Figures and figure supplements

A locomotor neural circuit persists and functions similarly in larvae and adult *Drosophila*

Kristen Lee and Chris Q Doe

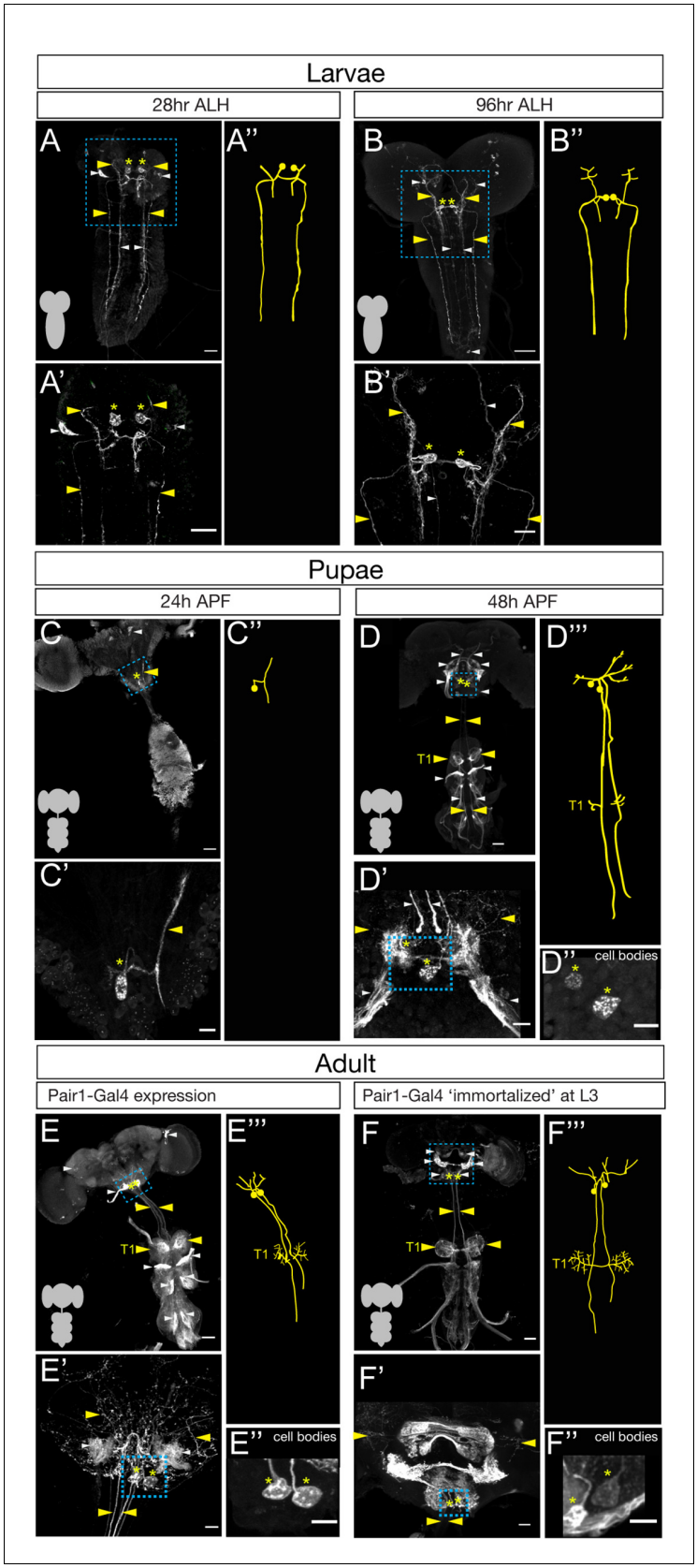

Figure 1. The Pair1 neuron persists from larval to adult stages. (**A–B**) Pair1 neurons (cell body: yellow asterisk; neurites: yellow arrowhead) in the larval CNS (gray outline) at 28 hr after larval hatching (ALH) (**A**) and 96 hr ALH

