

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 EPU Software, PatchMaster 2.90;

Data analysis MotionCor2 v1.4.7, Gctf v1.18, CryoSPARC v3.3.2, PHENIX v1.18.2, Coot v0.9.8.1, PyMOL v2.5.2, UCSF ChimeraX v1.3, Prism v9.1.1; Igor Pro; OriginPro 9.0.0; AMBER 20;

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 three-dimensional cryo-EM density maps of the $\alpha 6\beta 4$ nic and $\alpha 6\beta 4$ teb complexes have been deposited in the Electron Microscopy Data Bank under the accession codes EMD-60401 (<https://www.ebi.ac.uk/emdb/EMD-60401>) and EMD-60400 (<https://www.ebi.ac.uk/emdb/EMD-60400>), respectively. The coordinates for the $\alpha 6\beta 4$ nic and $\alpha 6\beta 4$ teb complexes have been deposited in the Protein Data Bank under accession codes 8ZRP (<https://www.rcsb.org/structure/unreleased/8zrp>) and 8ZRN (<https://www.rcsb.org/structure/unreleased/8zrn>), respectively. The PDB codes of $\alpha 4\beta 2$, $\alpha 3\beta 4$, and soluble $\alpha 7$ -AChBP nicotinic

receptors are 6cnj (<https://www.rcsb.org/structure/6CNJ>), 6pv7 (<https://www.rcsb.org/structure/6PV7>), and 3sq6 (<https://www.rcsb.org/structure/3SQ6>), respectively.

DNA sequence of human a6b4 and its chaperons are available in the Universal Protein Resource (UniProt) databases under accession code and link below:

Q15825 [<https://www.uniprot.org/uniprotkb/Q15825/entry>]

P30926 [<https://www.uniprot.org/uniprotkb/P30926/entry>]

O75460 [<https://www.uniprot.org/uniprotkb/O75460/entry>]

O00204 [<https://www.uniprot.org/uniprotkb/O00204/entry>]

Q8N350 [<https://www.uniprot.org/uniprotkb/Q8N350/entry>]

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	As for the cryo-EM analysis, the number of micrographs is determined by the available microscope time. As for the molecular dynamics simulations, $n \geq 3$ independent experiments were performed, which are usually sufficient to represent the trajectory of the molecules. As for the electrophysiological experiments, $n \geq 3$ independent experiments were performed, which are generally sufficient for electrophysiological experiments of ligands.
Data exclusions	No data were excluded from analysis.
Replication	Sample preparation-related experiments including protein purification and SDS-PAGE gel electrophoresis were reproduced at least three times independently. Whole-cell voltage-clamp experiments were replicated at least three times. All attempts at replication were successful.
Randomization	Randomization is not relevant to cryo-EM, whole-cell voltage-clamp experiments, and any other experiments in this study, because the samples were not allocated into experimental groups during data acquisition and analysis.
Blinding	Blinding is not relevant to this study. The parameters for biochemistry, cryo-EM, whole-cell voltage-clamp experiments, and any other experiments in this study did not require subjective assessments of the treatments.

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

Eukaryotic cell lines

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

Cell line source(s)	FreeStyle 293-F cells (Gibco, USA); Sf9 cells (Gibco, USA); Oocytes were harvested from <i>Xenopus laevis</i> ;
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	The cells were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	<i>Xenopus laevis</i> sourced from Nasco, 2 years old, weighing about 200g.
Wild animals	N/A
Reporting on sex	The information has not been collected.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	No ethical approval was required for this study as it involved standard laboratory procedures with <i>Xenopus laevis</i> and did not involve invasive procedures.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

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