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

Orbitrap Fusion Lumos Tune Application (3.4), Xcalibur (4.2)

Data analysis

BLAST (2.9.0), ColabFold (1.2.0), Crystallography and NMR system (CNS) (1.3), Cytoscape (3.8.0), FusionCapt advance (0.84), Ggplot2 (3.3.2), GraphPad Prism (6.01), HADDOCK (2.2), Mascot (2.5.1), MaxQuant (2.0.3.0), Openxlsx (4.1.5), PCDIIS (07/2018), Proteome discoverer (2.4), PSIPRED (4.0), PyMol (2.3), R (4.0.2), RStudio (1.3.1056), SCWRL (4.0), Spectronaut (14.7.20), STRING (11.0), SWISS-MODEL (09/2021), Tidyverse (1.3.0), TopoLink (05.06.2019), UniProt (05/2019), Viridis (0.5.1), XiView (07/2019)

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 MS-data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository and are publicly available as of the date of publication.
The integrative cross-link models (PDBs) for PPT1 and FLOT1-FLOT2, as well as all docking input/output parameters can be accessed from the supplementary file. The remaining data are available within the article, supplementary information. Source data are provided with this paper.

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

| Reporting on sex and gender | 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.

- [x] 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 | No sample size calculation was performed. Numbers of individual replicates were chosen to allow for calculation of statistical significance using appropriate tests. |
| Data exclusions | No data were excluded from data analysis. |
| Replication | Reproducibility of experimental findings was verified by performing replicate measurements and/or by experiments with other experimental approaches. |
| Randomization | No randomization was performed. Individual SCX fractions of the same sample were measured together to prevent carry over between biological samples, for single shot analyses of enrichment experiments, samples were analyzed according to their complexity. |
| Blinding | For mass spectrometry data analysis, blinding was not possible, as sample groups have to be defined during data analysis, and necessary, as determination of significance is solely based on automated procedures. For western blotting and microscopy blinding was not possible as samples were prepared and analyzed in small batches by the same investigator. |

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 | [x] |
| Eukaryotic cell lines | [x] |
| Palaeontology and archaeology | [x] |
| Animals and other organisms | [x] |
| Clinical data | [x] |
| Dual use research of concern | [x] |

Methods

| n/a | Involved in the study |
| - | - |
| ChIP-seq | [x] |
| Flow cytometry | [x] |
| MRI-based neuroimaging | [x] |

Antibodies

| Antibodies used | Goat anti LIMP2 (1:2000) R&D systems # AF1966-SP |
Goat anti mouse IgG HRP coupled (1:5000) Dianova # 115-035-044
Goat anti rabbit IgG (H+L)-Cy3 (1:400) Dianova #111-165-144
Goat anti rabbit IgG HRP coupled (1:5000) Dianova # 111-035-003
Goat anti-mouse IgG (H+L)-Alexa Fluor 488 (1:400) Thermo Fisher Scientific # A-10129
Mouse anti ACT2 (1:4000) Sigma-Aldrich # A5316
Mouse anti ATP6V1B2 (1:1000) Santa Cruz # SC166045
Mouse anti CANX (1:20,000) Proteintech # 66903-1-AP
Mouse anti FLOT1 (1:200) BD Biosciences # 610821
Mouse anti FLOT2 (1:1500) Proteintech # 66881-1-lg
Mouse anti FLOT2 (1:200) BD Biosciences # 610383
Mouse anti FZD9 (1:1500) Proteintech # 67023-1-lg
Mouse anti GAPDH (1:2500) Cell signaling # 5174
Mouse anti GM130 (1:1000) BD Biosciences # 610822
Mouse anti LAMPP2 (1:1000) Hybridoma Bank # H4B4
Rabbit anti ATP6V1A1 (1:2000) Thermo Fisher Scientific # PA5-29191
Rabbit anti ATP6V1D (1:1000) Proteintech # 14920-1-AP
Rabbit anti CTSD (1:1000) Proteintech # 21327-1-AP
Rabbit anti DSSO (1:5000) Self-made (Singh et al., 2021-https://doi.org/10.1021/acs.analchem.Dc04034)
Rabbit anti EEA1 (1:200) Cell signaling # 2411
Rabbit anti FLOT1 (1:2000) Proteintech # 15571-1-AP
Rabbit anti FLOT1 (1:200) Cell signaling # 18634
Rabbit anti GB4 (1:2000) Proteintech # 11978-2-AP
Rabbit anti LAMPP2 (1:400) Thermo Fisher Scientific # PA1-655
Rabbit anti LAMTOR1 (1:1000) Sigma-Aldrich # HPA02997
Rabbit anti LPHN1 (1:200) Thermo Fisher Scientific # PA5-77475
Rabbit anti LPHN2 (1:100) Novus biologicals #NBP2-58704
Rabbit anti LPHN3 (1:200) Novus biologicals #NLS1138
Rabbit anti RRAGA (1:1000) Cell signalling # 4357
Rabbit anti SDHA (1:800) Proteintech # 14865-1-AP
Rabbit anti ATP6V1A1 (1:2000) Thermo Fisher Scientific # PA5-29191
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Rabbit anti RRAGA (1:1000) Cell signalling # 4357
Rabbit anti SDHA (1:800) Proteintech # 14865-1-AP
Rabbit anti TUBA (1:2000) Rockland # 600-401-880
Mouse anti LAMP2 (1:1000) BD Biosciences # 610822
Mouse anti FLOT2 (1:200) BD Biosciences # 610383
Mouse anti FZD9 (1:1500) Proteintech # 67023-1-lg
Mouse anti ATP6V1B2 (1:1000) Santa Cruz # SC166045
Mouse anti CANX (1:20,000) Proteintech # 66903-1-AP
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Rabbit anti RRAGA (1:1000) Cell signalling # 4357
Rabbit anti SDHA (1:800) Proteintech # 14865-1-AP
Rabbit anti TUBA (1:2000) Rockland # 600-401-880
Rat anti FLAG-HRP coupled (1:10,000) Sigma-Aldrich # SAB4200119
Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| Cell line source(s) | HEK293 (ATCC, CRL-1573)  
|                      | HeLa (ATCC, CCL-2)  |
| Authentication       | None of the cell lines used were authenticated. |
| Mycoplasma contamination | All cell lines were tested negative for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified lines were used. |