

Supplementary Information for

Neuronal segmentation in cephalopod arms

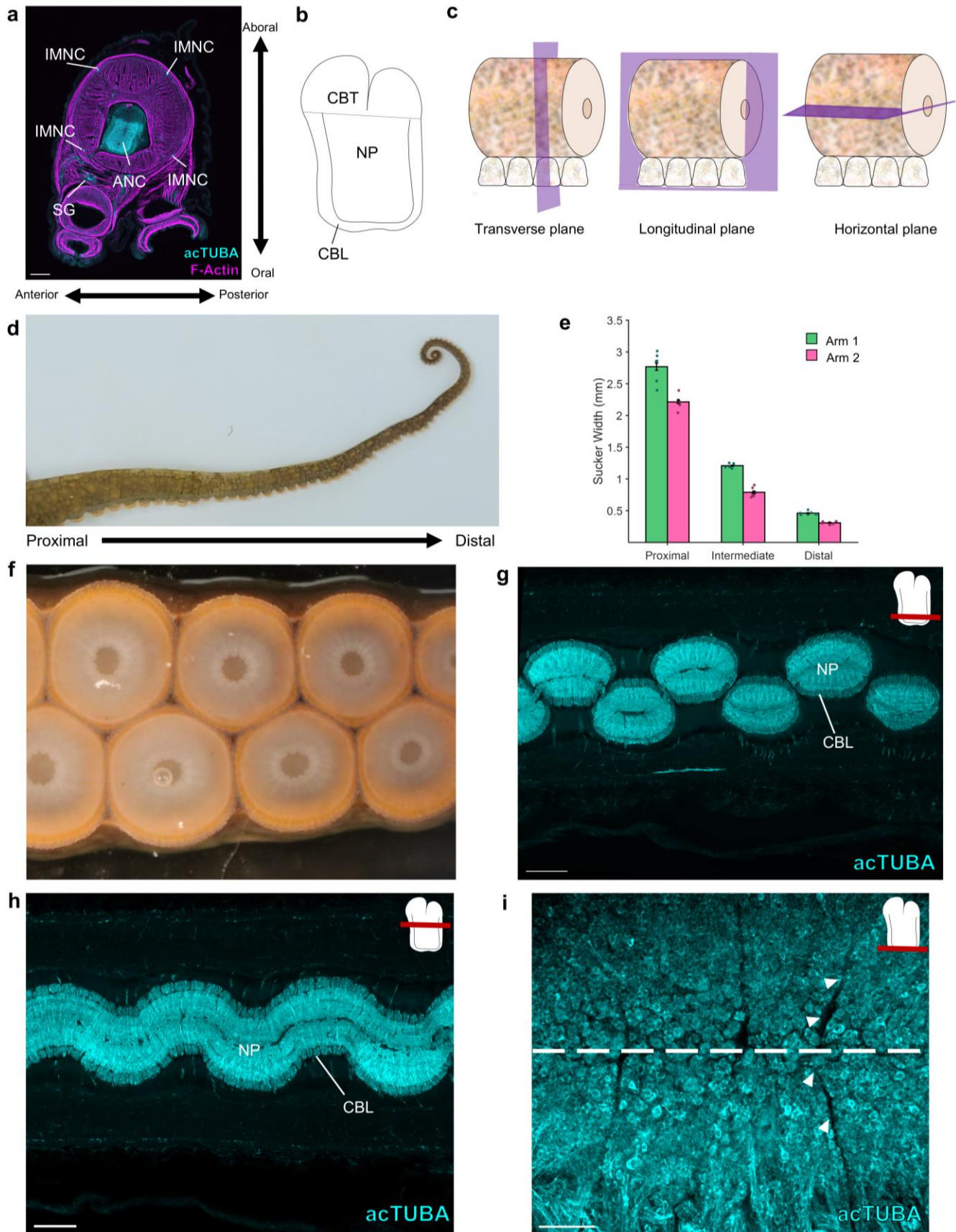
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