

Reporting Summary

Nature Research 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 Research 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

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ 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)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), 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 Nikon NIS elements 5.21.03 for interneuron strain recordings, custom matlab acquisition code as described in Venkatachalam et al. 2015 PNAS for pan-neuronal strain recordings.

Data analysis Targettrack 1.0 algorithm presented in this manuscript

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 Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data for key-point tracking are freely available for download and use at:

https://drive.google.com/drive/folders/1_qVKS09Cb4HuZeHzCG63ftxX1cgUimO

Videos illustrating key-point tracking are freely available for download and use at:

<https://drive.google.com/drive/folders/1uMUJu0Jed5HA1f3NbpZIAPDqPdxzI4su>

Sample data for 3D volume tracking and videos freely available for download and use at:

<https://drive.google.com/drive/folders/1-El9nexOvwNGAJw6uFFENGy1DqQ7tvxH>

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 sizes were not pre-calculated. We applied the algorithm to 3 worms for each mode (key-point tracking or volumetric tracking) because that was a reasonable number of tests to run.
Data exclusions	None
Replication	Our article is mainly about the development of an algorithm for image analysis. We thoroughly test the algorithm on the data we collected. There are no experimental conclusions that would suggest we may involve replicating experimental findings. We indicate number of tests / experiments / animals run in all figure captions.
Randomization	Subsets of ground-truth annotations were randomly selected for training the neural networks. Fig. 2 shows the number of such random subsets drawn.
Blinding	Data was put through the automated Targettrack pipeline, which does not involve further manual intervention except for annotating the initial ground-truth images.

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
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

All strains are derived from the N2 Bristol strain. The male tail recordings are from ref. (12). All other worms in this study were young adult hermaphrodites. To record 23 neurons in 26 worm heads, we used the strain ZM9624 hpls675[Prgef-1 GCaMP6::3xNLS::mNeptune + lin-15(+)] X.

The genotypes of the ADS1041, ADS1042, ADS27 and ADS1018 strains are as follows:

ADS27: aeals003[Pmod-1s::GCaMP6s Pttx-3::RFP Punc-122::mCherry]; hpls471[Pglr-1::GCaMP6::wCherry]; lite-1(xu7).

ADS1018: syls317[nlp-40p::NLS::GAL4SK::VP64::unc-54 3'UTR + myo-2p::NLS::mCherry + pBlueScript]; syls423[15xUAS::pes-10::GCaMP6s::SL2::mKate2::let-858 3'UTR + myo-2p::NLS::mCherry + 1kb DNA ladder(NEB)].

ADS1041: csbls30: [Pift-20::BFP + Peat-4::cyOFP1 + Punc-17::mScarlet + Pacr-5::BFP + Prgef-1::mNeptune injected into juSi164]; aeals008[pJH4039 Pift-20 GCaMP6s::3xNLS]; lite-1(ce314) X.

ADS1042: hpls675[Prgef-1 GCaMP6::3xNLS::mNeptune+lin-15(+)] X.; rpls3[gcy-33p::GFP]; vsIs33[dop-3::RFP] V.; egl-46(rp4).

The following interneuron-labeled strains were used:

SJR16 (used in Fig. 3 B-E): aeals009[Prgef-1::GCaMP6s::3xNLS, lin-15]; sjxls9[Pglr-3::mNeptune::GCaMP6s[90ng/ul], Psto-3::NLS-mNeptune[90ng/ul], Pcex-1::NLS-wrmScarlet[90ng/ul], Punc-122::dsRed[20ng/ul], lin-15[40ng/ul]]; lin-15(n765).

SJR17 (used in Fig. S7 C): aeals009; sjxls8[Pnpr-9::NLS-Scarlet[80ng/ul], Pttx-3::Neptune::GCaMP6s[80ng/ul], Pceh-16(extended)::Neptune::GCaMP6s[80ng/ul], Pgcy-28d::mCherry::GCaMP6s[80ng/ul], lin-15 [40ng/ul]]; lin-15(n765).

SJR15 (used in Fig. 2 D-F): aeals009; sjxls8; sjxls9; lin-15(n765).

The ADS1018 strain is a cross of two cGAL strains from ref. (44,45).

Wild animals

None

Field-collected samples

None

Ethics oversight

Not needed for *C. elegans*.

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