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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	N/A
Data analysis	Bowtie v2.2.6; TopHat v2.1.0; HTSeq-count v2.0.3; python 3.10; samtools v1.9; deeptools v3.1.1; DeepTMHMM; RibORF 2.0;

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

Sequencing data will be available the at the NCBI Gene Expression Omnibus (GEO) repository with accession number GSE297497. All other data are available in the main text or supplementary materials.

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

Life sciences study design

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

Sample size	Sample size for each analysis is indicated in figure legends and methods
Data exclusions	One RPN2 replicate failed at the library preparation stage and was discarded.
Replication	Rfoot-seq was performed in two biological replicates for Flag-TMCO1, Flag-CCDC47, Flag-Nicalin, and Flag-OST48, and a single replicate for Flag-RPN2. Except where noted, the Flag-TMCO1, Flag-CCDC47 and Flag-Nicalin samples were combined into a single sample ("MPT") (n=6); similarly, Flag-OST-48 and Flag-RPN2 were combined into a single sample ("OST-A") (n=3). Flow cytometry experiments were performed at least twice, in biological replicates that showed similar results.
Randomization	N/A
Blinding	N/A

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	Antibodies are from the following sources: rabbit anti-TMCO1 (PMID: 29281821); rabbit anti-Sec61β (PMID: 12578908); rabbit anti-Nicalin (A305-623A-M) and rabbit anti-CCDC47 (A305-100A) antibodies from Bethyl Laboratories; mouse anti-HRP (ab6728) antibody from Abcam; rabbit anti-peroxidase (SAB3700863) antibody from Sigma; rabbit anti-RPN2 (10576-1-AP) antibody from Proteintech; rabbit anti-uL22 antibody from Abcepta (AP9892b); mouse anti-OST48 (sc-74408) antibody from Santa Cruz Biotechnology; mouse anti-STT3A (H00003703-M02) antibody from Novus Biologicals. Antibodies were used at 1:1000 dilution.
Validation	Antibodies were validated for specificity against the human antigen by the manufacturer or in earlier published work as follows: Anti-TMCO1 (PMID: 29281821), anti-Nicalin (Bethyl Laboratories) (PMID: 36261522), anti-CCDC47 (Bethyl Laboratories) (PMID: 36261522), anti-STT3A (Novus Biologicals) (PMID: 36261522) and anti-Sec61beta (PMID: 29281821) Anti-RPN2 (Proteintech) and anti-OST48 (Santa Cruz Biotech) were also validated in experiments using tagged cell lines, as described in this manuscript.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Flp-In T-REx 293 cell line are from Invitrogen
Authentication	Flp-In T-REx 293 cell line was authenticated by the antibiotic resistance markers within its genome. All knockouts were validated by PCR amplification of the genomic locus and immunoblotting for the absence of protein.
Mycoplasma contamination	Cells were checked approximately every six months for mycoplasma contamination using the Universal Mycoplasma Detection kit (ATCC), and were found to be negative.
Commonly misidentified lines (See ICLAC register)	None used

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

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	Samples consisted of Flp-In T-REx 293 cells transiently transfected with the desired reporter construct. Cells were treated with 200 nM of Apratoxin or the same volume of DMSO as a control.
Instrument	LSRFortessa
Software	FlowJo version 10.10.0
Cell population abundance	Signals from a total of at least 10,000 fluorescent and live cells were analysed.

Gating strategy

A four-step gating strategy was used to analyze dual color fluorescent reporters. In step one, forward and side scatter was used to select cells. In step two, height vs. area forward scatter was used to select for single cells. In step three, live cells (i.e., DAPI-negative) were selected. In step four, transfected cells were selected by gating on expression of the translation reporter (GFP or RFP, depending on the construct). Cells meeting all these criteria are plotted in the figures as scatter plots.

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