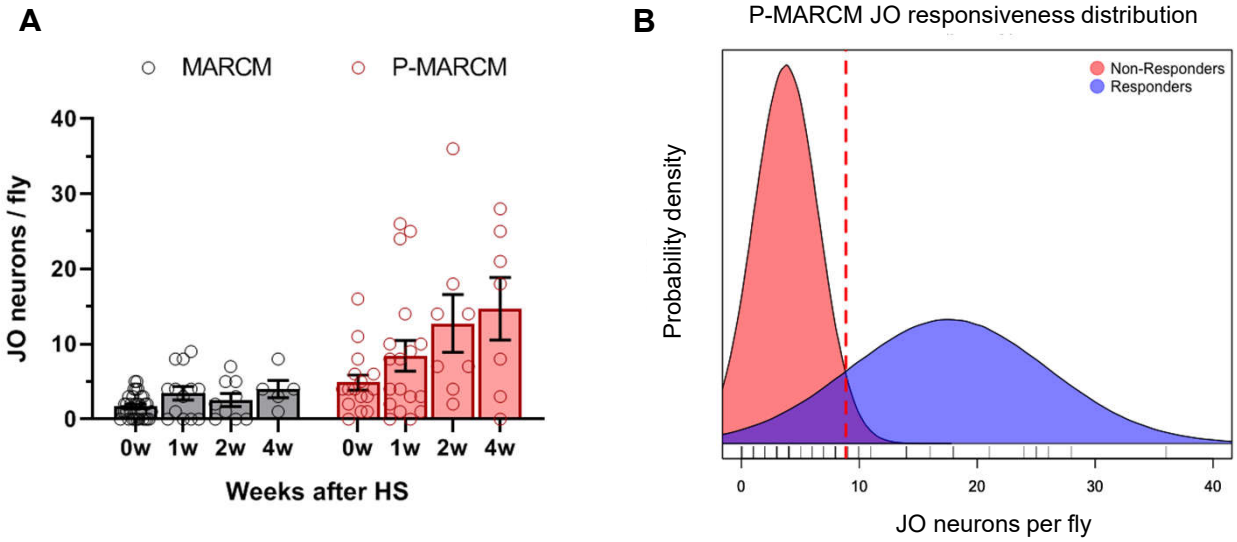


Fig. S4. JO neurogenesis detection and distribution

(A) Quantification of newborn JO neurons in MARCM and P-MARCM lineage tracing approaches. JO neurogenesis increases over time upon P-MARCM labeling. Error bars represent s.e.m.

(B) Gaussian mixture model classifies flies into “Responders” and “Non-responders” indicating the presence of adult JO neurogenesis beyond background levels.

