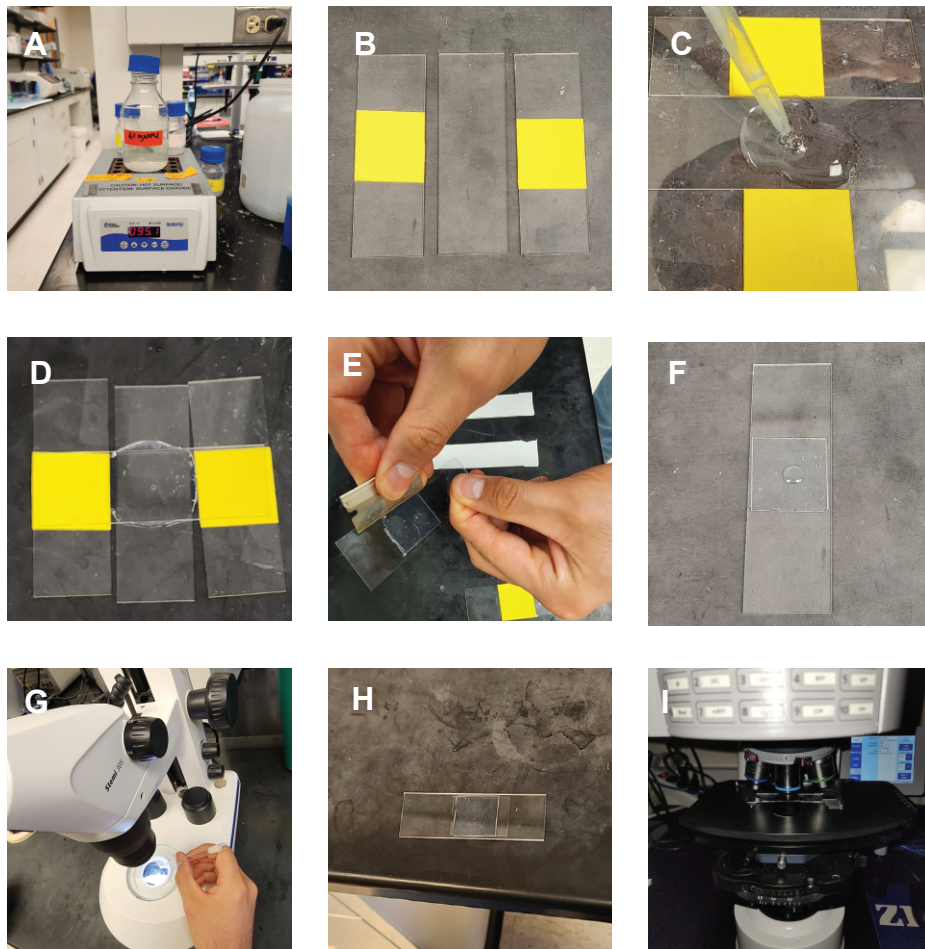


**Figure S1: Preparation of NGM-auxin and NGM-control plates and OP50 seeding, related to Step 1.**

**A.** Prepare nematode growth medium (NGM). Autoclave the NGM and let it cool down at 55 °C (water bath or hot plate) until ready to use. **B.** Add buffers and cholesterol to the autoclave NGM and divide into two bottles for ethanol and auxin. **C.** Prepare fresh auxin (stored at -20 °C). **D.** Prepare the 400 mM auxin (IAA or K-NAA) stock dissolved in ethanol (for IAA) or dH<sub>2</sub>O (for K-NAA). The final solution should be crystal clear as shown above in **E**. **E.** Shield the auxin with aluminum foil throughout. **F.** 400 mM auxin stock solution can be stored at 4 °C for up to a week. **G.** Add the 400 mM auxin to the cooled down (55 °C) NGM to make the final concentration of 4 mM auxin as well as an equal volume of ethanol (or dH<sub>2</sub>O) to the NGM as a control. **H.** Pour 10 mL of auxin-NGM and ethanol-NGM into the respective 55 mm culture petri dish. **I.** Shield the plates of ethanol and auxin and let them dry for a day. **J.** Use concentrated OP50 to allow good bacterial growth on auxin and ethanol plate. **K.** Next day seed the plate with 200 μL of OP50 and let them dry for 2 more days at the room temp and in a sterile place. **L.** Shield the ethanol and auxin plates, that can be stored at 4 °C for up to a month.



