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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 (*n*) 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 *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 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. *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

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	Cryo-EM data collection used SerialEM (version 3.8.8) in Titan Krios.
Data analysis	RELION-3.1, MotionCorr2-1.4.0, CTFFIND 4.1, cryoSPARC-3.1, ChimeraX 1.2.5, Coot (version 0.9.5), Phenix (version 1.19.2-4158-000), Pymol (version 2.4.0), MolProbity (version 4.5), ImageJ software (Fiji, version 1.53n).

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

The 3D cryo-EM maps in the active and inhibited states at 3.1 Å and 3.3 Å have been deposited in the Electron Microscopy Data Bank under accession code EMD-24784 and EMD-24785, respectively. The atomic model based on the two 3D maps has been deposited in the Protein Data Bank under accession code PDB ID 7S05 and 7S06, respectively. Source data are provided with this paper.

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

Life sciences study design

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

Sample size	Sample size was not predetermined. We collected a dataset of 13,320 movie stacks, leading to 5,186,047 raw particle images. After 2D classification, a total of 1,166,625 particle images were retained for 3D classification. 436,166 particles were selected after 3D classification. Further 3D classification with C2 symmetry led to a selection of 365,915 particles, resulting in the 3.1 Å 3D map in the active state. Focused 3D classification in the hockey-stick density region selected 66,173 particles and the 3.3 Å 3D map of the inhibited state. The sample size was deemed sufficient when the obtained 3D maps had enough details for atomic model building.
Data exclusions	"Bad" raw particle images of the PTase complex that did not produce 2D class averages or 3D class maps with defined features were excluded after 2D and 3D classifications. This criteria is empirical but is a standard image processing practice in the cryoEM community.
Replication	No replicate experiments were performed. Reproducibility resides in the large number of particles used to derive at the final 3D maps or 2D averages. The reliability and the resolution is measured by gold-standard Fourier shell correlation. Replication efforts with multiple refinement runs yielded was successful, yielding similar 3D maps.
Randomization	The raw particles were automatically selected by computer program (RELION-3.1). Randomization is not needed in cryo-EM analysis.
Blinding	The investigators were not blinded to the specific data points during data collection and analysis, because visual inspection is necessary to ascertain the data quality.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	We used six primary antibodies in this study for western blots/immunoprecipitation/immunofluorescence, four against the epitope tags (V5, myc and FLAG), and two against the endogenous proteins giantin and GAPDH. The anti-V5 mouse monoclonal antibody (IgG2a) is from Invitrogen (Cat# R960CUS, Lot # 2106407) and purchased from Thermo Fisher Scientific. Invitrogen does not provide a clone name for this anti-V5 antibody but instead provides an RRID (AB_2556564). The anti-V5 rabbit monoclonal antibody (IgG) is from Cell Signaling (Cat# 13202, clone D3H8Q, Lot# 4). The anti-myc mouse monoclonal antibody was affinity purified in the lab using Protein-A agarose beads (CaptivaA PriMAB Protein A Affinity Resin from Repligen, Cat# CA-PRI-1000) from the supernatant of the hybridoma cell line (clone 9E10) obtained from the Development Studies Hybridoma Bank (Iowa, City, IA, USA). The anti-FLAG mouse monoclonal antibody was purchased from Stratagene (Cat# 200472-21, clone M2, Lot# 1240390). The anti-giantin rabbit polyclonal antibody (IgG) was purchased from BioLegends (Cat# 909701, clone Poly 19087, RRID AB_2565099)(previously Covance Cat# PRB-114P) and aliquoted for storage. Unfortunately, we do not have the Lot# for this antibody. The anti-GAPDH mouse monoclonal antibody was purchased from Sigma-Aldrich (Cat# G8795, Lot# 092M4820V). The anti-mouse HRP-linked IgG secondary antibody used for Western blots was purchased from GE Healthcare (Cat# NA931V, Lot# 17212127). For immunofluorescence studies, the goat anti-mouse Alexa 488+ antibody from Invitrogen (Cat# A32723, Lot # VL315964) and the goat anti-rabbit Alexa 555 antibody, also from Invitrogen (Cat# A21429, Lot# 2298171), were both purchased from Thermo Fisher Scientific.
Validation	The validity of the anti-V5 mouse monoclonal antibody was demonstrated in four previous publications from our laboratory (ref

Validation	10-13) by western blotting. In all four studies, we saw no signal with this antibody in mock transfected cells but a robust signal in cells transfected with GNPT-ab-V5 wt and mutant constructs. The validity of the anti-V5 rabbit monoclonal antibody is demonstrated in this study, as shown in both Figure 2c and Supplementary 6c, where neither GNPT-ab-myc (Figure 2c, lower panel) nor GNPT-ab-FLAG (Supplementary 6c, lower panel) were immunoprecipitated when expressed alone. The hybridoma cell line (Clone 9E10) is the source of many commercially available anti-myc monoclonal antibodies although we affinity purified this antibody by ourselves using the same clonal cell line. The validity of this anti-myc antibody is ascertained by the western blot shown in Figure 2c (upper panel) where no signal is observed in the cell lysate from the first lane (transfection of GNPT-ab-V5) but a signal is observed in the cell lysates from all the other lanes (transfection of GNPT-ab-myc). The validity of the anti-FLAG antibody is ascertained by the western blot shown in Supplementary Figure 6b (upper panel) where no signal is observed in the cell lysate from the first lane (transfection of deltaS1-S3-V5) but a signal is observed in the cell lysates from all the other lanes (transfection of deltaS1-S3-FLAG). The validity of the anti-giantin rabbit polyclonal antibody used in this study was demonstrated in human retinal pigment epithelial cells where both alleles of the giantin gene were inactivated using the CRISPR technology (Stevenson et al. J Cell Sci. 2017 130:4132-4143).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The HEK 293 and parental HeLa cells used in this study were obtained from ATCC many years ago and have been used in numerous published studies from our lab. The GNPTAB null cell line was generated at the Genome Engineering Center at Washington University School of Medicine (St. Louis, MO) and confirmed to be a bona fide knockout by genome sequencing.
Authentication	The validity of the GNPTAB cell line was ascertained by sequence data, and confirmed by complete loss of endogenous lysosomal enzyme phosphorylation as determined by failure to bind to the cation-independent mannose 6-phosphate receptor beads (Ref 13: van Meel et al, JBC, 2016).
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.