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Figure 1 continued

(**B**). Here and in subsequent panels are maximum intensity projections of confocal sections containing the Pair1 neurons; anterior, up; dorsal view. Significant 'off-target' expression marked with white arrowheads. Scale bar, 50 μm. (**A'–B'**) Enlargement of the brain regions boxed in A,B. Scale bar, 20 μm. (**A"–B"**) Tracing to show Pair1 neuron morphology. Genotype: *+; UAS-myr::GFP; R75C02-Gal4*. (**C–D**) Pair1 neurons (cell body: yellow asterisk; neurites: yellow arrowhead) in the pupal CNS (gray outline) at 24 hr after pupal formation (APF) (**C**) and 96 hr APF (**D**). Significant 'off-target' expression marked with white arrowheads. Scale bar, 50 μm. (**C'–D'**) Enlargement of the brain regions boxed in C, D; cell body: yellow asterisk, neurites: yellow arrowhead. Scale bar, 10 μm. (**C"**) Tracing to show Pair1 neuron morphology. (**D"**) Focal plane showing Pair1 cell bodies (region boxed in D', cell body marked with yellow asterisks). Scale bar, 10 μm. (**D"'**) Tracing to show Pair1 neuron morphology. Note that Pair1 can be followed to T1 in the 3D confocal stack but is difficult to represent here due to fasciculation of Pair1 with off-target neurons. Genotype: *+; UAS-myr::GFP; R75C02-Gal4*. (**E**) Pair1 neurons (cell body: yellow asterisk; neurites: yellow arrowhead) in the 4-day adult CNS (gray outline) Significant 'off-target' expression marked with white arrowheads. Scale bar, 50 μm. (**E'**) Enlargement of the brain region boxed in E. Scale bar, 10 μm. (**E"**) Focal plane showing Pair1 cell bodies (region boxed in E', cell body marked with yellow asterisks). Scale bar, 10 μm. (**E"'**) Tracing to show Pair1 neuron morphology. Genotype: *+; UAS-myr::GFP; R75C02-Gal4*. (**F**) Pair1 neurons (cell body: yellow asterisk; neurites: yellow arrowhead) permanently labeled at 96 hr ALH and visualized in the 4-day old adult. See Materials and methods for details. Significant 'off-target' expression marked with white arrowheads. Scale bar, 50 μm. (**F'**) Enlargement of the brain region boxed in F; Pair1 cell body: yellow asterisk; Pair1 neurites: yellow arrowhead. Scale bar, 10 μm. (**F"**) Focal plane showing Pair1 cell bodies (region boxed in F', cell body marked with yellow asterisks). Scale bar, 10 μm. (**F"'**) Tracing to show Pair1 neuron morphology. Genotype: *Hs-KD,3xUAS-FLP; 13xLexAop(KDRT.Stop)myr:smGdP-Flag/+; 13xLexAop(KDRT.Stop)myr:smGdP-V5, 13xLexAop(KDRT.Stop)myr:smGdP-HA, nSyb(FRT.Stop)LexA::p65*.