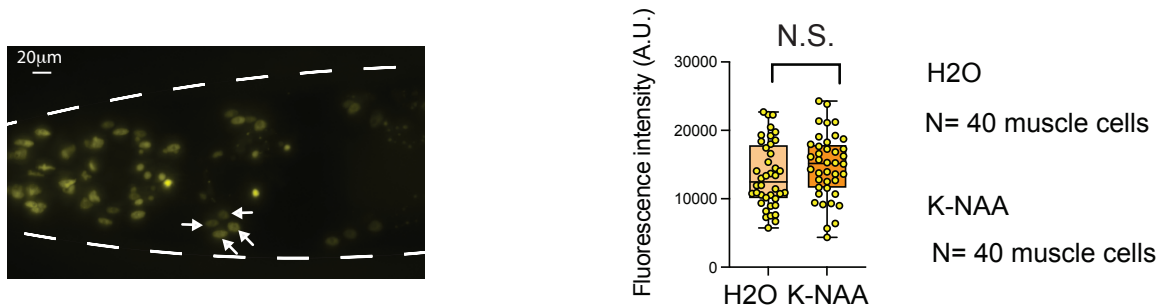
**Figure S2: Preparation of imaging slides, related to Steps 3-5.**

**A.** Prepare 4% agarose solution. Keep the melted agarose on a heat block, at approximately 95 °C. **B.** Place the support slides on each side of a blank slide. **C.** Place one drop (~600L) of warm 4% agarose onto the blank slide using a laboratory pipette with a cut blue tip. **D.** Quickly place a second blank slide on the top of the agarose drop, at a 90° angle to the three slides, and gently press the edges of this slide such that the agarose spreads uniformly. Let the agarose dry for a minute. **E.** Separate the glass slides without perturbing the agarose pad. Use a razor blade to trim excessive parts and adjust the agarose pad. **F.** Place a 10  $\mu$ L droplet of 100mM sodium azide ( $\text{NaN}_3$ ) on the agarose pad. **G.** Pick the worms to be imaged under the dissection scope and put them in the  $\text{NaN}_3$  droplet. **H.** Gently cover the droplet with a coverslip. **I.** Place imaging slide on the microscope stage with the glass coverslip facing the objective lens.



