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 version 2.5
Data analysis	MotionCor2 1.6.3; CTFFIND-4.1.13; cryoSPARC v4; Phenix-1.19.2; Coot-0.8.9; AlphaFold 2; UCSF ChimeraX 1.17.3; UCSF ChimeraX 1.6.1; MolProbity 4.2 included in Phenix-1.19.2; HADDOCK 2.4; ClustalW 2; GraphPad Prism 9.0; GROMACS 2023.4; DeepEMhancer 1.0; Phyre2 2.0; PPM 3.0; CHARMM-GUI 3.8; CCP4i 8.0; WebLogo 3; Max-Planck Institute Bioinformatics Toolkit (no version); ClustalΩ.

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 coding sequence of human VMAT1 is from UniProt P54219 (https://www.uniprot.org/uniprotkb/P54219/entry). The cryo-EM maps have been deposited into the Electron Microscopy Data Bank under accession numbers EMD-41238 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41238) (Unbound/reserpine), EMD-41241 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41241) (reserpine/reserpine), EMD-41237 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41237) (dopamine/reserpine), EMD-41240 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41240) (norepinephrine/reserpine), EMD-41242 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41242) (serotonin/reserpine), EMD-41239 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41239) (histamine/reserpine), EMD-41236 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41236) (amphetamine/reserpine), and EMD-41235 (https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41235) (MPP+/reserpine). The coordinates have been deposited at the Protein Data Bank under accession numbers 8TGJ (https://www.rcsb.org/structure/8TGJ) (Unbound/reserpine), 8TGM (https://www.rcsb.org/structure/8TGM) (reserpine/reserpine), 8TGI (https://www.rcsb.org/structure/8TGI) (dopamine/reserpine), 8TGL (https://www.rcsb.org/structure/8TGL) (norepinephrine/reserpine), 8TGN (https://www.rcsb.org/structure/8TGN) (serotonin/reserpine), 8TGK (https://www.rcsb.org/structure/8TGK) (histamine/reserpine), 8TGH (https://www.rcsb.org/structure/8TGH) (amphetamine/reserpine), and 8TGG (https://www.rcsb.org/structure/8TGG) (MPP+/reserpine).

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	No statistical methods were used to predetermine sample size. The data size for cryo-EM experiments was determined by the availability of microscope time and the particle density on the grids. Sufficient cryo-EM data were collected to achieve adequate map resolutions, which is sufficient for model building. For functional experiments, the sample size was at least three, adhering to common practice in the field and striking a reasonable balance between statistical robustness and practicality.
Data exclusions	No data were excluded from analyses.
Replication	Each experiment was repeated at least three times in independent experiments. Experimental findings were reproduced reliably.
Randomization	No group allocation was needed for functional experiments in this study.
Blinding	Investigators were not blinded to group allocation, because no grouping was needed for this study.

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	antibody name; supplier name; clone name; catalogue number; lot number; dilution; vendor website rabbit-anti-beta-Actin mAb; Cell Signaling Techonology; D6A8; 8457S; 9; 1:1000; https://www.cellsignal.com/products/primary-antibodies/b-actin-d6a8-rabbit-mab/8457 rabbit-anti-flag mAb; Cell Signaling Techonology; D6W5B; 14793S; 7; 1:1000; https://www.cellsignal.com/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/14793 goat anti-rabbit IgG HRP conjugated; Cell Signaling Techonology; -; 7074S; 32; 1:3000; https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
Validation	The antibodies used in western blot were bought from commercial vendors and were validated by the manufacturers or relevant literature was cited on their websites (see vendor websites listed above) From the manufacturers: Specificity confirmed: Detects a single band of protein (β-actin) on a western blot from various cell lines. Specificity confirmed: Detects a single band of protein (Flag-GFP) on a western blot from 293T cells transfected with DYKDDDDK-GFP.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293-T cells (ATCC CRL-3216)
Authentication	No further authentication was performed for commercially available cell lines.
Mycoplasma contamination	Tested Negative
Commonly misidentified lines (See ICLAC register)	None of the cell lines used is listed in the database of commonly misidentified cell lines maintained by ICLAC.