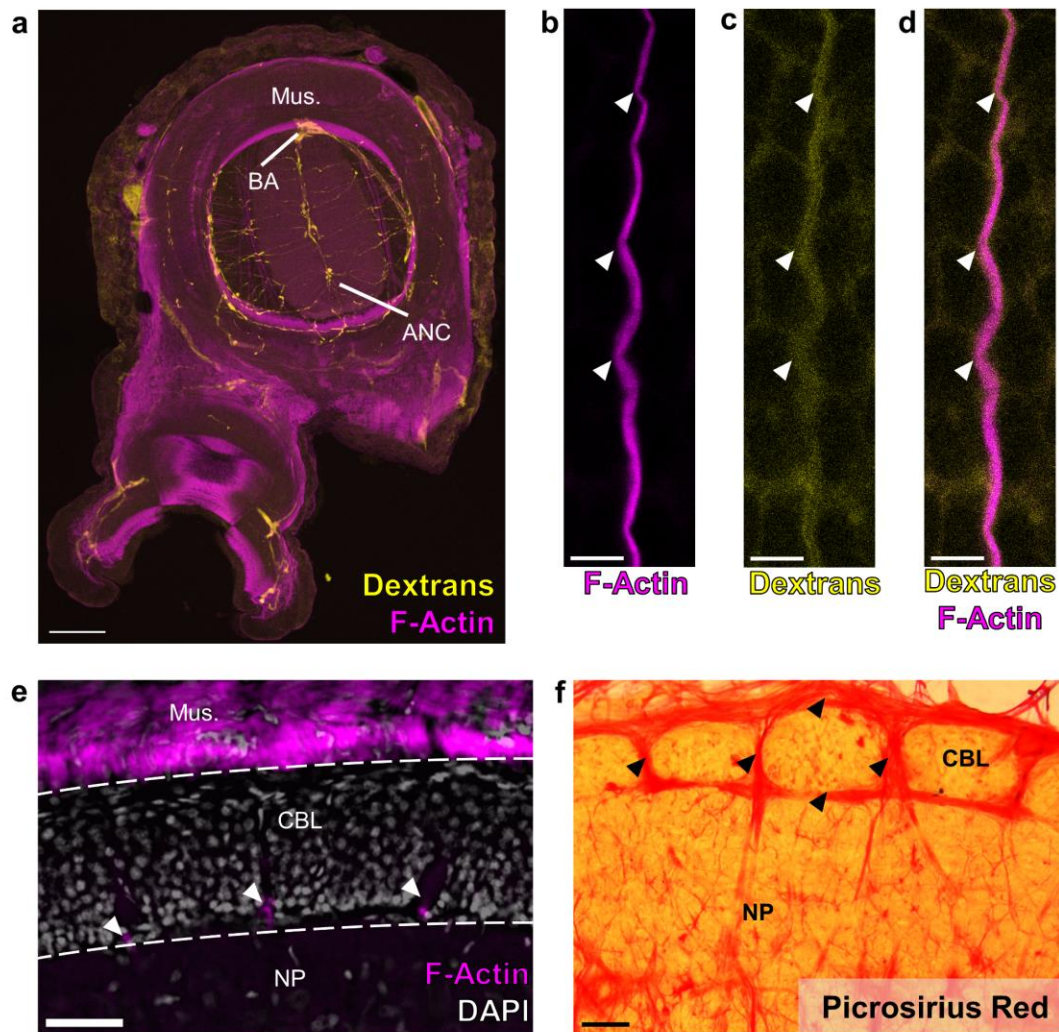
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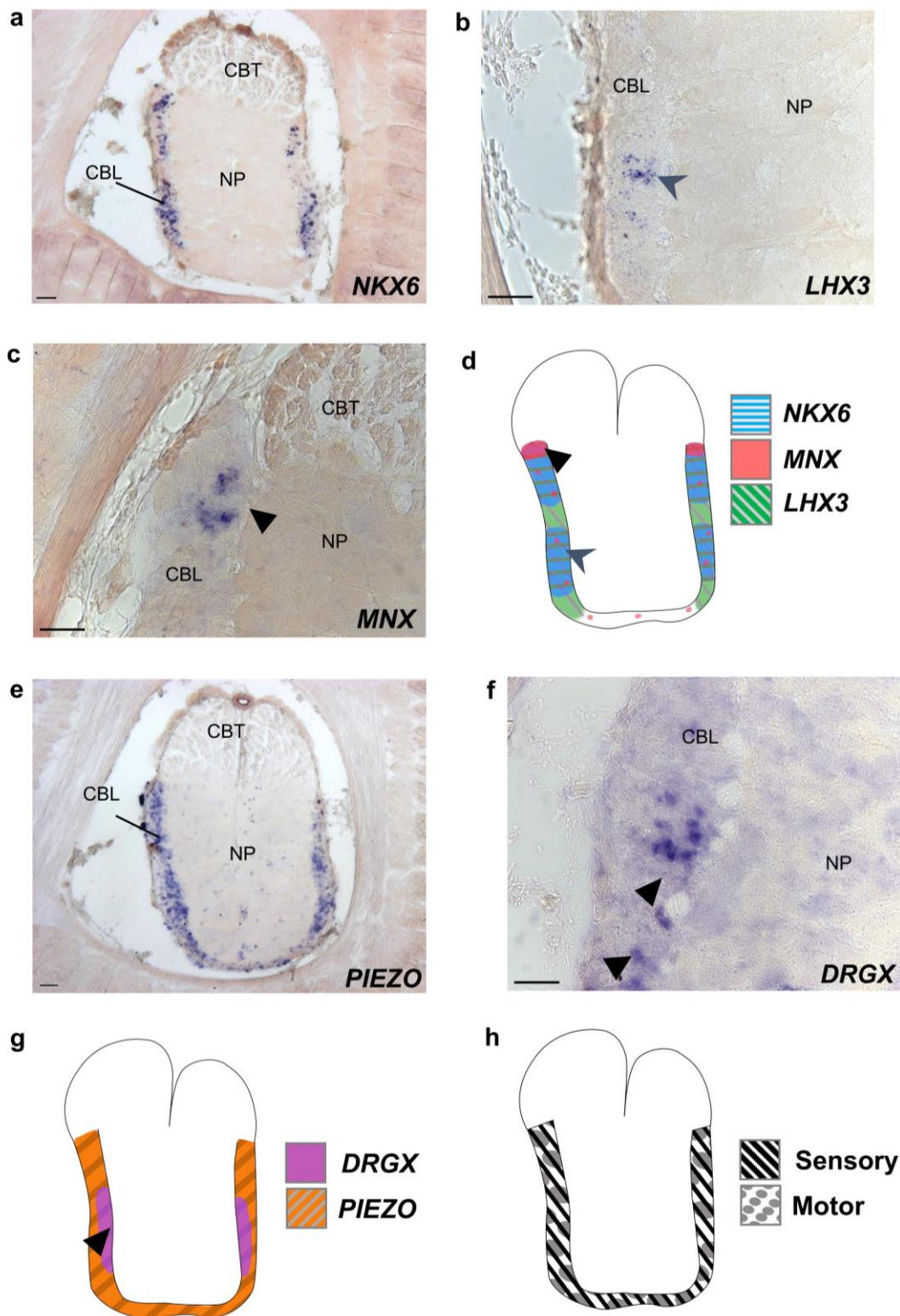