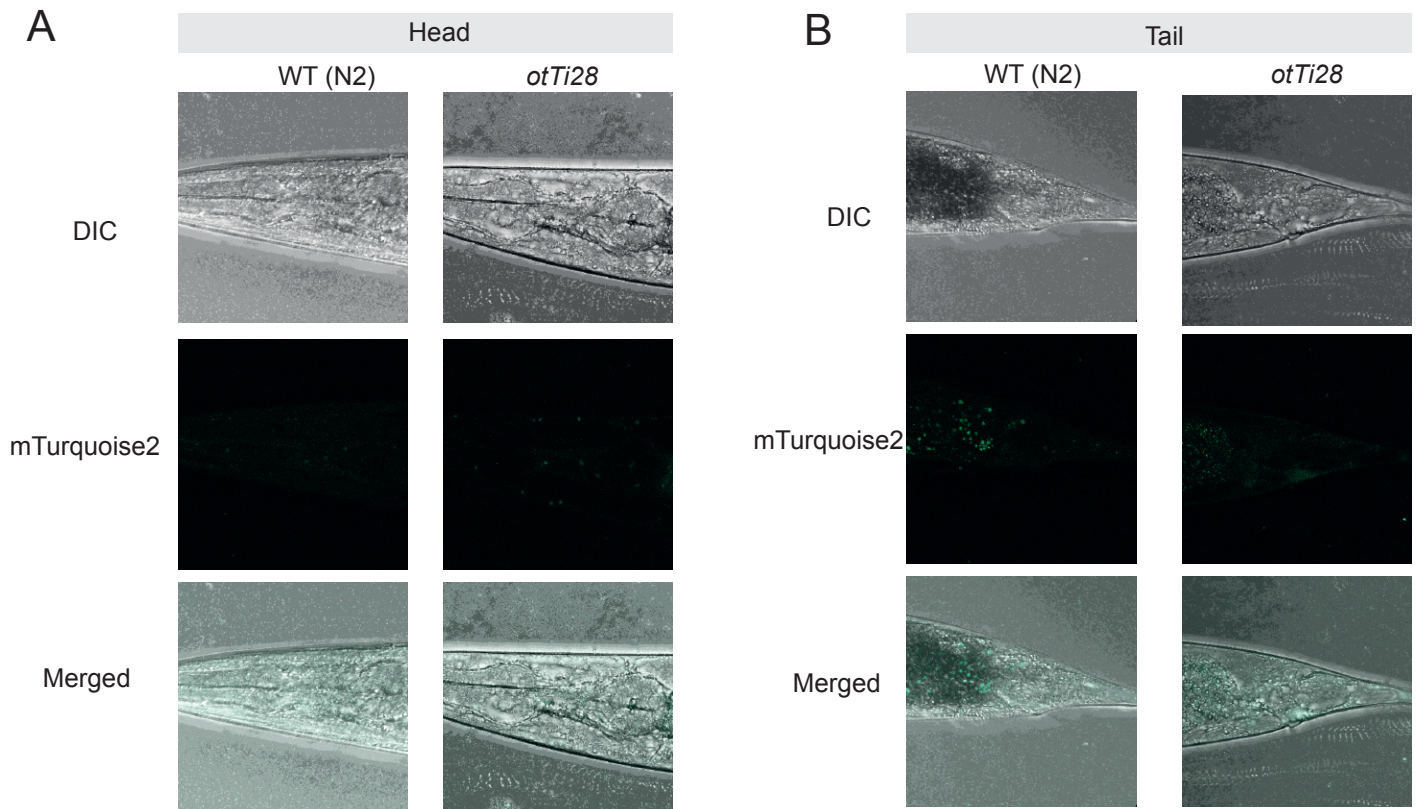
Panneuronal TIR1 expression (*otTi28*)

Nuclear protein: CFI-1 (ARID3) mNG::AID::3xFLAG::CFI-1



**Figure S4: Quantification of fluorescent signal in head muscle cells, related to Steps 15-24.**

Representative image showing mNG::AID::3xFLAG::CFI-1 expression in the *C. elegans* head. In every animal, the same four ventral muscle cells (arrows) expressing the reporter were quantified for fluorescence intensity with Fiji. The panneuronal *otTi28* panneuronal TIR1 line was used. In the fluorescence intensity quantification graph, each dot represents a single muscle cell quantification. All images are lateral and the animal's head faces left. Fluorescence intensity was calculated using the "sum of stacks" function. Box and whisker plots were adopted to represent the quartiles in each graph. The box includes data points from the first to the third quartile value with the horizontal line in box representing the median value. Upper and lower limits indicate the max and min, respectively. N = number of muscle cells analyzed in total per condition. Scale bar: 20 μm.



**Figure S5: Imaging of the panneuronal TIR1-expressing line, related to Steps 6-14 .**

**A-B:** Larval stage 4 (L4) control (N2 strain) and *otTi28* [*unc-11prom8+ehs-1prom7+rgef-1prom2::-TIR1::mTurquoise2::unc-54 3'UTR*] animals were imaged with confocal microscopy. mTurquoise2 expression was not detected in head or tail neurons. This data combined with the modest effects on mNG::Al-D::3xFLAG::CFI-1 protein depletion (Figure 3) suggest that the *otTi28* line expresses TIR1, but at low (undetectable) levels.