

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 [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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> 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	Slidebook 6.0.22 (3i Intelligent Imaging Innovations), Visiview v.4.4.0.11 (Visitron Systems), LAS X version 4.9.0.30221 (Leica Microsystems), softWoRx-DV-AcquireUltra-1.2.3-RC1x86_64 (Cytiva), Tomography 5 Software (Thermo Fisher Scientific), MetaXpress (Molecular Devices)
Data analysis	Fiji v1.54f, Origin 2024b v10.1.5.132, JPKSPM Data Processing, WSxM software, BIOP JaCoP plugin (https://github.com/BIOP/ijp-jacob-b), IMOD (Mastrorade and Held, 2017; Kremer et al., 1996), Dynamo (Castaño-Díez et al., 2012), MetaXpress (Molecular Devices)

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.

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Source data for graphs is provided in this manuscript. Fluorescence microscopy data are provided at FigShare image repository (doi: 10.6084/m9.figshare.30901727) upon publication. Original Western blot images are provided as supplementary data files. Subtomogram averages (Access code EMD-56110) and tomograms (Access code: EMD-56112) are provided upon publication at the Electron Microscopy Data Bank (EMDB). All other data is available upon reasonable

request from the corresponding authors.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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

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	No statistical methods were used to determine the sample size. The sample size in each experiment was determined based on our previous experience and previous publications. All in vitro reconstitution assays and cell biology experiments were repeated at least three times. PREM experiments and AFM experiments were repeated at least twice. In high content imaging experiments, at least 1000 cells were imaged, and experiments were repeated three times.
Data exclusions	No data were excluded
Replication	All replicates were successful.
Randomization	In vitro reconstitution assays were randomized by measuring samples in different order in each experimental replicate. High content samples were randomized by plating cells in different order in 96-well imaging plate.
Blinding	In vitro reconstitution assays, sample blinding during sample preparation step was not possible, but samples were analysed blinded. PREM samples were blinded after sample preparation step and before imaging. Immunofluorescence staining samples were blinded before imaging.

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	Methods
n/a	n/a
<input type="checkbox"/> <input checked="" type="checkbox"/> Involved in the study	<input checked="" type="checkbox"/> <input type="checkbox"/> Involved in the study
<input type="checkbox"/> <input checked="" type="checkbox"/> Antibodies	<input checked="" type="checkbox"/> <input type="checkbox"/> ChIP-seq
<input type="checkbox"/> <input checked="" type="checkbox"/> Eukaryotic cell lines	<input checked="" type="checkbox"/> <input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/> <input type="checkbox"/> Palaeontology and archaeology	<input checked="" type="checkbox"/> <input type="checkbox"/> MRI-based neuroimaging
<input checked="" type="checkbox"/> <input type="checkbox"/> Animals and other organisms	
<input checked="" type="checkbox"/> <input type="checkbox"/> Clinical data	
<input checked="" type="checkbox"/> <input type="checkbox"/> Dual use research of concern	
<input checked="" type="checkbox"/> <input type="checkbox"/> Plants	

Antibodies

Antibodies used	HRS, Anti-HGS Abcam Ab72053
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EEA1, Anti-EEA1 BD Biosciences 610457
 LBPA, LBPA antibody serum Jean Gruenberg lab, University of Geneva -
 LAMP1, Anti-LAMP1 (H4A3) Developmental Studies Hybridoma Bank (DSHB) H4A3
 Actin, Anti-actin Sigma-Aldrich A2066

Validation

anti-HGS: <https://www.abcam.com/en-us/products/primary-antibodies/hgs-antibody-ab72053>
 anti-EEA1: https://www.bdbiosciences.com/en-eu/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-eea1.610457?tab=product_details
 LAMP1: <https://dshb.biology.uiowa.edu/H4A3>
 anti-actin: <https://www.sigmaaldrich.com/Fl/en/product/sigma/a2066>
 anti-LBPA: <https://doi.org/10.1038/32440>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

HeLa MZ cells were obtained from ACCESS core facility of the University of Geneva, Switzerland
 HeLa Kyoto cell lines were obtained from Prof Harald Stenmark lab, University Hospital Oslo, Norway

Authentication

HeLa cell lines were authenticated by Microsyth, and they had 100% identity to reference HeLa cells (ATCC: CCL-2)

Mycoplasma contamination

Cell were tested negative for mycoplasma infection by Eurofins

Commonly misidentified lines
(See [ICLAC](#) register)

HeLa cell lines are not listed as a commonly misidentified cell lines.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a