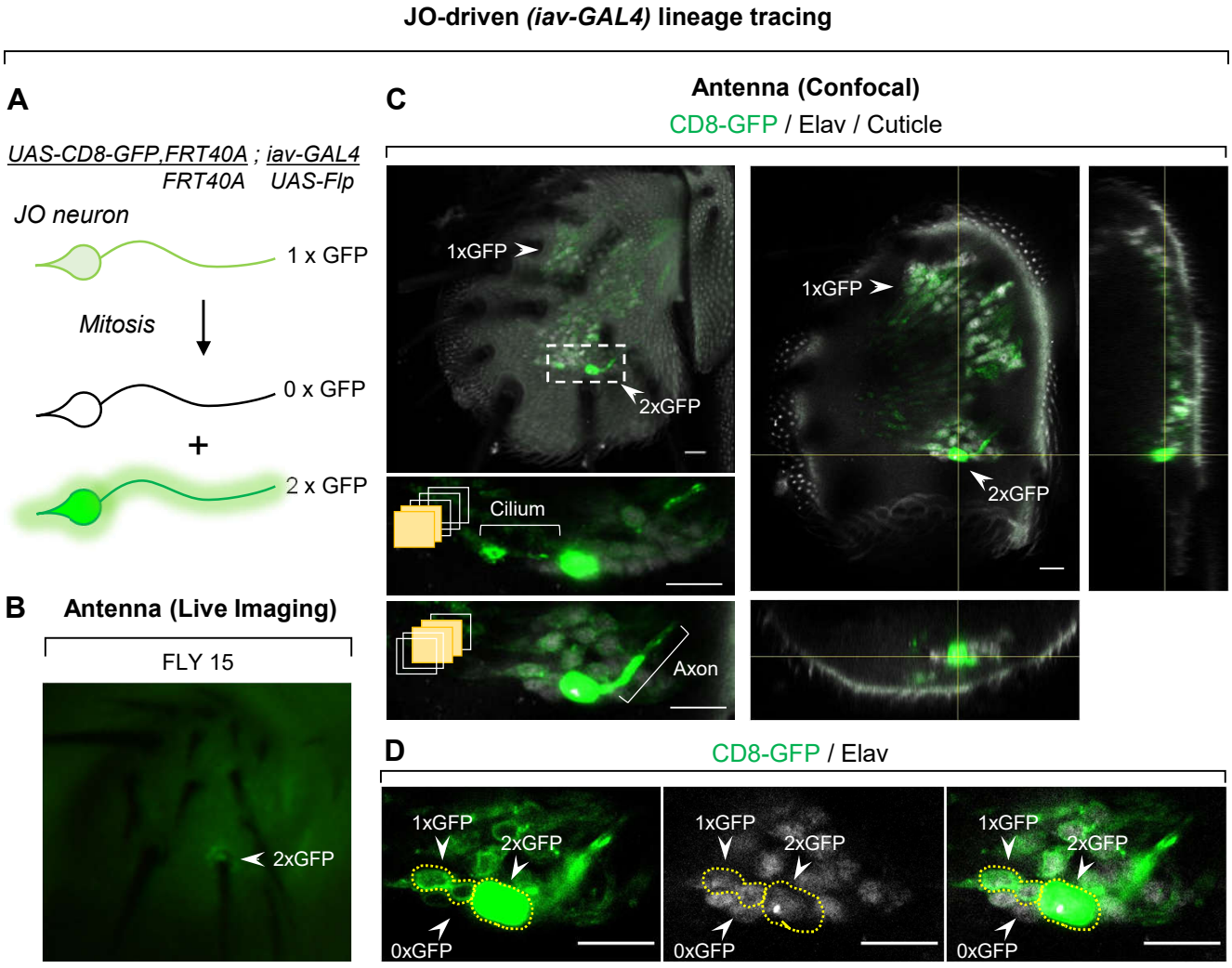


Fig. S5. Neurogenic clones from JO neurons are captured at single-cell resolution *in vivo*.

(A) *.iav-GAL4*-driven lineage tracing system to assess neurogenesis from JO neurons. Daughter cells express 2xGFP and 0xGFP, while non-dividing JO neurons contain 1xGFP.
(B) A single JO neuron expressing 2xGFP detected by fluorescent microscopy on an intact, alive fly.
(C) Confocal microscopy confirms proliferation of a single neuron on the anterior part of the antenna. Captions show maximum intensity projections of discrete planes to visualize the JO neuron cilium and axon.
(D) Neurogenesis from JO neurons detected by twin-spots of 2xGFP/Elav+ neurons and 0xGFP/Elav+ neurons among non-dividing 1xGFP/Elav+ JO neurons. Scale bars: 10μm

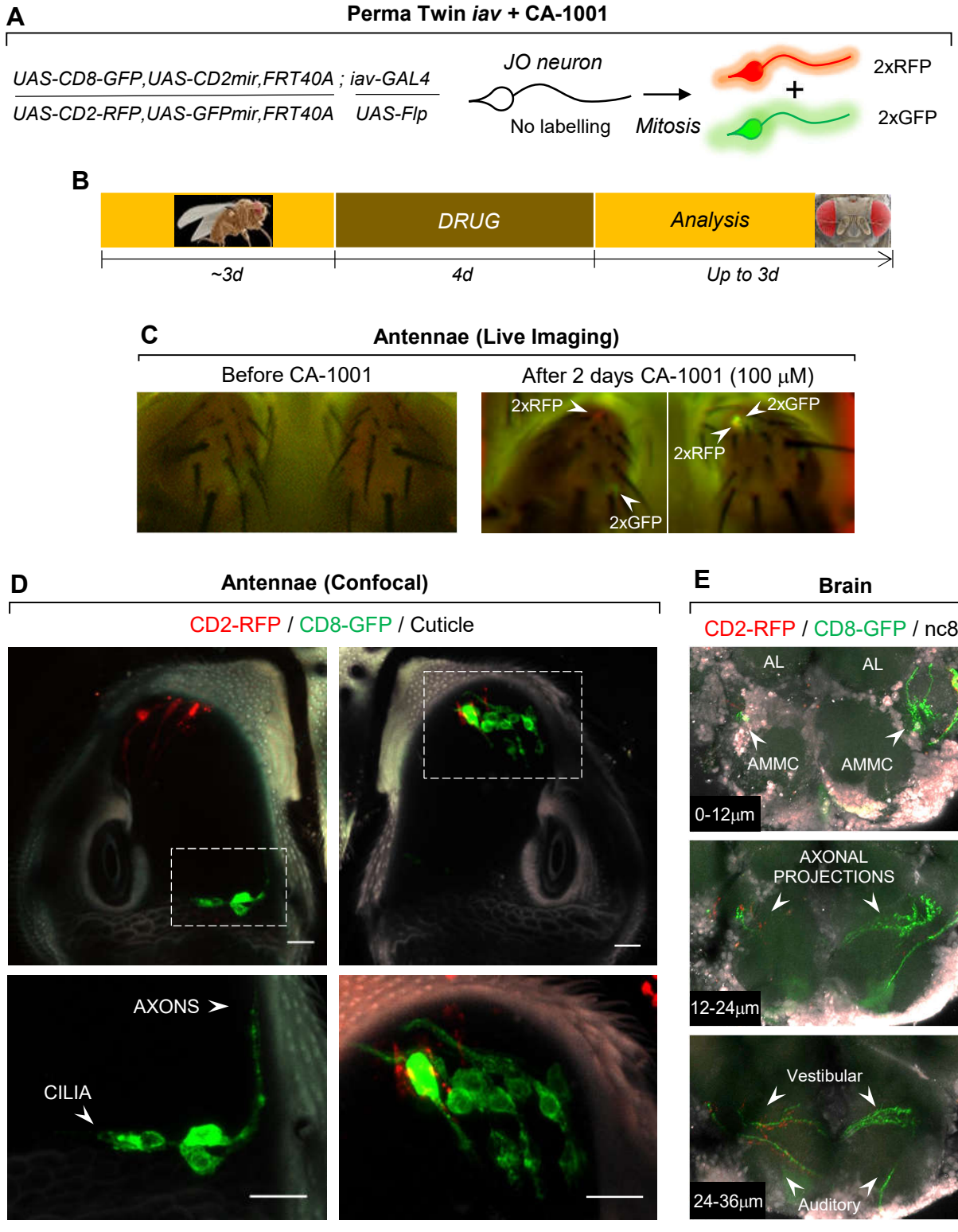
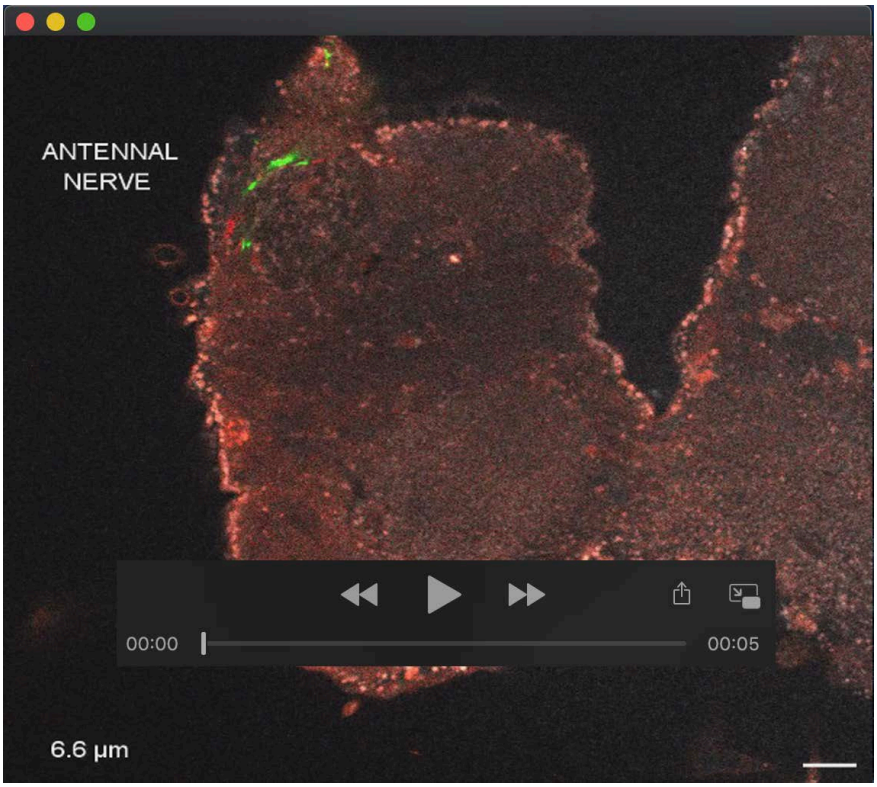


Fig. S6. The calcium ionophore CA-1001 increases neurogenesis from JO neurons.

(A) Perma Twin-iav lineage tracing system to assess neurogenesis from JO neurons.
(B) Experimental strategy to capture neurogenesis from JO neurons. Three-day old PT-iav flies receive oral drug administration for 4 days and are analyzed up to 3 days later.
(C) New JO neurons are detected by fluorescent microscopy on intact, alive flies as soon as 2 days after administration of CA-1001 at 100μM.
(D) Newly-generated JO neurons develop cilia and extend axons to the brain. AMMC: Antennal Mechanosensory and Motor Center; AL: Antennal Lobe
(E) New JO neurons target the brain in the Auditory and Vestibular circuit pattern as early as 2 days following drug administration. Scale bars for all panels: 10μm.



Movie 1. Axonal projections of regenerated Johnston Organ (JO) neurons induced by CA-1001. Annotated confocal stack of newborn auditory and vestibular JO neuron axonal projections after 2 days of CA-1001 administration to Perma Twin-iav flies. Scale bar: 10 mm.