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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
<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. 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> |
| ☒ | ☐ | 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 images of OST α / β were collected using EPU 2.0 software.

Data analysis Cryo-EM image analyses were performed using software: MotionCor2, CTFFIND4, cryoSPARC 4.7.1. Model building was conducted in Coot (v. 0.8.9), followed by structural refinement and validation in PHENIX (v. 1.20.1). Structural figures were prepared with UCSF ChimeraX (v. 1.8), UCSF Chimera 1.18 and PyMOL (v. 3.1.4.1). The data of SPR were fitted with steady-state affinity binding model using Biacore Insight Evaluation software and the graphs were plotted using OriginPro 2023 software. Protein sequences were aligned using Multalin (<https://multalin.toulouse.inra.fr/multalin/>) and the sequence-alignment figures were generated using ESPript3 sever (<https://esprict.ibcp.fr/ESPript/ESPript/index.php>). Nonlinear curve fitting and unpaired, two-sided t tests were performed using Graphpad Prism 8.0.2 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](#)

The cryo-EM maps and coordinates have been deposited at the Electron Microscopy Data Bank (EMD-67632 for apo-OST α/β , EMD-67668 for TC bound-OST α/β), and PDB (21FS for apo-OST α/β , 21HR for TC bound-OST α/β), 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	This information has not been collected.
Reporting on race, ethnicity, or other socially relevant groupings	No socially constructed or socially relevant categorization variable is used in our manuscript.
Population characteristics	No human research participants are involved
Recruitment	No participants were recruited
Ethics oversight	Not applicable

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	All the cell-based taurocholic acid transport assays were performed with at least three technical triplicates and details are mentioned in the figure legends.
Data exclusions	No data was excluded in the analysis.
Replication	All of functional assays were repeated independently at least three times and all attempts at replication were successful. The number of replications are described in the text.
Randomization	The protein samples for the biochemical assays were randomly allocated into experimental groups and control groups. Imaging of samples was randomly performed between control/mock and experimental treatments.
Blinding	Blinding was not applicable, because it is not technically or practically feasible to implement blinding for either the cryo-EM structure determination or the biochemical assays between wild type and mutants of OST α/β .

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	mouse monoclonal anti-ATP1A1, mouse monoclonal anti-FLAG, HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L).
Validation	mouse monoclonal anti-ATP1A1(1:10,000,) and mouse monoclonal anti-FLAG(1:3,000,) antibody was used as the primary antibody. HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L)(1:10000) was used as secondary antibody. All three antibodies were obtained from Proteintech.

Eukaryotic cell lines

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

Cell line source(s)	HEK293F(Human Embryonic Kidney 293 Cells)
Authentication	No further authentication was performed for commercially available cell lines.
Mycoplasma contamination	Periodically test negative.
Commonly misidentified lines (See ICLAC register)	None of cell lines used is listed in the database of commonly misidentified cellines maintained by ICLAC.

Plants

Seed stocks	No seed stocks or plant material is used
Novel plant genotypes	No plant genotypes were produced.
Authentication	No seed stock is used, and no novel genotype is generated