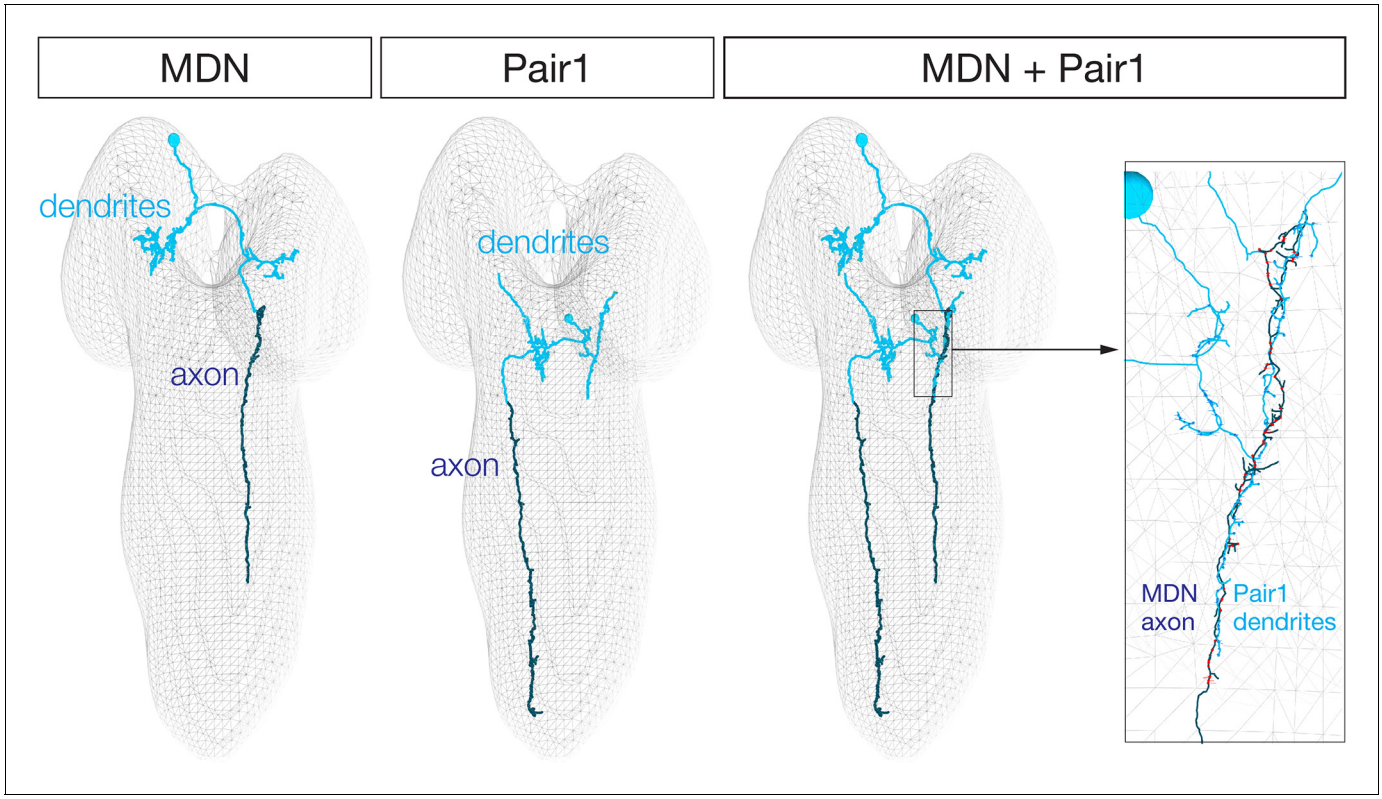

Figure 1—figure supplement 1. Moonwalker descending neuron (MDN) axon and Pair1 dendrite target the same neuropil in the larval brain. The TEM volume of the newly hatched larva 'Seymore' showing the axon (green) and dendrite (blue) domains of a single MDN and Pair1 neuron defined by the location of pre- and post-synapses. Left: MDN axon and dendrite domains. Middle: Pair1 axon and dendrite domains. Right: MDN axon and Pair1 dendrite are closely entwined the same region of neuropil (white bracket).

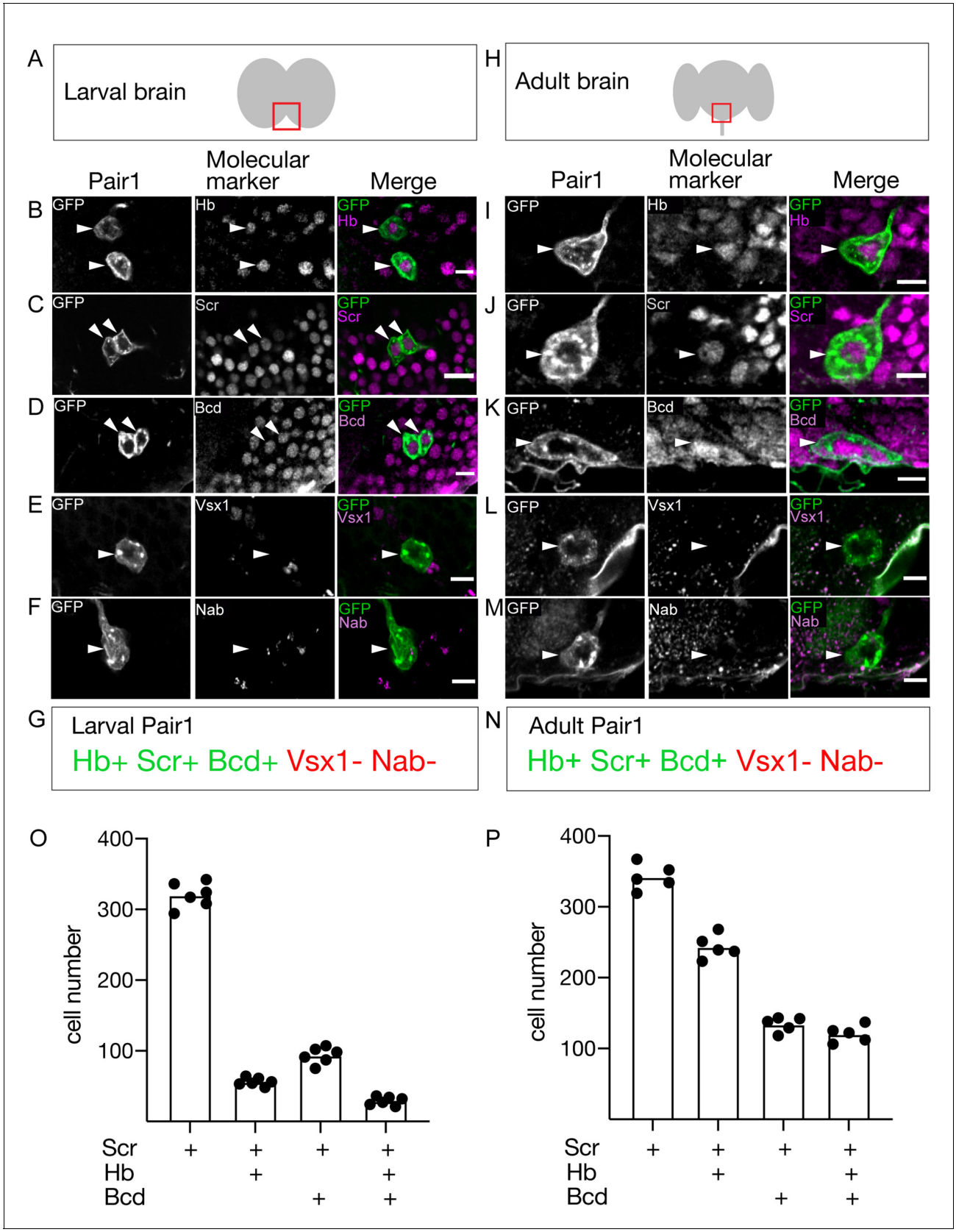

Figure 2. The Pair1 neuron expresses the same molecular markers at larval and adult stages. (**A**) Schematic of the larval brain showing region of Pair1 neurons (red box) enlarged in panels below. Anterior up, dorsal view. (**B–G**) Larval Pair1 neurons (left column), indicated markers (middle column), and

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merge (right column) at 28 hr after larval hatching (ALH). In some cases the second Pair1 neuron is out of the focal plane, but both Pair1 neurons always have the same gene expression profile. Markers detect the following transcription factors: Hb, Hunchback; Scr, Sex combs reduced; Bcd, Bicoid; Vsx1, Visual system homeobox 1; and Nab. Scale bar, 5 μm. (**G**) Summary: marker expression matches that in adults. Genotype: *+; UAS-myr::GFP; R75C02-Gal4*. (**H**) Schematic of the adult brain showing region of Pair1 neurons (red box) enlarged in panels below. Anterior up, dorsal view. (**I–N**) Adult Pair1 neurons (left column), indicated markers (middle column), and merge (right column) in 4-day old adult. Scale bar, 5 μm. (**N**) Summary: marker expression matches that in larvae. Genotype: *+; UAS-myr::GFP; R75C02-Gal4*. (**O–P**) The number of cells expressing Scr (first column), Scr/Hb (second column), Scr/Bcd (third column), and Scr/Hb/Bcd (fourth column) in larvae (**O**) and adults (**P**). n = 5–6 whole brains.

