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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	The Zeiss Crossbeam 540 system was controlled by Atlas5 software. The STED microscope was controlled by Imspector software.
Data analysis	Immuno-EM micrographs were analyzed manually on print outs. Confocal image acquisition was performed with a Leica SP8 microscope using LAS X 3.1.1.15751. Nansocopy image acquisition was performed on an Abberior STED microscope using Imspector 0.12.9862 – 0.14.13919. FIB-SEM image acquisition was performed with Atlas 5 software. Segmentation and 3D reconstruction of the FIB-SEM data set was done by semiautomatic line tracking utilizing the software package IMOD (Version 4.9) (http://bio3d.colorado.edu/imod/). Contour lengths of CMs in FIB-SEM slices were analyzed using Image J/Fiji (version of 2017) and Microsoft Excel 2016.

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 author upon reasonable 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

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	For the immuno-gold analysis, no statistical method was used to determine sample size. Sample size was chosen based on previous experience and standards in the field. For most processes (i.e. early or late assembly, etc.) the localizations of several different proteins were analyzed. We measured at least 100 localization data points for each protein. Increasing the number of data points (to up to 450 data points) did not change the conclusions drawn on 100 data points.
Data exclusions	No data were excluded from the analysis.
Replication	All light microscopy experiments were done in triplicate. For immuno-gold EM, at least three individual slices were decorated and analyzed. At least 100 gold particles were assigned to the CM or the IBM for each protein localization reported. All attempts of replication were successful and gave similar results.
Randomization	Randomization of the immunolocalization data was not required, as we measured a physical value, i.e. the distance of the gold particle from the IBM, which is not influenced by the observer. Still, a subset of the data sets has been analyzed in a double-blind approach, resulting in the same distribution values.
Blinding	The persons performing sample preparation and EM imaging were unaware of the sample identity. A subset of the data sets has been analyzed in a double-blind approach, resulting in the same distributions as in the non-blinded analysis. All localization data have been documented and are available upon reasonable request.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	Cor1 and Cox2 antisera were provided by Prof. H. Herrmann, University of Kaiserslautern.

Antibodies

Antibodies used	Detailed information about the antibodies used in this study are provided in Suppl. Tab. 2.
Validation	In addition to the information provided in Suppl. Tab. 2, further validation of the antibodies (Western blots on yeast cells lysates) is shown in Suppl. Fig 6.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging