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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Centrioles were imaged using a Leica TCS SP8 using a 63x 1.4 NA oil objective, with the HyVolution mode2 to generate deconvolved images, with the following parameters. 'HyVolution Grade' at max Resolution, Huygens Essential as 'Approach', water as 'Mounting Medium' and Best Resolution as 'Strategy'. STED imaging was performed on a commercial STED microscope (Expert Line, Abberior-Instruments, Germany) working at repetition rate of 40 Mhz. dSTORM imaging was conducted on an inverted microscope (Zeiss Axio Observer.Z1, Carl Zeiss Microscopy) equipped with a 100x oil-immersion objective (alpha Plan-Apochromat 100x/1.46 Oil DIC, Carl Zeiss Microscopy) and a 63x water objective lens (LD C-Apochromat 63x/1.15 W Corr M27, Carl Zeiss Microscopy).

Data analysis	The extraction of individual particles in input volumes was realized manually with the software ImageJ, version 1.51s. A drift correction was applied in the acquired stacks of images with the ImageJ plugin StackReg: http://bigwww.epfl.ch/thevenaz/stackreg/. The code for particle averaging was developed by the authors and is available on the GitHub repository: https://github.com/dfortun2/U-ExM. This is a Matlab code, with a graphical interface for each step of the reconstruction. We refer to the README file of the repository for more details. 2D Super-resolution images were reconstructed using the ImageJ plugin ThunderSTORM 5 and for 3D images the open source software rapidSTORM 3.36 was used . The Intensities of clathrin-coated pits were normalized to the maximum intensity value and double guassian fits were fitted to the Intensity profiles using the software Origin (OriginLab, Northampton, MA).

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/reviewers upon request. 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

The data that support the findings of this study are available from the corresponding authors upon request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	We analyzed in most experiments around 30 isolated centrioles (10 centrioles per each three independent experiments). Note that we stated in the manuscript how many centrioles were analyzed in each experiment. We did not use a predetermined sample size. Considering the time of acquisition, we acquired 10 centrioles per experiment.
Data exclusions	To measure centriole diameter, we solely quantify nearly perfect top view centrioles to avoid any biais due to tilted centrioles. We stated this in the online methods section (Measurements of centriole diameter) and made a supplementary figure to explain this choice (Supplementary Fig.2). For the in cellulo flagella analysis, we analyzed only fully expanded flagella and we specified this in the online methods section.
Replication	All experiments, imaging and analysis were carried out 3 times independently, unless specified otherwise for Figures 2b, 3b-f, 4c, which were performed once. This is reported in the Online method (statistics and reproducibility).
Randomization	This is not relevant for our study because we selected only nearly perfect top and lateral views of centrioles. In a given gel, centrioles are found in many orientations.
Blinding	Blinding is not relevant for our study for the same reasons as specified above.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

-rabbit polyclonal anti-polyglutamate chain (PolyE, IN105), reference AG-25B-0030-C050, Adipogen, dilution 1/500
-mouse monoclonal anti-alpha tubulin (DM1alpha), reference T6199, Sigma, dilution 1/500
-rat monoclonal anti-alpha tubulin (YL1/2), Abcam, ab6160, dilution 1/500
-goat anti-rabbit Alexa Fluor 488 IgG (H+L), Invitrogen A11008, dilution 1/400
-goat anti-mouse Alexa Fluor 488 IgG (H+L), Invitrogen A11029, dilution 1/400
-goat anti-mouse Alexa Fluor 568 IgG (H+L), Invitrogen A11004, dilution 1/400
-anti-rabbit STAR 580, Aberrior, S-12-2015Hp, dilution 1/400
-anti-mouse STAR RED, Aberrior, S-08-2016Hp, dilution 1/400
-A1647 conjugated F(ab')2 fragment of goat anti-rabbit, reference A-21246, ThermoFisher, dilution 1/200
-MitoTracker red CMXRos, M7512, InVitrogen, 100nM
-rabbit monoclonal anti-TOMM20 (EPR15581-39), ab186734 Abcam, dilution 1/200
-anti-rat Cy3, Jackson ImmunoResearch, 712-166-153, dilution 1/400
-mouse anti-alpha tubulin (B-5-1-2) Sigma T5168, dilution 1/500 (6.7mg/ml)
-rabbit anti-clathrin heavy chain, Abcam, dilution 1/500
-Alexa Fluor 488 F(ab`)2 of goat anti rabbit IgG (2mg/ml, 1:200, A11070, ThermoFisher)
-Se Tau-647-NHS (K9-4149, SETA BioMedicals) conjugated to F(ab') 2 of goat anti-Rabbit IgG (SA5-10225, ThermoFisher), 1.5mg/ml, 1/200
-DNA-dye Hoechst 3342, C10340, Invitrogen, 10mg/ml, 1/1000
-Alexa Fluor 647 F(ab`)2 of goat anti rabbit IgG, A-21246, ThermoFisher, 2mg/ml, 1/200

Validation

-PolyE antibody (IN105) recognizes specifically glutamate chains of four or more glutamates. https://adipogen.com/ag-25b-0030-anti-polyglutamate-chain-polye-pab-in105.html/
-DM1A antibody recognizes the following epitope in alpha tubulin: aa 426-450. https://www.sigmaaldrich.com/catalog/product/sigma/t6199?lang=fr&region=CH

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

U2OS: cell line provided by Erich Nigg (Biozentrum, Basel, Switzerland). COS-7 were purchased from CLS Cell Line Service GmbH.

Authentication

None of the cell lines used were authenticated

Mycoplasma contamination

U20S cell lines were negative for mycoplasma contamination. COS-7 were not tested for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The cell wall-less Chlamydomonas strain CW15- used in this study was grown in liquid TAP (Tris-acetate-phosphate) buffer at ~22°C or on solid TAP plates with 1.5% agar.

Wild animals

no wild animals were used in this study.

Field-collected samples

no field-collected samples were used in this study.