nature portfolio

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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	EPU v2.11, 3.1 and 3.8 for cryo-EM data collection
Data analysis	cryoSPARC v2.15, 4 and 4.3 for density map reconstruction. Phenix v1.20.1 for refinement. Coot v0.9.8 for model building. ChimeraX v1.9 for visualization.

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

Coordinates for the structures generated in this study have been deposited in the Protein Data Bank (PDB) under accession codes: 1) 9RON [https://doi.org/10.2210/pdb9ron/pdb], 9ROO [https://doi.org/10.2210/pdb9roo/pdb], 9ROP [https://doi.org/10.2210/pdb9rop/pdb], and 9ROQ [https://doi.org/10.2210/pdb9roq/pdb] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively, of α1β1-FXYD1 at Na+ turn-over conditions; 2) 9RO9 [https://doi.org/10.2210/

pdb9ro9/pdb], 9ROA [https://doi.org/10.2210/pdb9roa/pdb], 9ROD [https://doi.org/10.2210/pdb9rod/pdb], and 9ROE [https://doi.org/10.2210/pdb9roe/pdb] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively, of α3β1-FXYD1 at Na+ turn-over conditions; 3) 9ROF [https://doi.org/10.2210/pdb9rof/pdb], 9ROG [https://doi.org/10.2210/pdb9rog/pdb], 9ROH [https://doi.org/10.2210/pdb9roh/pdb], and 9ROI [https://doi.org/10.2210/pdb9roi/pdb] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and [K2]E2.P states, respectively, of α3β1-FXYD1 at full turn-over conditions (Na+ and K+); and 4.) 9ROJ [https://doi.org/10.2210/pdb9roj/pdb], 9ROK [https://doi.org/10.2210/pdb9rok/pdb], 9ROL [https://doi.org/10.2210/pdb9rol/pdb], and 9ROM [https://doi.org/10.2210/pdb9rom/pdb] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively of α3-Q140Lβ1-FXYD1 at Na+ turn-over conditions. The cryo-EM maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession codes 1.) EMD-54125 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54125], EMD-54126 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54126], EMD-54127 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54127] and EMD-541228 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54128] for 9RPQ for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively, of α1β1-FXYD1 at Na+ turn-over conditions; 2.) EMD-54113 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54113], EMD-54114 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54114], EMD-54115 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54115] and EMD-54116 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54116] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively, of α3β1-FXYD1 at Na+ turn-over conditions; 3) EMD-54117 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54117], EMD-54118 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54118], EMD-54119 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54119], EMD-54120 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54120] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and [K2]E2.P states, respectively, of α3β1-FXYD1 at full turn-over conditions (Na+ and K+); and 4.) EMD-54121 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54121], EMD-54122 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54122], EMD-54123 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54123] and EMD-54124 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-54124] for Na2E1-ATP, [Na3]E1P-ADP, [Na3]E2P and E2P states, respectively of α3-Q140Lβ1-FXYD1 at Na+ turn-over conditions.. Source data are provided with this paper.
For alignments of α3 structures obtained in this study with published structures from Nguyen at a. [16] presented in supplementary figure 5 the following accession codes were used 8D3V [https://doi.org/10.2210/pdb8d3v/pdb] (E1), 8D3W [https://doi.org/10.2210/pdb8d3w/pdb] (E1-AMPPCP), 8D3U [https://doi.org/10.2210/pdb8d3u/pdb] ([Na3]E1-AlFx-ADP), 8D3Y [https://doi.org/10.2210/pdb8d3y/pdb] (E2BeFx), 8D3X [https://doi.org/10.2210/pdb8d3x/pdb] ([K2]E2-MgFx).
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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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	Number of micrographs collected per dataset was determined by available time and particle density on grid. All biochemical experiments were performed at least three times using biological replicates. T-tests were used to determine statistical significance.
Data exclusions	EM micrographs and particles were excluded using established classification parameters within cryoSPARC. 2D classification and heterogeneous refinement was used to eliminate particles that represent contaminations. 3D classification was used to remove particles that could not be classified as defined states. Biochemical characterization: no data was excluded
Replication	One cryo-EM dataset was obtained per sample. At least biological repeats were analyzed for biochemical experiments as indicated in the respective figure legends. T-tests were used to determine statistical significance ($p<0.05$ was deemed significant).
Randomization	Not relevant, Na,K-isoforms and mutant were deliberately chosen. All samples were treated identical within the respective analysis without pre-selection of material.
Blinding	EM data collection was automated and processing was carried out free of external references.

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	Anti-KETYY, antibody raised against the C-terminus of Na,K-ATPase (see ref 86 in paper) used as 1:5'000 dilution
Validation	By immunoblotting using membranes from yeast producing the target vs non-transformed strains.

Plants

Seed stocks — *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*

Novel plant genotypes — *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

Authentication — *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication. — *For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*

Files in database submission — *Provide a list of all files available in the database submission.*

Genome browser session
(e.g. UCSC) — *Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates — *Describe the experimental replicates, specifying number, type and replicate agreement.*

Sequencing depth — *Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*

Antibodies — *Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Peak calling parameters — *Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*

Data quality — *Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*

Software — *Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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 — *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*

Instrument — *Identify the instrument used for data collection, specifying make and model number.*

Software — *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.