# nature portfolio

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Last updated by author(s):	Aug 15, 2022

## **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 <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	Confirmed
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
x	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
X	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.
X	A description of all covariates tested
x	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 <i>Give P values as exact values whenever suitable.</i>
X	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	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 <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

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

Data collection

Leginon (J Struct Biol 151, 41-60)

Data analysis

Appion 3.3, Relion 3.0, Gctf 1.06, Motioncor 2 1.1.0, PyMOL 2.2.3, Chimera 1.13, Coot 0.8.9.1, Phenix 1.18, cryoSPARC v1, ChimeraX 1.2.5, MetaMorpheus V0.0.320, xVis online server (https://xvis.genzentrum.lmu.de/login.php), PyXlinkViewer v3, Clustal Omega 1.2.1

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.

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

Cryo-EM reconstruction of the augmin complex has been deposited in the Electron Microscopy Data Bank under accession number EMD-25387. Coordinates for the atomic model of augmin have been deposited in the Protein Data Bank under accession number 7SQK. Mass spectrometry data has been deposited in the PRIDE Database under submission number PXD031411 [https://www.ebi.ac.uk/pride/archive/projects/PXD031411]. The source code of "rockstar.py" is available on GitHub [https://github.com/zhuangli200/Rockstar]. Single protein predictions for HAUS1-8 are available from the AlphaFold Protein Structure Database.

#### Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

### Field-specific reporting

Life sciences

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

Ecological, evolutionary & environmental sciences

Behavioural & social sciences For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

#### Life sciences study design

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

Sample size	No sample-size calculation was performed. For cryo-EM, the actual sample size was increased until no further improvement in resolution was obtained in the cryo-EM maps.
Data exclusions	Cryo-EM particle images that fall into 2D averages or 3D classes with poor features were excluded from final reconstruction.
Replication	The crosslinking experiments were carried out as triplicates and reliably reproduced.
Randomization	Randomization is not relevant for the crosslinking experiments in this study. For cryo-EM, randomization was internally used by data processing software in initial particle assignment for 2D classifications and generation of half maps for resolution estimation.
Blinding	Blinding was not relevant to this study. Blinding is not typically performed for the crosslinking and structural studies reported in this paper.

#### 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 sy	ystems Methods		
n/a Involved in the study	n/a Involved in the study		
X Antibodies	ChIP-seq		
Eukaryotic cell lines	Flow cytometry		
Palaeontology and archaeology	pgy     MRI-based neuroimaging		
Animals and other organism			
Clinical data			
Dual use research of concern	ı		
•			
Eukaryotic cell lines			
Policy information about <u>cell lines</u>	and Sex and Gender in Research		
Cell line source(s)	Sf9, clonal isolate derived from the parental Spodoptera frugiperda cell line IPLB-Sf-21-AE, Expression Systems, LLC Cat. # 94-001F		
	High Five™ (BTI-TN-5B1-4), ThermoFisher Cat. # B85502		
Authentication	No cell lines were authenticated		
Mycoplasma contamination	The cell lines were not routinely tested for mycoplasma contamination.		
Commonly misidentified lines (See ICLAC register)	None		