

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	AxioVision v. 4.5 , ImSpector Pro v. 7_124, OlyVIA Viewer software
Data analysis	QuPath v. 0.4.2, Fiji v. 2.1.0/1.53c, Arivis Vision4D software v. 3.1, Python 3.8.17, PyImageJ 1.4.1, SNT v. 4.2.0, Numpy 1.24.4, Matplotlib 3.7.3, MATLAB 2021b Custom ImageJ Macro for calculating cell density, custom MATLAB code for analyzing measurements, and custom python code using PyImageJ and SNT for nerve fiber analysis is available at https://github.com/olsoncs/Neuronal-Segmentation-Cephalopod-Arms

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Raw data for nerve fiber traces and measurements are available at <https://github.com/olsoncs/Neuronal-Segmentation-Cephalopod-Arms> (DOI 10.5281/zenodo.14064124). Example image stacks are available at <https://doi.org/10.5281/zenodo.14064132>. Source Data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Sample size was determined by prior experience and field standards. Statistical methods were not used to determine sample size.

Data exclusions If tissue sections were ripped or otherwise damaged, they were excluded.
For measurements, data were collected along a length of six serial suckers to ensure equal number of samples per group. If the tissue section had more than six suckers, a subset of six serial suckers were selected and the rest excluded. This was a pre-established exclusion criterion.

Replication Data presented were confirmed by at least two markers and at least 2 animals.

Randomization All individuals were randomly selected for each experimental protocol out of a pool of animals.

Blinding Experimenters were not blinded to experiments or results, which is typical for this kind of study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Mouse monoclonal antibody 6–11B-1 (1:500 dilution of ascites fluid; Sigma-Aldrich, Cat#: T6793), mouse monoclonal antibody SMI-31 (1:500 dilution; BioLegend, Cat# 801601), Alexa Fluor® 488 AffiniPure Goat Anti-Mouse IgG (1:500 dilution; Jackson ImmunoResearch, Cat#: 115-545-003) and Cy™3 AffiniPure Donkey Anti-Mouse IgG (1:500 dilution; Jackson ImmunoResearch, Cat#: 715-165-151), Anti-DIG-Alkaline phosphatase (Sigma-Aldrich, Cat#: 11093274910)
Validation	Clone 6–11B-1 was isolated following immunization with sea urchin sperm flagella protein preparations (Piperno, Gianni and Fuller, Margaret T. 1985). It recognizes an acetylated α -tubulin (acTUBA) epitope found broadly but not universally across microtubules, and has been extensively employed to identify axon tracts in vertebrate and invertebrate nervous systems (Chitnis and Kuwada 1990; LeDizet and Piperno 1991; Shigeno and Yamamoto 2002; Baratte and Bonnaud 2009). Clone SMI-31 reacts with a phosphorylated epitope of neurofilament in mammals and neurofilament 220 in squid (Sternberger and Sternberger 1983; Grant et al. 1995; Grant and Pant 2016). Anti-DIG-AP is polyclonal antibody from sheep specific to digoxigenin and digoxin, plant steroids, and shows no cross-reactivity with other steroids, such as estrogens and androgens.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Octopus bimaculoides, adult (1-2 years) and juvenile (6 weeks post hatching).
Wild animals	Wild caught adult <i>Doryteuthis pealeii</i> (n = 3) were obtained from the Marine Biological Laboratory, Woods Hole, MA. Animals were kept in circulating, filtered containment tanks for several days before being deeply anesthetized in 7.5% MgCl ₂ /ASW and dissected. Arm crowns were immersion fixed in 4% PFA/PBS. Arms and tentacles were dissected and cut into 2-4 cm pieces for further processing.
Reporting on sex	Sex was recorded in sexually mature animals, but not studied. Sample includes both sexes.
Field-collected samples	N/A
Ethics oversight	These cephalopod experiments were performed in compliance with the EU Directive 2010/63/EU guidelines on cephalopod use, the University of Chicago Animal Resources Center and the MBL and UChicago Institutional Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A