Supplementary Figure 1: Arm anatomy overview. **a**, Transverse section of the arm stained with acTUBA (cyan) and phalloidin (F-Actin, magenta). The main components of the arm nervous system are highlighted. Scale bar: 500 μ m. **b**, Cartoon depiction of the ANC. **c**, The three perpendicular planes of sectioning through the arm. The transverse plane is perpendicular to the long axis of the arm. The longitudinal plane is parallel to the long axis of the arm. The horizontal plane is parallel to the long axis of the arm and to the suckers. **d**, Photograph of the arm of *O. bimaculoides*. From proximal to distal, the girth of the arm decreases. **e**, Sucker width decreases from proximal to distal. Arm 1 (green) and Arm 2 (pink). $n = 6$ suckers per condition, error

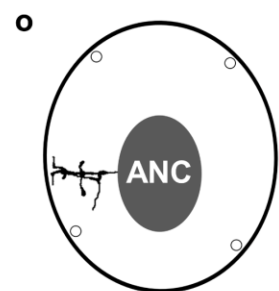
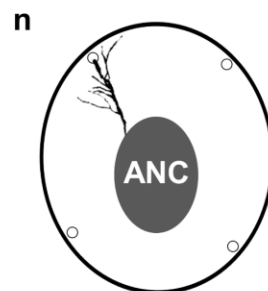
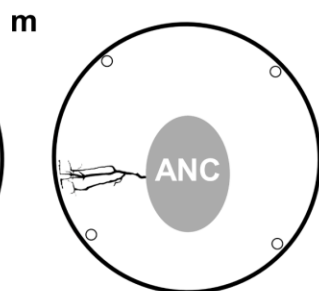
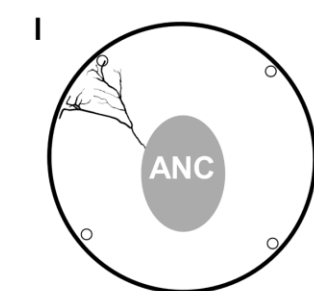
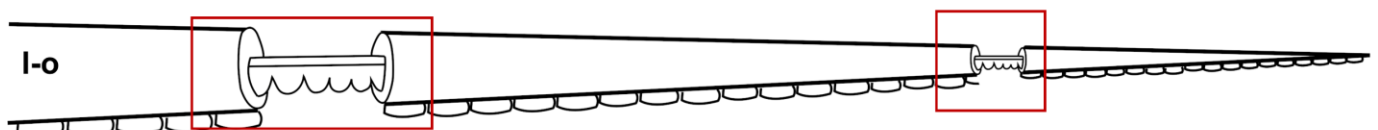
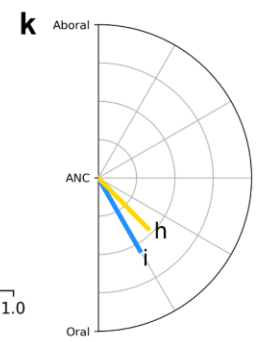
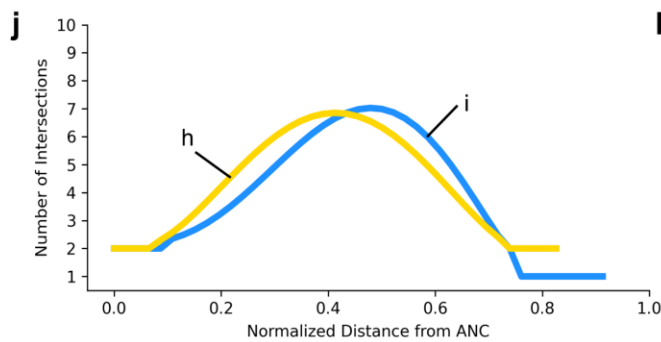
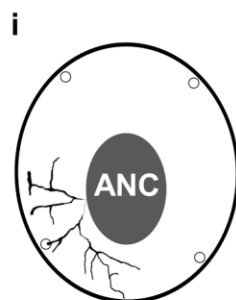
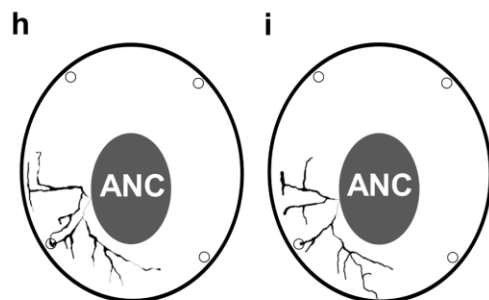