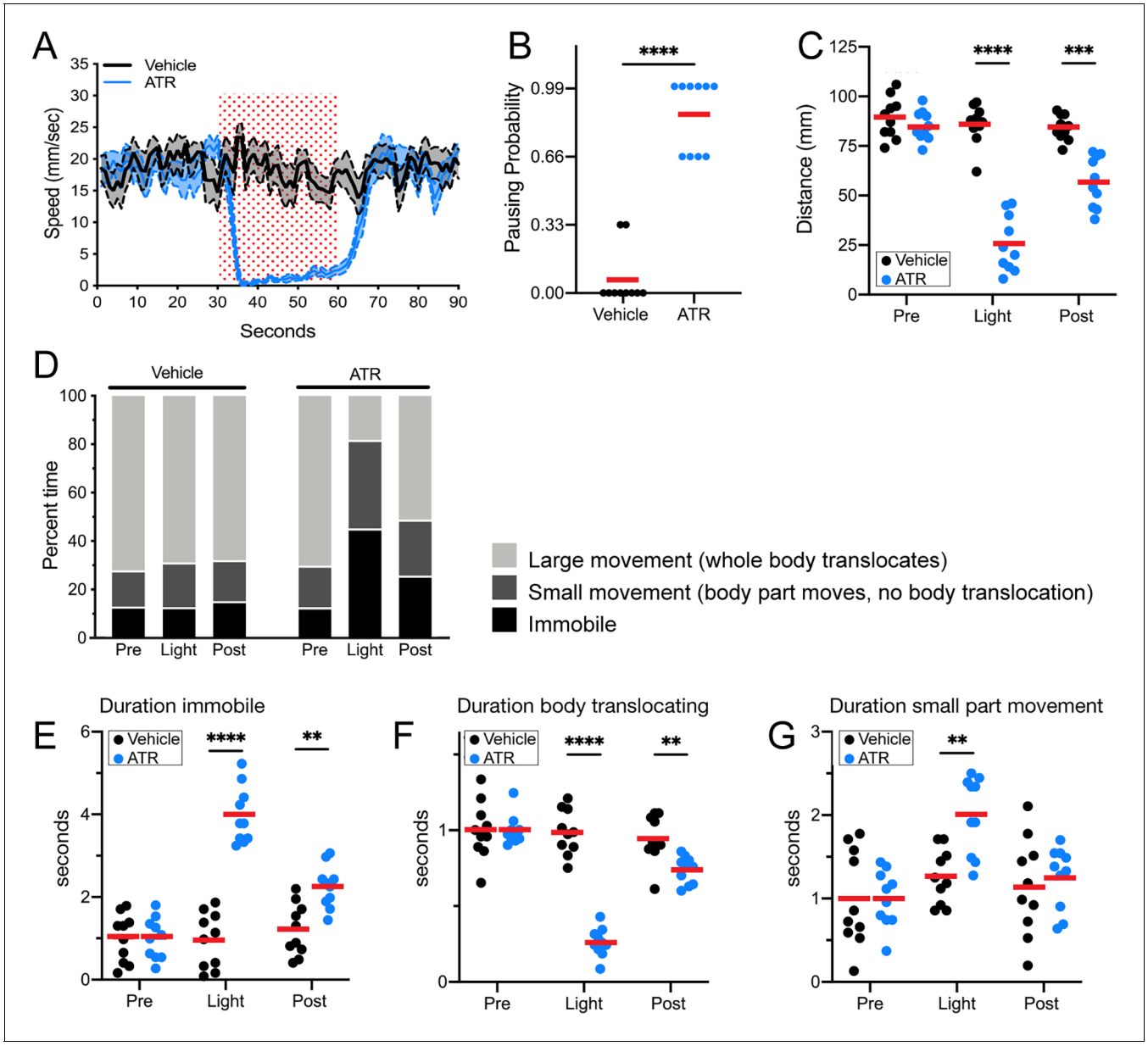

Figure 3. Pair1 activation for 30 s arrests forward locomotion but does not cause paralysis in adults. (**A**) Speed (mm/s) of adult flies expressing Chrimson in Pair1 neurons following neuronal activation (+ATR [all-*trans* retinal] , blue) or no activation (vehicle control, black) in a closed loop arena. Speed was recorded for the 30 s prior to activation, the 30 s light-induced activation (red stipple), and 30 s after activation. Mean ± SEM, n = 10. Genotype for this and all subsequent panels: *UAS-CsChrimson::mVenus; +; R75C02-Gal4*. (**B**) Probability of forward locomotion pausing upon light-induced Pair1 activation (ATR treatment, blue) compared to vehicle control (black). Statistics: t-test, $p < 0.001$; n = 10. (**C**) Total distance traveled pre-light stimulus ('pre'), during the light stimulus ('light') and post-light stimulus ('post') (terminology used here and in subsequent panels) of flies fed ATR (Pair1 activation, blue) compared to controls (fed vehicle, no Pair1 activation, black). Statistics: two-way ANOVA: drug treatment, $F(1, 18) = 111.3$, $p < 0.0001$; time, $F(1.867, 33.61) = 47.03$, $p < 0.0001$; interaction $F(2, 26) = 38.24$, $p < 0.001$; Bonferroni's multiple comparisons between drug treatments within each timepoint: pre, $p > 0.9999$; light, $p < 0.0001$; post, $p = 0.0001$; n = 10. (**D**) Percent time doing large movements (whole body translocation, light gray), small movements (body part movement but no translocation, dark gray) or no movements (immobile, black) of flies fed vehicle (left side) or ATR (right side) during each time phase (pre, light, post). (**E**) Normalized duration of time spent immobile during each timepoint (pre, light, post) for flies fed ATR (Pair1 activation, blue) compared to controls fed vehicle (black). Statistics: two-way ANOVA: drug treatment, $F(1, 18) = 112.8$, $p < 0.0001$; time, $F(1.930, 34.74) = 25.55$, $p < 0.0001$; interaction, $F(2, 36) = 27.81$, $p < 0.0001$; Bonferroni's multiple comparisons between drug treatments within each timepoint: pre, $p > 0.9999$; light, $p < 0.0001$; post, $p = 0.0022$; n = 10. (**F**) Normalized duration of time spent doing small movements during each timepoint (pre, light, post) for flies fed ATR (Pair1 activation, blue) compared to controls fed vehicle (black). Statistics: two-way ANOVA: drug treatment, $F(1, 18) = 5.111$, $p = 0.036$; time, $F(1.923, 34.62) = 10.82$, $p = 0.0003$; interaction, $F(2, 36) = 4.225$, $p = 0.0225$; Bonferroni's multiple comparisons between drug treatments within each timepoint: pre, $p > 0.9999$; light, $p = 0.0022$; post, $p > 0.9999$; n = 10. (**G**) Normalized duration of time spent doing large

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movements during each time phase (pre, light, post) for flies fed ATR (Pair1 activation, blue) compared to controls fed vehicle (black). Statistics: two-way ANOVA: drug treatment, $F(1, 18) = 53.56$, $p < 0.0001$; time, $F(1.869, 33.64) = 53.44$, $p < 0.0001$; interaction, $F(2, 36) = 52.20$, $p < 0.0001$; Bonferroni's multiple comparisons between drug treatments within each timepoint: pre, $p > 0.9999$; light, $p < 0.0001$; post, $p = 0.0074$; $n = 10$.

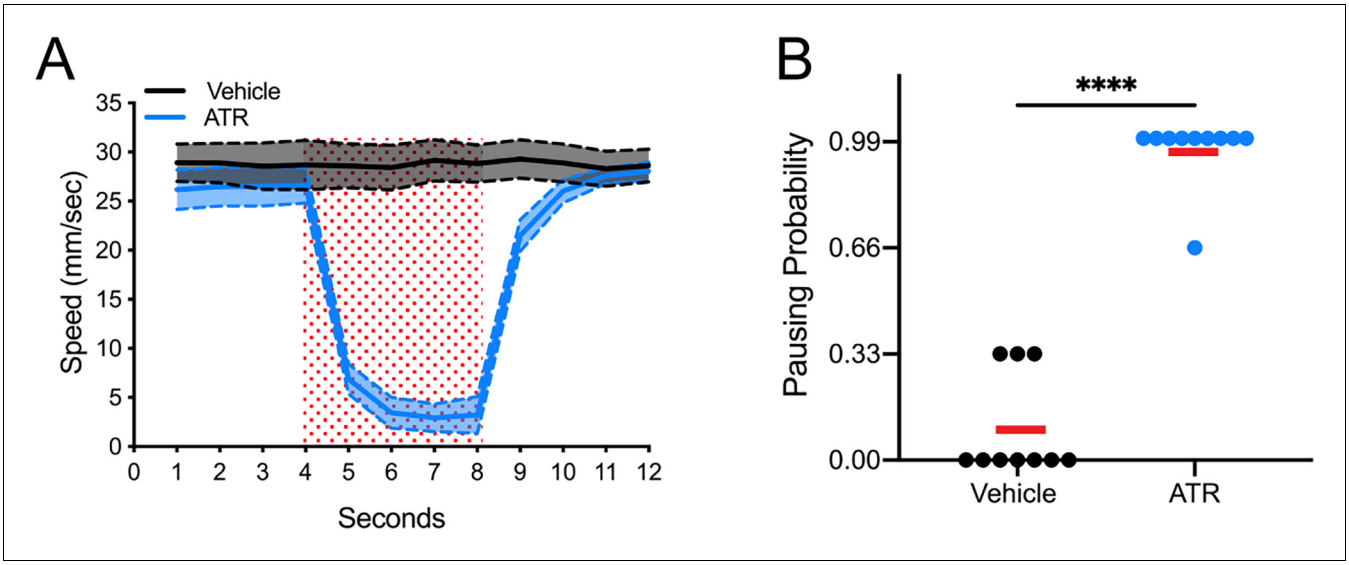

Figure 3—figure supplement 1. Pair1 activation for 4 s arrests forward locomotion but does not cause paralysis in adults in an open field arena. (**A**) Speed (mm/s) of adult flies in an open field arena. Flies were fed food supplemented with all-*trans* retinal (ATR) (blue) or ethanol (vehicle, black). Red square represents the presentation of the light stimulus. (**B**) Probability of pausing upon light activation of Pair1 (ATR treatment, blue) compared to controls (vehicle treatment, black) (t-test, $p < 0.001$; $n = 12$).

