nature portfolio

Corresponding author(s): Jonathan F. Fay and Bryan L. Roth

Last updated by author(s): May 15, 2022

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	JbluIce-EPICS V2013.1 build 5287 (Crystallography), SerialEM V3.8.2 (CryoEM)
Data analysis	Structure determination and refinement: HKL3000, cryoSPARC V3.1.0, Topaz 0.2.2, DeepEMhancer, Relion3.1, UCSF Chimera 1.15, cisTEM 1.0.0-beta, PyMol 2.4.1, Coot 0.9.5, MolProbity (built in phenix 1.18.2), Phenix 1.18.2 Functional data analysis: GraphPad Prism 9.2 Ligand illustrator: ChemDraw 20.0. FEP analysis: Schrödinger modeling suite (v2020-4), PROPKa, Force Field Builder, FEP+

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 structures of the 5-HT5AR-AS2674723, 5-HT5AR-miniGo-5-CT, 5-HT5AR-miniGo-Lisuride, and 5-HT5AR-miniGo-Methylergomertrine have been deposited with the PDB (EMDB) under accession code 7UM4, 7UM5 (EMD-26597), 7UM6 (EMD-26598), and 7UM7 (EMD-26599). The cryoEM micrographs of 5-HT5AR-miniGo-5-

CT, 5-HT5AR-miniGo-Lisuride, and 5-HT5AR-miniGo-Methylergomertrine have been deposited in the EMPIAR database (https://www.ebi.ac.uk/empiar/) with accession numbers EMPIAR-11033, EMPIAR-11036, and EMPIAR-11039, respectively.

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	For cryoEM studies, the number of micrographs is determined by the available microscope time. For BRET and radioligand binding assays, number of technical replicates and biological replicates are reported in the figure legends. Sample size was determined based on variability of the response deviating from the mean as indicated by the standard error of the mean (SEM), which is also represented in the figures. Typically, at least three biological replicates were performed so that the SEM was within at least 20% of the mean, but exact number of replicates are indicated per result in figure legends.
Data exclusions	No data were excluded.
Replication	For BRET and radioligand binding assays, three biologically independent experiments (n=3) were performed. All attempts at replication were successful.
Randomization	For cryoEM study, meshes on the grids with good ice thickness were randomly allocated into experimental groups for data collection. For BRET and radioligand binding assays, randomization is not relevant as no group allocations were performed.
Blinding	No blinding was performed in this study. For both cryoEM structure determination and functional studies, blinding is not necessary due to the nature of these experiments do not requires subject assessment of the data that may influence the validity of the results

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	gp64-PE antibody; anti-Flag–HRP-conjugated antibody
Validation	gp64-PE (R-PHYCOERYTHRIN (R-PE)-conjugated mouse anti-baculovirus monoclonal antibody is from mouse clone AcV1 and used for baculovirus titration. Detailed information can be found at: https://expressionsystems.com/product/gp64-pe-antibody/. anti-Flag–HRP-conjugated antibody is from mouse clone M2 and used for measuring protein expression on the surface of cells. Detailed information can be found at: https://www.sigmaaldrich.com/US/en/product/sigma/a8592.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells were purchased from the American Type Culture Collection (ATCC, ATCC CRL-11268). Sf9 cells were obatined from Expression System (Cat 94-001S)

Authentication	HEK293T cells were authenticated by the supplier (ATCC) using morphology and growth characteristics, and STR profiling. Sf9 cells are commercial and obtained from vendors as indicated in the manuscript.
Mycoplasma contamination	HEK293T cells have been tested and shown to be free from mycoplasma (Hoechst DNA strain and Direct Culture methods employed). Sf9 cell line was certified as mycoplasma-free by the source company.
Commonly misidentified lines (See ICLAC register)	None