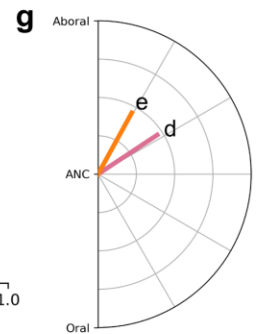
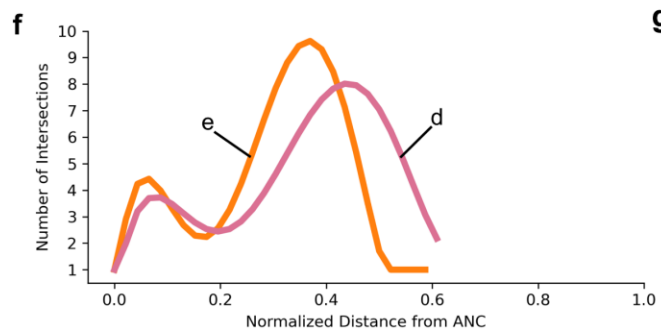
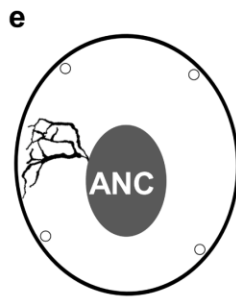
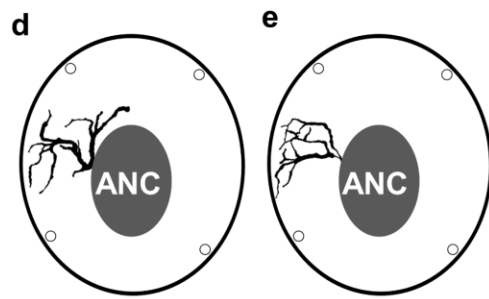
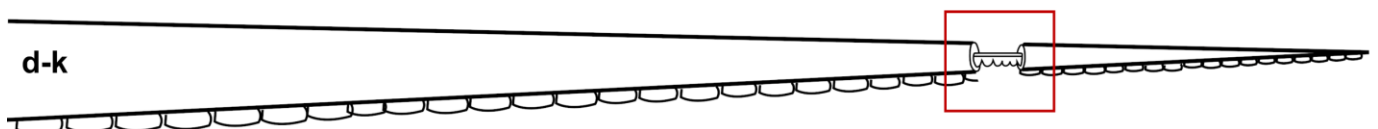
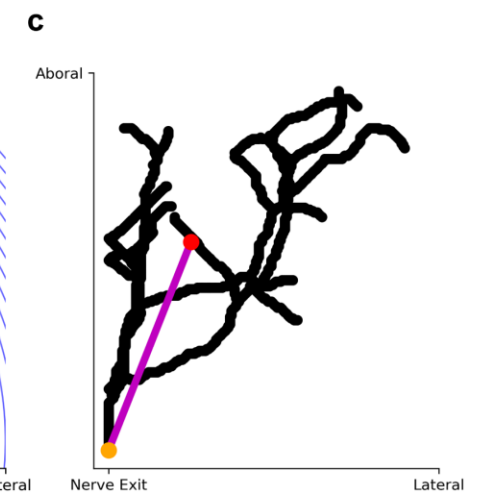
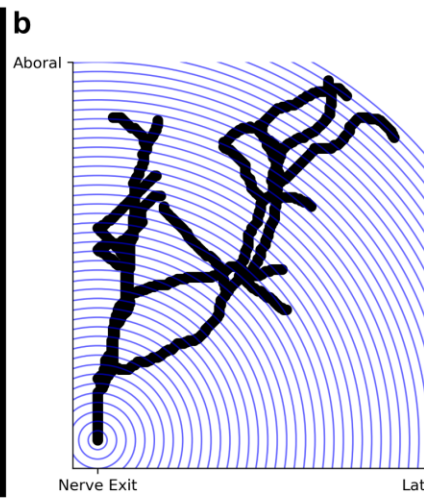
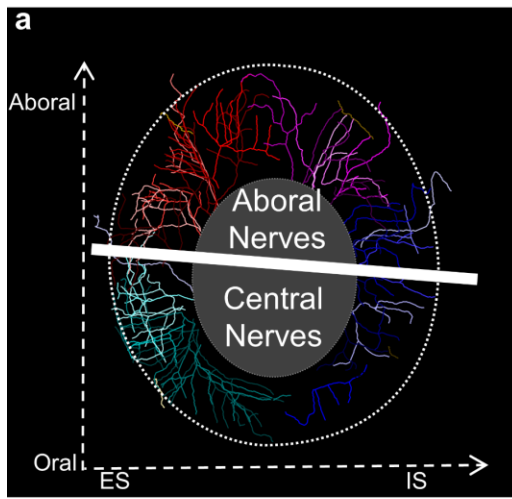
bars +/- sem. **f**, An en face photograph of the suckers shows that the suckers are arranged in two offset rows. **g**, Horizontal section of the ANC through the sucker territory labeled with acTUBA (cyan). The ANC oscillates to the side overlying the sucker. Scale bar: 500 μ m. **h**, Horizontal section of the ANC through the brachial territory labeled with acTUBA (cyan). Scale bar: 500 μ m. **i**, Maximum projection of a horizontal slice whole mount immunolabeled with acTUBA (cyan) through the oral CBL. Segments extend across the midline (denoted with the dashed line, arrowheads indicate septum crossing the midline). Scale bar: 100 μ m. ANC, axial nerve cord; IMNC, intramuscular nerve cord; SG, sucker ganglion; CBT, cerebrobrachial tract; CBL, cell body layer; NP, neuropil.