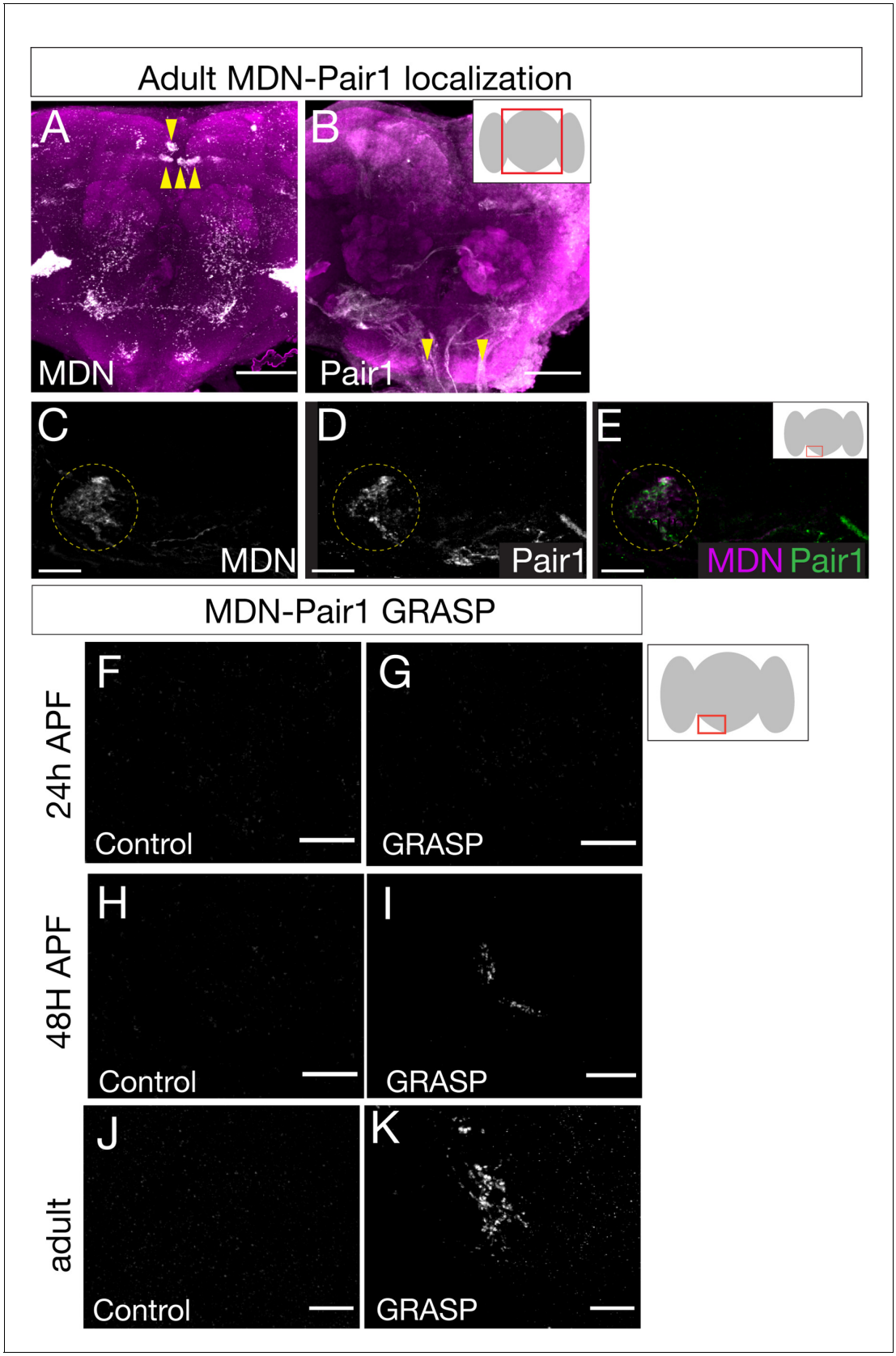

Figure 4. Moonwalker descending neurons (MDN) and Pair1 are synaptic partners in adults. (**A–E**) MDN and Pair1 show close membrane apposition. (**A, B**) MDN neurons (**A**) and Pair1 neurons (**B**) in the adult central brain. Neurons are in white, nc82 counterstain in magenta for whole brain

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orientation; cell bodies marked by yellow arrowheads. Here and in subsequent panels shows maximum intensity projection of volume; anterior, up; dorsal view. Scale bar, 50 μm. Genotype: *UAS-mCD8::RFP, LexAop-mCD8::GFP; VT044845-LexA; R75C02-Gal4*. (**C–E**) MDN neurites (**C**), Pair1 neurites (**D**), and merge (**E**) in the left subesophageal ganglion (red box in schematic). Scale bar, 10 μm. Genotype: *UAS-mCD8::RFP, LexAop-mCD8::GFP; VT044845-LexA; R75C02-Gal4*. (**F–K**) t-GRASP (targeted GFP reconstitution across synaptic partners) between MDN and Pair1. In all panels: Scale bar, 10 μm. Genotype:;; *LexAop-pre-t-GRASP, UAS-post-t-GRASP/R75C02-Gal4*. (**F–G**) Pupal t-GRASP at 24 hr after pupal formation (APF). (**F**) No detectable t-GRASP signal was observed in the subesophageal ganglion without expression of the pre-t-GRASP fragment in MDN. (**G**) t-GRASP signals between MDN and Pair1 were lacking in the subesophageal ganglion. (**H–I**) Pupal t-GRASP at 48 hr APF. (**H**) No detectable t-GRASP signal was observed in the subesophageal ganglion without expression of the pre-t-GRASP fragment in MDN. (**I**) t-GRASP signals between MDN and Pair1 were observed in the subesophageal ganglion. (**J–K**) Adult t-GRASP. (**J**) No detectable t-GRASP signal was observed in the subesophageal ganglion without expression of the pre-t-GRASP fragment in MDN. (**K**) t-GRASP signals between MDN and Pair1 were observed in the subesophageal ganglion.

