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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For all	statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a C	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <u>statistics for biologists</u> contains articles on many of the points above.
Soft	ware and code

Policy information about <u>availability of computer code</u>

Data collection Image data was collected with a custom program written in National Instruments (NI) LabVIEW 2016 64-bit, NI DAQmx, and NI Vision Development Module.

Data analysis

Image data was analyzed with custom code written in MatLAB 2016 or later (64-bit) using resources from CUDA toolkit 10.2 (64-bit), and DIPimage (64-bit). Vutara SRX 6.04.07 was used to visulize reconstructed 3D images. ImageJ 1.50c was used for line profile generation.

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 <u>availability of data</u>

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

Software and hardware plans are available from the following online repositories: CAD File Repository (Autodesk Inventor): https://github.com/4Pi-SMS-consortium/CAD-files; Parts List: https://github.com/4Pi-SMS-consortium/CAD-files/blob/master/PartsList.xlsx; Hardware Control Software: https://github.com/Gurdon-Super-Res-Lab/Microscope-Control. Example Data (Figure 26a) is current available via the following online repository: https://drive.google.com/open?id=1l3vBu9UyKaMJ6h1eGCE8DSaslcNGrG9X. The corresponding authors are available to address any technical questions upon reasonable request.

Field-spe	ecific re	porting			
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\times Life sciences	s Behavioural & social sciences Ecological, evolutionary & environmental sciences				
For a reference copy of t	the document with a	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces stu	udy design			
All studies must dis	sclose on these	points even when the disclosure is negative.			
Sample size	N/A				
Data exclusions	N/A				
Replication	N/A				
Randomization	N/A				
Blinding	N/A				
Reportin	g for sr	pecific materials, systems and methods			
<u> </u>	<u> </u>	about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,			
system or method list	ted 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 & ex		·			
n/a Involved in th	•	n/a Involved in the study			
Antibodies Karyotic		ChIP-seq			
	logy and archaeol	Flow cytometry MRI-based neuroimaging			
	nd other organism				
	search participant	35			
Clinical dat	ta				
Dual use research of concern					
Antibodies					
Antibodies used	Mouse	Mouse Anti-α-Tubulin (Sigma Aldrich, T5168)			
Validation	Comm	mmercial antibodies were consistant with specifications provided by vendors, where over 2000 references are listed.			
Eukaryotic c	ell lines				
Policy information	about <u>cell lines</u>				
Cell line source(s	·)	COS7 ATCC batch 63624240			
Authentication		Cell lines were purchased directly from ATCC and not independently authenticated.			
Mycoplasma contamination		No contamination tests were carried out.			

Commonly misidentified lines (See <u>ICLAC</u> register)

N/A