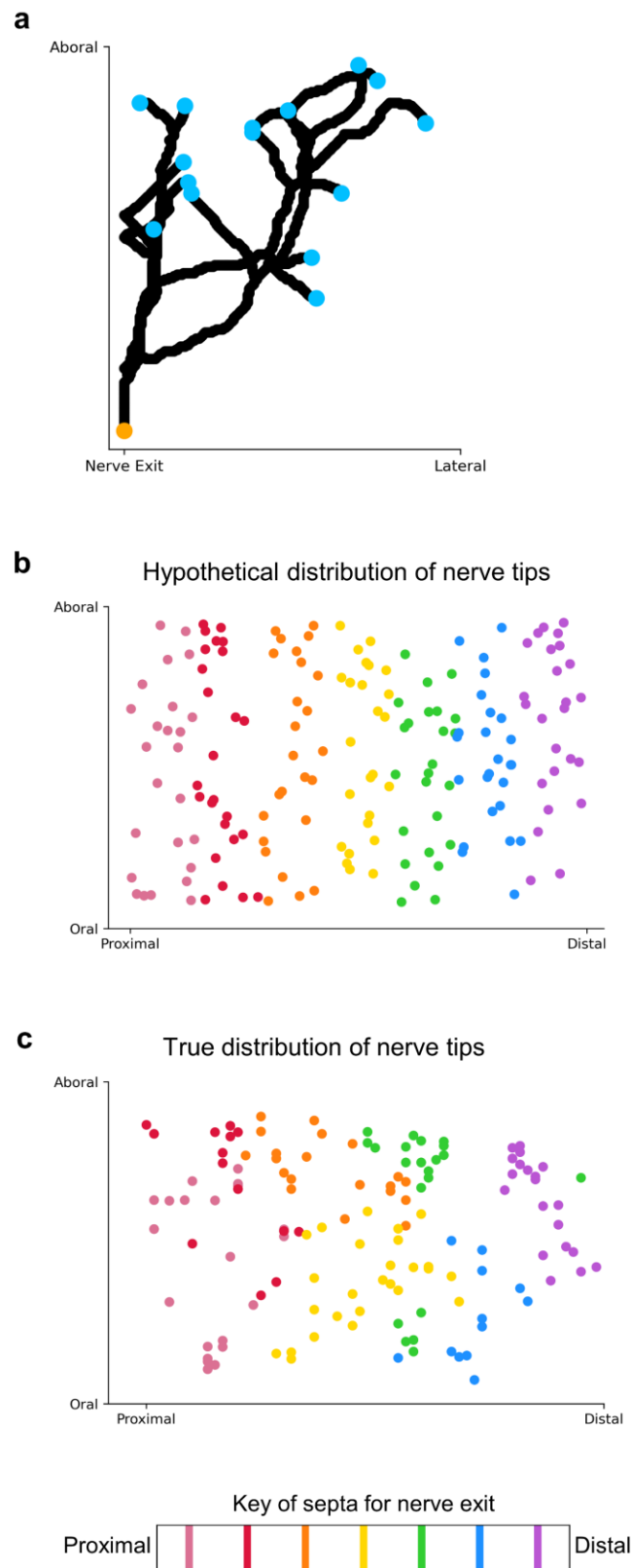
Supplementary Data Figure 2: Septal localization of vasculature and collagen. **a**, Transverse section of arm with dextran labeling (yellow) of the vasculature. F-actin and dextran delivery clearly identify the large brachial artery (BA). Scale bar: 100µm. **b-d**, Maximum projection of a single blood vessel. **(b)** F-actin (magenta) and **(c)** dextran (yellow) **(d)** colocalize. Arrowheads point to bumps in the blood vessel that are highlighted in both labelings. Scale bar: 5 µm. **e**, Horizontal section through the axial nerve cord (ANC) labeled with F-actin (magenta) and DAPI (gray). The vessels are located in the cell body layer (CBL) at the interface between the CBL and the neuropil (NP), indicated by arrowheads. Scale bar: 50 µm. **f**, Horizontal section through the ANC labeled with Picrosirius Red. Arrowheads point to collagen, labeled in red, which wraps around the CBL segments and extends within the full length of the septa. Scale bar: 50 µm. Mus., brachial musculature.



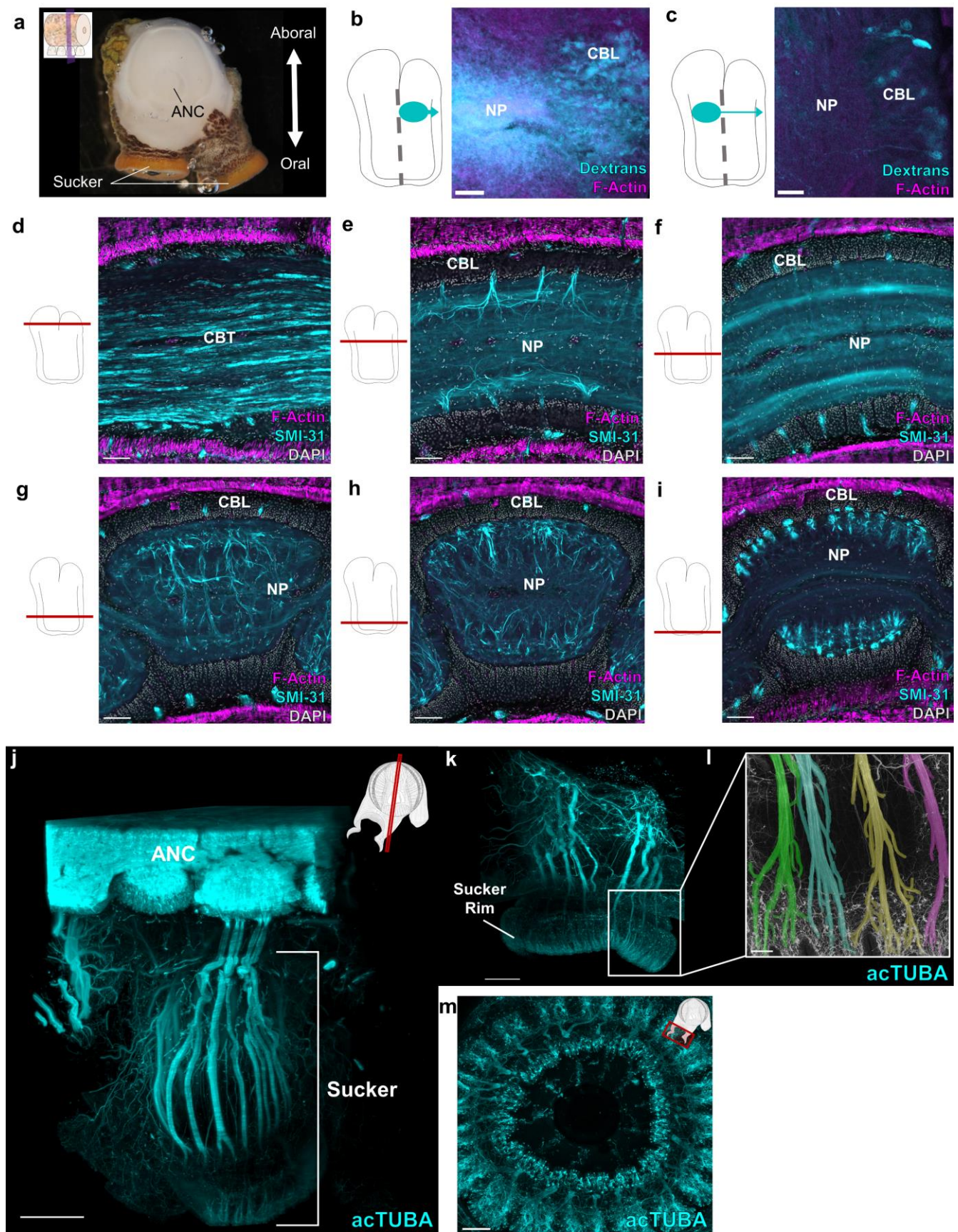
Supplementary Figure 3: Markers of sensory and motor neurons label overlapping territories in the ANC. **a-d**, ISH for motor neuron markers in ANC transverse sections. **(a)** *NKX6* (NK6 homeobox), **(b)** *LHX3* (LIMB homeobox 3), arrowhead points to expression, **(c)** *MNX* (Motor neuron and pancreas homeobox), arrowhead points to expression **(d)** Cartoon summary of motor neuron marker distributions. Expression is extensive in the lateral walls of the CBL. Arrowheads indicate where expression in **b** and **c** was found. **e-g**, Expression of sensory neuron markers in ANC transverse sections. **(e)** *PIEZO* (Piezo type mechanosensitive ion channel component), **(f)** *DRGX* (Dorsal root ganglia homeobox), arrowhead indicates expression. **(g)** Cartoon summary of sensory neuron marker distribution. Expression is also strong in the CBL lateral walls. Arrowhead indicates where expression in **e** was found. **h**, Diagram of overlapping sensory and motor neuron territories in the CBL. Scale bars: 50 μ m. ANC, axial nerve cord; CBL, cell body layer.



Supplementary Figure 4: Nerve fiber analysis. **a**, Brachial nerves segmented out of transverse whole mount slice immunolabeled with acTUBA. Brachial nerve fibers can be split into aboral nerves and central nerves based on ANC exit location. **b**, Diagram depicting Sholl analysis on an example nerve. Branching is characterized as the number of intersections between the nerve fiber and a series of spheres centered at the nerve exit, with an increasing radius of 10 μm . Three-dimensional data were analyzed, with example portrayed here in two-dimensions. **c**, Schematic of the average trajectory, which is a vector created from the nerve exit point to the center of the processes. **d-k**, Within the same slice, similarities in nerve fibers are found separated by more than one segment. **d, e**, Transverse diagrams with two aboral nerves that have similar morphologies. These nerves also have **(f)** similar Sholl profiles and **(g)** average trajectories. **h, i**, Transverse diagrams with two central nerves that have similar morphologies. These nerves also have **(j)** similar Sholl profiles and **(k)** average trajectories. **l-o**, Similarities in nerve fiber morphologies can be found when comparing proximal and distal slices. An aboral nerve from a proximal slice in **(l)** is similar to a selected aboral nerve from a distal slice in **(n)**. A central nerve from a proximal slice in **(m)** is similar to a selected central nerve from a distal slice in **(o)**.

