

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 |
| ☐ | ☒ The exact sample size (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Commercial software: EPU v3.7 (cryoEM data collection); Digital Micrograph v3; Zeiss ZEN 2.1 (fluorescence microscopy imaging); Olympus VS200 ASW v4.2.1 (slide scanning imaging); Nikon Elements v5.42.07 (live cell fluorescence microscopy); BioRad Image Lab Touch v2.4 (imaging of blots and gels); UNICORN v5.0 (AKTA for protein purification); BD FACS Diva v9.0 (flow cytometry); FlexControl v3.3 (MALDI-TOF); LAS X v3.9.1.28433 and iFLM v1.4.1 (cryo-fluorescence); AutoTEM v2.4 (cryo-FIB milling); Tomography 5 (cryo-ET) No custom software.
Data analysis	Open source and commercial software: RELION v5.0, cryoEF v1.2, pyEM v0.4, cryoSPARC v4.2-4.6 (cryoEM data analysis); AlphaFold 3, ChimeraX v1.7-1.10, ISOLDE v1.10, coot v0.9, Phenix v1.21 (atomic model building, refinement, and visualization); Fiji v1.54p (fluorescence image display); GraphPad Prism v10, Anaconda v3 for Python v3.6 (graphs); FlowJo v10 (flow cytometry); Qupath v0.6 (slide scanner image display and analysis); ConSURF web server (2022 version) (conservation analysis); FlexAnalysis v3.3 (MALDI-TOF); Fiji v11.18.39 (cryo-fluorescence); IMOD v4, UCSF ChimeraX v.1.10 (cryo-ET reconstructions and segmentations); Icy software with ec-CLEM plugin v1.1 (correlation cryo-fluorescence to cryo-ET) No custom software.

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](#)

All data are included in the Article and its Supplementary Information. Gel source images are provided in Supplementary Fig. 1. Source data are provided with this paper. The cryo-EM maps and the refined atomic models have been deposited in Electron Microscopy Data Bank under accession codes EMD-55686 to 55701, EMD-59437 to 59444, and the Protein Data Bank under accession codes 9T8H, 9T8I, and 33HM respectively.

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	No human participants involved in the research.
Reporting on race, ethnicity, or other socially relevant groupings	No human participants involved in the research.
Population characteristics	No human participants involved in the research.
Recruitment	No human participants involved in the research.
Ethics oversight	No human participants involved in the research.

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 sample size. For cryoEM, the sample size was determined by the data collection time available. The resulting resolution of the refined maps indicated that sufficient data had been collected. The numbers of micrographs and particles are listed in the main text and Extended Data Table 1. For flow cytometry and cell counting, the sample sizes are similar to those generally used in the field and are stated in the figure legends; the statistical results from biological replicates show that the sample sizes chosen were sufficient for statistical significance and reproducibility. For flow cytometry, analysing 10,000 relevant cells per sample is sufficient to ensure that the error on the Poisson event counting is less than or equal to 1%, which is smaller than the typical differences between the analysed samples and the standard deviation between biological replicates. For cell counting, the sample size was sufficient to ensure that the counting error is less than the standard deviation between biological replicates.
Data exclusions	No technical replicates were excluded. For cryoEM data processing, particles were excluded using standard classification algorithms and those excluded corresponded to contaminants or false picks.
Replication	Experiments in the manuscript are reliably reproducible. The number of independent biological repeats are stated in the figure legends.
Randomization	Cells from common pools were split randomly for the different experiments. For the cryoEM data analysis, the particles were split into random subsets (mini-batches) or half-sets during 2D/3D classification and refinement.
Blinding	Blinding was not relevant to this study as the experiments as phenotypes were obvious thus revealing the sample identity.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Primary antibodies used for immunofluorescence:

FK2 (Enzo Life Sciences, LSI-AB-0120, 1:400),
anti-RNF213 (homemade, 1:400),
anti-p62 (MBL Life Science, PM045, 1:1000),
anti-LC3 (Cosmo Bio USA, CAC-CTB-LC3-2-IC, 1:50),
anti-HA (Covance, MMS-101P, 1:400),
anti-LAMP1 (Abcam, ab25245, 1:400),
anti-GFAP (Abcam ab4674, 1:1000),
anti-linear ubiquitin (Genetech 1F11/3F5/Y012L, 1:400)
anti-optineurin (Cayman Chemical, #100000, 1:400)
anti-TAX1BP1 (gift from Kendrick-Jones lab, 1:400)
anti-HOIP (Abcam, ab315162, 1:400)

Secondary antibodies: AF488-Goat-anti-Rabbit IgG (Invitrogen A11034, 1:400), AF647-Goat-anti-Mouse IgG (Invitrogen A21235, 1:400), AFPlus405-Goat-anti-Rat IgG (Invitrogen A48261, 1:400)

Primary antibodies used for immunoblots:

VU-1 (Enzo Life Sciences, LSI-VU-0101, 1:2000),
anti-RNF213 (homemade, 1:1000),
anti-HA (Cell Signaling Technology, C29F4, 1:2000),
anti-actin (Abcam, ab8227, 1:5000),
anti-GYG1 (Abnova, H00002992-M07, 1:1000),
anti-GYS1 (Abcam, ab230422, 1:1000),
anti-PYGB (antibodies.com, A15212, 1:1000),
anti-GBE1 (Proteintech, 20313-1-AP, 1:1000),
anti-linear ubiquitin (Merck, clone 1E3, 1:1000),
anti-HOIL-1 (Abcam, ab309104, 1:1000),
anti-HOIP (Abcam, ab315162, 1:1000),
anti-Sec61 β (gift from Hegde lab, 1:5000),
anti-Halo (Promega, G9211, 1:5000),
anti-FLAG-M2-Peroxidase (HRP) (Sigma, A8592, Sigma)

Secondary antibodies: Goat anti-Mouse Immunoglobulins/HRP (Dako P0447, 1:5000), Goat anti-Rabbit Immunoglobulins/HRP (Dako P0448, 1:5000)

Validation

FK2 – validated by manufacturers on ubiquitin chains (K29, K48, K63-linked)
anti-RNF213 - validated by us by blotting against WT or RNF213-KO MEF cells.
anti-p62 - validated by manufacturers on cell lysate from various cell lines and species
anti-LC3 - validated by manufacturers by immunofluorescence microscopy on starved MEF cells
anti-HA - validated by manufacturers on cell lysate transfected with HA-tagged protein
anti-LAMP1 - validated by manufacturers by flow cytometry and immunohistochemistry
anti-GFAP – validated by manufacturers by immunofluorescence in brain sections
anti-linear ubiquitin – validated by manufacturers by blotting against linear, K11, K48 or K63 ubiquitin chains
anti-optineurin – validated by manufacturers by blotting in cell lines and immunohistochemistry
anti-TAX1BP1 – previously used (PMID 17635994)

VU-1 - validated by manufacturers on cell lysate treated with bortezomib or MG132
anti-HA - validated by manufacturers on cell lysate transfected with HA-tagged protein
anti-actin - validated by manufacturers on HeLa cell lysate
anti-GYG1 – validated by manufacturers by blotting in transfected lysate
anti-GYS1 – validated by manufacturers by blotting from cell lines and tissues
anti-PYGB – validated by manufacturers by blotting against various cell lines and tissues
anti-GBE1 – validated by manufacturers by blotting against various cell lines and tissues
anti-linear ubiquitin – validated by manufacturers by blotting against linear, K11, K48 or K63 ubiquitin chains

anti-HOIL-1 – validated by manufacturers by blotting against cell lines
 anti-HOIP – validated by manufacturers by blotting against various cell lines and tissues
 anti-Sec61 β – previously used (PMID: 12578908)
 anti-Halo – validated by manufacturers by blotting against Halo fusion protein
 anti-Flag-M2-Peroxidase - validated by manufacturers by blotting against Flag-tagged protein

Secondary antibodies were validated by the manufacturer using ELISA with immunoglobulins from various species.

Eukaryotic cell lines

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

Cell line source(s)	MEF cells - provided by Chihiro Sasakawa. RNF213 KO MEF cells are previously described (Otten et al, Nature, 2021). C8-D1A cells - purchased from ATCC. HEK293ET cells - provided by Brian Seed (Massachusetts General Hospital, Richard B. Simches Research Center).
Authentication	No authentication was performed.
Mycoplasma contamination	All cells regularly tested monthly negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	C57BL/6J0la controls are bred in the MRC-LMB Ares animal facility. RNF213 ^{-/-} and RNF213(C4462S) mice were generated in the C57BL/6J0la background in MRC-LMB Ares animal facility. All mice were maintained in the Medical Research Council ARES animal facility under specific-pathogen-free conditions at 19–23°C and 45–65% humidity with a 12/12h light/dark cycle. In individual experiments, mice were matched for age, sex and background strain. All mice used in the study were between 2-12 months old.
Wild animals	No wild animals were used in the study.
Reporting on sex	Both male and female mice were used since the brain polyglucosan accumulation phenotype was qualitatively similar across both sexes.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All experiments undertaken in this study were performed with the approval of the LMB Animal Welfare and Ethical Review Body and the UK Home Office.

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

Plants

Seed stocks	No plants were involved in the research.
Novel plant genotypes	No plants were involved in the research.
Authentication	No plants were involved in the research.

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	MEF cells that produce polyglucosan. Where indicated in the methods, the cells were treated with autophagy inhibitors. The cells were fixed and permeabilized using BD Fix/Perm kit, as described in the methods section, then stained with the Cy5-labelled glycogen probe.
Instrument	BD LSRFortessa
Software	Data collection: BD FACS Diva v 9.0. Data analysis: Flowjo v10.
Cell population abundance	A total of 10,000-20,000 single cells (gated for by FSC-A/SSC-A and FSC-H/FSC-A) that were positive for the Cy5-labelled glycogen probe were analyzed.
Gating strategy	Gating strategy is described in Extended Data Figure 10. Cells were first gated by FSC-A and SSC-A, then by FSC-A and FSC-H for single cells. Subsequently, cells that are positive for the Cy5-labelled glycogen probe were gated, followed by analysis of TMR-labelled Halo-GYG1 levels. The raw scatter plots of the entire cell population after the single cell gating for a representative experiment is shown in Extended Data Fig 10.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.