

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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
Data analysis	Relion 4.0, Gctf, Gautomatch, cryoSPARC, PHENIX, Coot, PyMOL, UCSF Chimera, SnapGene, HOLE

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 map of Vangl1 has been deposited in the Electron Microscopy Data Bank (EMDB) under the accession code EMD-60540 (<http://www.ebi.ac.uk/pdbe/entry/emdb/EMD-60540>). The structural model of Vangl1 has been deposited in the Protein Data Bank (PDB) under the PDB ID 8ZXD (<http://doi.org/10.2210/pdb8ZXD/pdb>). The deposited map and model will be released to public upon publication of the manuscript.

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](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	In the single particle cryo-EM analysis, 192,766 particle images from 6,248 micrographs were used in the final reconstruction. Reducing the number of particles resulted in decreases in the resolution of reconstruction.
Data exclusions	In the single particle cryo-EM analysis, defective particle images were removed after 2D- and 3D- classifications.
Replication	All attempts at replication of western blots/native-PAGE were successful. Numbers of replications were indicated in supplementary figure legends where applicable.
Randomization	In single particle cryo-EM reconstructions, particle images were randomly split into two groups for independent reconstructions, between which the Fourier shell correlation was calculated for the estimation of the overall resolution. This process has been widely accepted in the cryo-EM field as a gold standard.
Blinding	The resolution estimation was performed by Relion or cryoSPARC software packages, where the splitting of particle images was unbiased and blinded from investigators.

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	His-Tag (D3I1O) XP® Rabbit mAb antibody(CST,12698S); GFP (D5.1) Rabbit mAb antibody(CST,2956S);Anti-rabbit IgG, HRP-linked Antibody(CST,7074S)
Validation	https://www.cellsignal.cn/products/primary-antibodies/his-tag-d3i1o-xp-rabbit-mab/12698?site-search-type=Products&N=4294956287&Ntt=12698s&fromPage=plp&_requestid=327670 https://www.cellsignal.cn/products/primary-antibodies/gfp-d5-1-rabbit-mab/2956 https://www.cellsignal.cn/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074?site-search-type=Products&N=4294956287&Ntt=7074s&fromPage=plp&_requestid=1267717

Eukaryotic cell lines

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

Cell line source(s)	Expi293F cells (Thermo Fisher); Sf9 cells (Sino Biological)
Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	None of the cell lines used were tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified line were used.

Plants

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