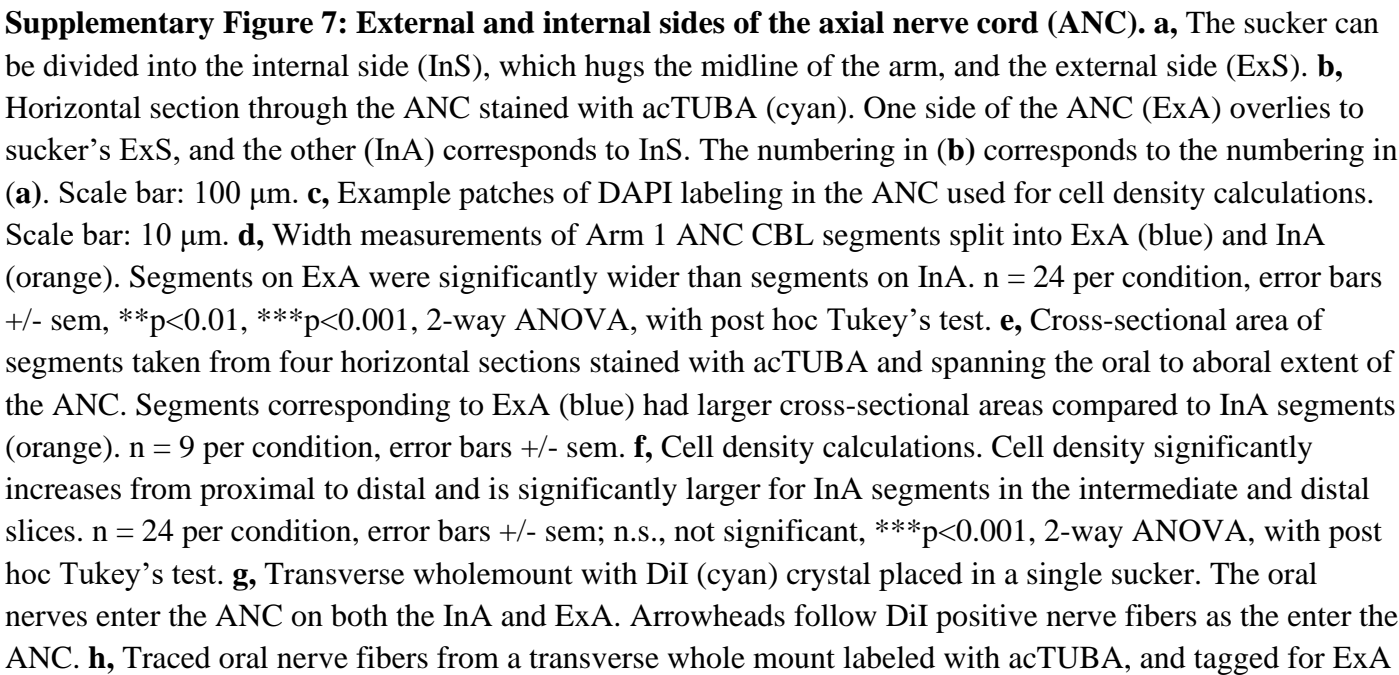


Supplementary Figure 5: Distribution of brachial nerve tips. **a**, Example brachial nerve with nerve tips labeled. **b**, Hypothetical distribution of nerve tips across the proximal-distal axis, colored by ANC exit point. Nerves exiting from one septum fully cover the aboral-oral extent of the brachial musculature and do not intercalate with nerves exiting from other septa. **c**, True distribution of nerve tips across the proximal-distal axis, colored by ANC exit point. Only nerve fibers from multiple septa added together cover the aboral-oral extent of the brachial musculature.



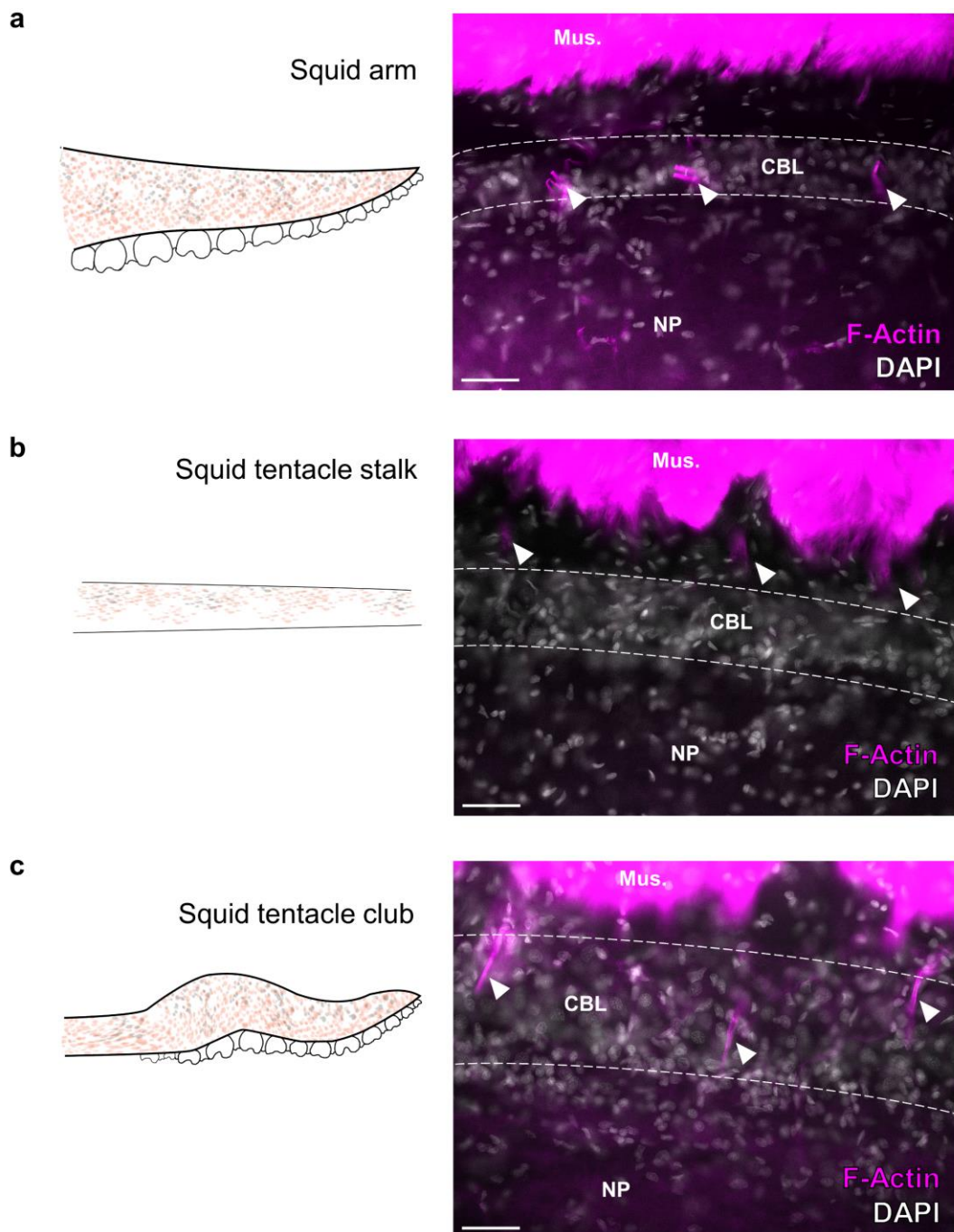
Supplementary Figure 6: Neuropil organization. **a**, Example transverse slice used for tracer injections. Injections of dextrans were made into the axial nerve cord (ANC). **b**, Transverse section of an injection of dextran (cyan) demonstrating ipsilateral connections to the cell body layer (CBL). Scale bar: 50 μ m. **c**, Transverse section of an injection of dextran (cyan) into the neuropil (NP) demonstrating contralateral connections arising from the CBL. Scale bar: 50 μ m. **d-i**, Horizontal series stained with SMI-31 (cyan), F-actin (magenta) and DAPI (gray) through the ANC from aboral (**d**) to oral (**i**). SMI-31 labels a