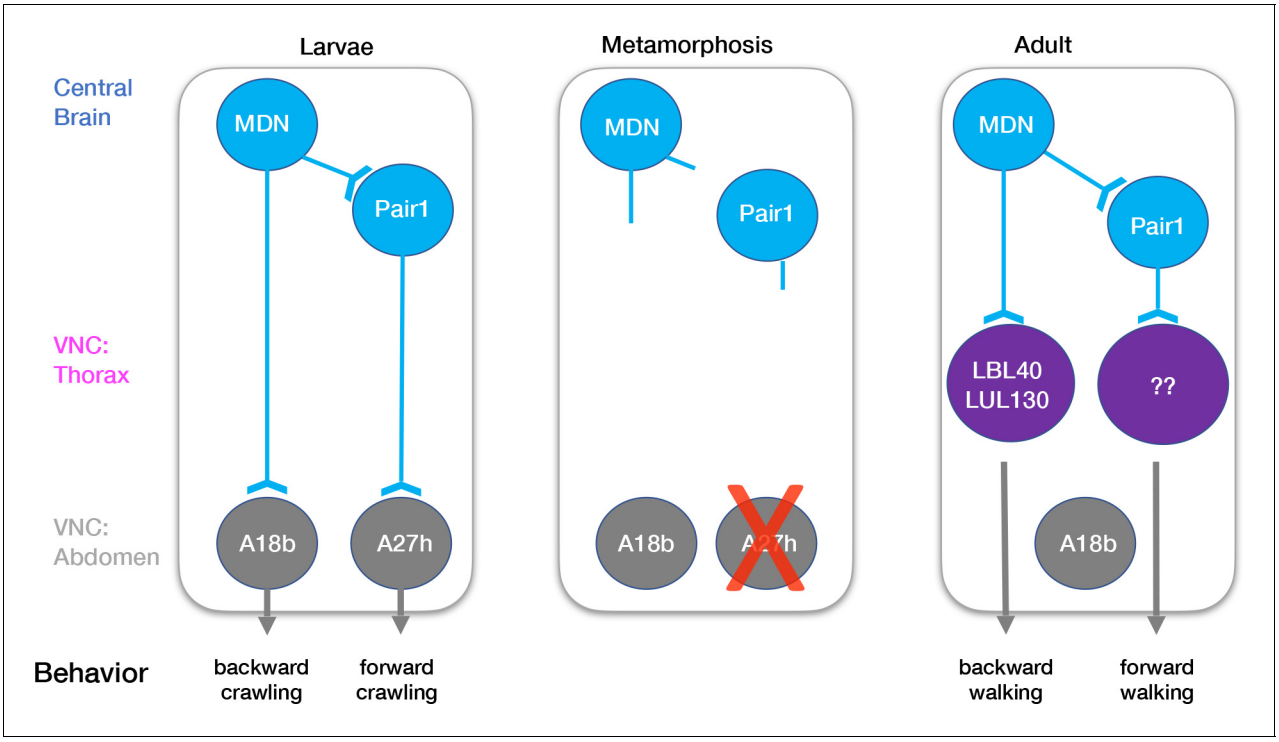

Figure 5. Model describing the moonwalker descending neuron (MDN)-Pair1 circuit in larval and adult stages. In the larvae, MDN and Pair1 neurons are located in the central brain. MDN and Pair1 neurons are synaptic partners. MDN neurons also extend axons into the abdominal region of the ventral nerve cord (VNC) and synapse onto A18b. A18b subsequently regulates backward crawling by synapsing onto motor neurons. Pair1 synapses onto and inhibits the pre-motor neuron A27h, which generates forward locomotion when activated. During metamorphosis, MDN and Pair1 neurons remain in the central brain, drastically prune their neurites, and survive. The A18b neuron and processes remain in the abdomen of the VNC and survives in the adult. The A27h neuron undergoes apoptosis. In the adult, synapses between MDN and Pair1 are re-established. MDN neurons also extend axons into the thoracic region of the VNC and synapse onto LBL40 and LUL130. Activation of LBL40 and LUL130 generates backward walking. MDN does not extend axons into the abdominal region of the VNC and is no longer synaptic partners with A18b. Pair1 neurons extend axons into the thoracic region of the VNC and synapse onto unknown neurons. Activation of Pair1 generates a pausing behavior, likely through the inhibition of neurons generating forward locomotion. In both larvae and adult, MDN and Pair1 neurons (blue) persist and function as a core circuit to regulate locomotion.