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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 *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	SerialEM 3.6 software was used for automated cryoEM data collection.
Data analysis	Data from radioligand binding, bimane fluorescence, and GTPase Glo assay were analyzed used GraphPad Prism v7. Flow cytometry data were analyzed using Prosort v.1.5.0.15 and FlowJo 10. CryoEM image processing and 3D reconstructions conducted using MotionCor2 1.3.0, Gctf 1.06, relion3.0 and cisTEM 1.0.0. Bsoft 2.0.5 was used for local resolution determination and Phenix 1.15 for map fsc curve calculation and model validation. Coot 0.8.9.1 was used for model building. Molecular dynamics experiments were run with NAMD v2.13 and analyzed with VMD v1.9.3. Chimera v1.12 was used for electrostatic analysis.

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

Atomic coordinates have been deposited in the Protein Data Bank (PDB) under accession number 6U1N and the cryoEM density maps in EMDB under number 20612 and 20948

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	Since no inferences were determined for a population based on a sample, no sample size determination was needed in this study,
Data exclusions	No data were excluded from analysis
Replication	A minimum of three independent experiments were conducted for radioligand binding, flow cytometry, bimane fluorescence, confocal microscopy, and GTPase Glo assays. All attempts at replication were successful with no data exclusions.
Randomization	The independent variables of the experimental design were well controlled and did not require randomization
Blinding	Experimental results were all quantitative and did not require any subjective analysis, thus no experiments were preformed with blinding

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	Anti-FLAG M1 used for receptor purification, flow cytometry (120nM), and confocal microscopy (30nM) was purified from ATCC hybridoma line HB-9259 (Clone 4E11). Anti-FLAG-M2-HRP (Sigma; A8592) and Tubulin (Sigma; T7816) were used in western blots at concentrations of 1:1000 and 1:5000, respectively.
Validation	Certificate of analysis of FLAG-M1 is available from ATCC website and was further validated by pull-down assays and flow cytometry. Specificity of FLAG-M2-HRP was determined by including control cell lysate not expressing FLAG-tagged receptor. Tubulin antibody was validated by western blot analysis and compared to that illustrated on the Sigma specification sheet

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293F cells: ThermoFisher. The deriative tetracycline-inducible Expi293F cell line used for M2R expression was reported in Staus et. al. Proc Natl. Acad. Sci. USA 115, 3834-3839 (2018). Beta-arrestin1/2 knockout HEK293 cell lines (Dr. Asuka Inoue, Tohoku University) were characterized and reported in Grundmann et. al. Nat Commun. 2018;9(1):341.
Authentication	No additional authentication of cell lines was performed
Mycoplasma contamination	Cells were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	beta-Arrestin1/2 null HEK293 cells (courtesy of A. Inoue) overexpressing GFP-beta-arrestin1 constructs were stained with AlexaFluor650-M1 (anti-FLAG) antibody.
Instrument	Bio-Rad S3e Cell Sorter
Software	Data were collected using ProSort software and analyzed in FlowJo 10.
Cell population abundance	N/A, cells were not sorted.
Gating strategy	N/A

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.