subpopulation of nerve fibers and is useful for illustrating key selected features of fiber architecture. Scale bars: 100 μm . **(d)** Cerebrobrachial tract (CBT) composed of two massive longitudinally running fiber bundles. **(e)** Brachial territory of the ANC. SMI-31 brachial nerves branch in proximal and distal directions, pooling over segments. **(f)** Interface between the brachial territory and the sucker territory of the ANC. This territory is dominated by longitudinally running tracts. **(g-i)** Sucker territory of the ANC, progressively showing **(g)** clear contralateral connections and links to adjoining suckers, **(h)** more restricted intermediate ipsilateral connections, and **(i)** highly local ipsilateral connections. **j**, Maximum projection of a longitudinally cut whole mount labeled with acTUBA (cyan). The oral nerves originate from a sucker enlargement and directly target the corresponding sucker. Scale bar: 500 μm . **k**, Maximum projection of a whole mount of a sucker labeled with acTUBA. The oral nerves can be traced to the sensory epithelium lining the sucker rim. Scale bar: 500 μm . **l**, Oral nerves false colored in their position in the sensory epithelium. Neighboring nerve fibers target adjoining territories along the sucker rim. Scale bar: 100 μm . **m**, Maximum projection of a horizontal slice through a sucker whole mount labeled with acTUBA (cyan). The sensory epithelium is evenly and densely innervated. Scale bar: 100 μm .



Supplementary Figure 7: External and internal sides of the axial nerve cord (ANC). **a**, The sucker can be divided into the internal side (InS), which hugs the midline of the arm, and the external side (ExS). **b**, Horizontal section through the ANC stained with acTUBA (cyan). One side of the ANC (ExA) overlies to sucker's ExS, and the other (InA) corresponds to InS. The numbering in **(b)** corresponds to the numbering in **(a)**. Scale bar: 100 μ m. **c**, Example patches of DAPI labeling in the ANC used for cell density calculations. Scale bar: 10 μ m. **d**, Width measurements of Arm 1 ANC CBL segments split into ExA (blue) and InA (orange). Segments on ExA were significantly wider than segments on InA. $n = 24$ per condition, error bars \pm sem, $**p < 0.01$, $***p < 0.001$, 2-way ANOVA, with post hoc Tukey's test. **e**, Cross-sectional area of segments taken from four horizontal sections stained with acTUBA and spanning the oral to aboral extent of the ANC. Segments corresponding to ExA (blue) had larger cross-sectional areas compared to InA segments (orange). $n = 9$ per condition, error bars \pm sem. **f**, Cell density calculations. Cell density significantly increases from proximal to distal and is significantly larger for InA segments in the intermediate and distal slices. $n = 24$ per condition, error bars \pm sem; n.s., not significant, $***p < 0.001$, 2-way ANOVA, with post hoc Tukey's test. **g**, Transverse wholemount with DiI (cyan) crystal placed in a single sucker. The oral nerves enter the ANC on both the InA and ExA. Arrowheads follow DiI positive nerve fibers as they enter the ANC. **h**, Traced oral nerve fibers from a transverse whole mount labeled with acTUBA, and tagged for ExA

(blue) and InA (orange) targeting. **i**, Distribution of oral nerve tips traces from a longitudinal whole mount stained with acTUBA. ExA covered 63%; InA, 37%. **j**, Distribution of oral nerve tips traces from a transverse whole mount stained with SMI-31. ExA covered 64.5%; InA, 35.5%.



Supplementary Figure 8: Localization of F-Actin in *D. pealeii* axial nerve cord (ANC). **a**, Horizontal section through the ANC in *D. pealeii* arm stained for F-actin (magenta) and DAPI (gray). F-actin projections are contained within the CBL, indicated by white arrowheads. **b**, Horizontal section through the ANC in *D. pealeii* tentacle stalk stained for F-actin (magenta) and DAPI (gray). F-actin projections are beyond the CBL, indicated by arrowheads. **c**, Horizontal section through the ANC in *D. pealeii* tentacle club stained for F-actin (magenta) and DAPI (gray). F-actin projections are again within the cell body layer, indicated by arrowheads. Scale bars: 100 μ m.