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# Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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<br>*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<br>*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 | Cryo-EM: Leginon 3.2, Gatan Microscopy Suite 3.3;<br>LC-MS/MS: Thermo Xcalibur v4.0.27.19;<br>ICP-MS: Syngistix v1.1.<br>Cell-based functional assay data collection: PHERAstar FS v3.10 R3;<br>MALDI-MS: FlexControl3.4.135. |
| Data analysis | Cryo-EM: MotionCor2 v2.1, Gctf v1.06, Relion 3.0, cryoSPARC v2.4, UCSF Chimera v1.13.1;<br>Model building and refinement: COOT v0.8.9.0, Phenix v1.14, Molprobity, EMRinger;<br>Structural alignment: LSQMAN v9.7.9;<br>Figures: Pymol v1.8.6.0 and v2.3.1, UCSF Chimera v1.13.1; UCSF ChimeraX v0.9;<br>Cell-based functional assay data analysis: PRISM v8.2.0;<br>SPA binding data analysis: SigmaPlot v13.0;<br>LC-MS/MS: MS-DIAL v3.40, LipidBlast v49. |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data are included in this article and its Supplementary Information. Cryo-EM density maps of GABA(B) receptor have been deposited in the Electron Microscopy

Data Bank under accession codes EMD-21685. Atomic coordinates for the GABA(B) receptor structure are deposited in the RCSB Protein Data Bank under the accession number 6WIV.

# 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 sample size calculation was performed.<br>Cell-based IP accumulation data represent average of multiple experiments (n = 3 - 15), each consisting of quadruplicate measurements.<br>ICP-MS data represent average of eight measurements within two experiments. |
| Data exclusions | No data was excluded from analysis. |
| Replication | Cell-based IP accumulation experiments were repeated three to fifteen times.<br>ICP-MS experiments were repeated twice. |
| Randomization | Randomization was not relevant to the experiments in our study. |
| Blinding | Blinding was not relevant to the experiments in our study since no subjective allocation was involved. |

# 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 |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

| | |
|---|---|
| Antibodies used | 1. Mouse monoclonal ANTI-FLAG® M2 Affinity Gel, Milliporesigma A2220.<br>2. Mouse monoclonal ANTI-FLAG® M1 antibody, MilliporeSigma F3040, clone M1, 1:300 dilution.<br>3. IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody, Li-Cor Biosciences 926-32210, 1:4,000 dilution. |
| Validation | 1. Mouse monoclonal ANTI-FLAG® M2 Affinity Gel: Validation by Milliporesigma; application included purification of FLAG fusion proteins.<br>2. Mouse monoclonal ANTI-FLAG® M1 antibody: Validation by Milliporesigma for detection of proteins with an N-terminal FLAG marker; application included immunocytochemistry (on-cell western). |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | 1. HEK293S GnTI- (ATCC® No. CRL-3022™); 2. HEK 293T/17 (ATCC® No. CRL-11268™). |
| Authentication | Cell lines were authenticated by ATCC. Cellular morphology was checked by microscope for each passage of cells. |
| Mycoplasma contamination | Cell lines were certified by ATCC, but not tested again for mycoplasma contamination during culturing. |

| | |
|---|---|
| Commonly misidentified lines<br>(See ICLAC register) | None. |