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<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 | Molecular docking was conducted using the Glide algorithm in Maestro 11.5 (Schrödinger 2018). Protein membranes were constructed with CHARMM-GUI 2.0. The ff14SB force field was employed for proteins, Lipid17 for lipids, and the TIP3P model for water molecules. All MD simulations were carried out on graphics processing units (GPUs) using the Compute Unified Device Architecture (CUDA) version of particle-mesh Ewald molecular dynamics (PMEMD) in AMBER20. |
| Data analysis | Data analysis was conducted using CPPTRAJ in the AMBER20 suite, VMD 1.9.3, PyEMMA 2.5.7, MSMBuilder 3.8.0, PyMOL 2.5.3, ChimeraX 1.3, and MATLAB 2021a. Cell experiment results were analyzed and visualized with GraphPad Prism 10. |

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

Relevant data supporting the findings of this study are available in the Source Data file provided with this paper. The associated collective variables of the VFT, CRD, and 7TM, as well as tICA data generated in this study, are included in the Source Data file. Initial structures for MD simulations were obtained from the RCSB PDB database (PDB ID: 6N51 and 6N52). Other GPCRs were also downloaded from the RCSB PDB database at https://www.rcsb.org/. Other possible data are available from the corresponding authors upon reasonable request. NEB calculations and MD simulations were based on AMBER suite, according to https://ambermd.org/. The analysis protocol for Markov State Model referred to http://www.emma-project.org/latest/ and http://msmbuilder.org/3.8.0/.

## 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 | The sample size for MD simulations was chosen based on the timescale of class C GPCR dimer arrangement (submillisecond level). In Section 2, SI, the simulation system has been converged in simulation time. Thus, the sample size has been enough for research.<br>For cell experiments, including IP1 accumulation assay and cell surface expression, the sample size was determined based on prior literature and common practices in the field. At least three independent experiments were performed, as described in the related Figure legends. Data were analyzed by fitting various ligand concentrations and readouts using appropriate equations in GraphPad Prism software. |
| Data exclusions | No data were excluded for this study. |
| Replication | Ten rounds of MD simulations (150 μs in total) were conducted to confirm our conclusions. All replication attempts were successful.<br>For cell experiments, at least three biologically independent experiments were performed. The exact number of biological replicates is reported in the figure legends. All experimental findings were reliably reproduced. |
| Randomization | In MD simulations, each run begins with a random velocity to ensure randomness.<br>For cell experiments, randomization was neither attempted nor needed. The present study did not involve animal experiments that would depend on randomization. |
| Blinding | For cell experiments, blinding was not performed since samples were allocated into pre-specified groups, and readouts were automated (e.g., plate reader). All data acquired in this study are included in the article and subjected to statistical analysis whenever necessary. |

# 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) | HEK293 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). |
| Authentication | All of the cell lines are maintained by the supplier. No additional authentication was performed in this study. |
| Mycoplasma contamination | All the cell lines were routinely tested and confirmed negative for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |

# Plants

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