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 *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	Data collection for cryo-EM data was conducted using SerialEM 3.7.
Data analysis	Cryo-EM data analysis/visualisation software: UCSF Chimera-v1.14/ChimeraX-v1.2.5, Relion v3.1, CryoSPARC-v3.3, cryOLO 1.5, MotionCor2, CTFFIND-4.1, Coot 0.9, Phenix 1.2, Molprobity v4, LigPlotPlus v2.24; Pharmacology data analysis software: GraphPadPrism 8.0

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

CryoEM maps and atomic model coordinate files for complexes determined in this study are available on the EMDB and PDB: AMY3R-San385 complex: PDB 8F0K / EMDB EMD-28759; AMY3R-San385 (the cluster 5) complex: PDB 8F2A / EMDB EMD-28812; AMY3R-San45 complex: PDB 8F2A / EMDB EMD-28810; CTR-San45: PDB 8F0J/ EMDB EMD-28758.
Atomic coordinates for structures not determined in this study but used for comparison or as a starting model are available from the PDB: 7TZF (AMY3R-rAmy), 7TYI (CTR-rAmy, CT-like), 7TYL (CTR-rAmy, bypass), 6NIY (CTR:sCT).
The chemical information for the lipid modification of the peptides in this study is available from the PDBeChem: D6M.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	Each GPCR complex was prepared once for structural studies, and one cryo-EM dataset comprising several thousand micrographs was collected. The number of micrographs in each dataset was determined based on the available microscope time and our experience with GPCR complexes. No statistical methods were used to predetermine sample size. All functional data (cAMP accumulation assay and cAMP duration of action studies) were obtained from a minimum three independent biological replicates which is common practice in this field.
Data exclusions	In accordance with standard cryo-EM practice, micrographs with low estimated CTF resolution were excluded from further processing. No data were excluded from the pharmacological assays.
Replication	No replication was performed for structural studies in complex preparation or data acquisition. Each cryo-EM dataset comprises millions of copies of the investigated GPCR complex and therefore has inherent replication by using random particle subsets during the analyses. For pharamcological assays (cAMP accumulation assay and cAMP duration of action studies) , each individual biological replicate was performed in duplicate or triplicate on different days. All findings were reliably reproduced.
Randomization	Cryo-EM - Random particle subsets were used during the 3D auto-refinement steps in Relion or cryoSPARC. Single particle cryo-EM data processing follows gold standard, that is to separate particle stacks by the order, even or odd, into two separate data sets, and processed separately. This separation is considered random. All biochemical experiments were initiated from independent aliquots of the frozen cells expressing the target proteins. Cells were mixed in suspension randomly distributing cells and providing homogeneous groups that were then subjected to indicated experimental conditions. No animals or human research participants are involved in this study.
Blinding	Blinding was not performed in this study, as protein samples are not required to be allocated into experimental groups in protein structural studies and neither for peptides in pharmacological studies. In addition, the raw data collection for cryo-EM and pharmacological assays was automated by relevant instruments. No animals or human research participants are involved in this study.

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

Methods

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

Antibodies

Antibodies used	Nanobody 35 (expressed and purified in-house)
Validation	This nanobody was used widely as a scaffold protein to stabilize Gs protein, originally validated in DOI:10.1038/nature10361. In our case, the density of this nanobody was observed in the EM map.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293-Flp-In cell lines was obtained by Sanofi company. COS-1 and COS-7 cells were obtained from ATCC. Sf9 and Trichoplusia ni cell lines (Expression systems) were purchased from Expression systems.
Authentication	Grown from original stocks purchased from suppliers. Cell lines were not authenticated in house.
Mycoplasma contamination	COS-1, COS-7 and HEK293-Flp-In cells were tested negative for mycoplasma contamination. Trichoplusia ni and Sf9 cell lines were not tested.
Commonly misidentified lines (See ICLAC register)	not relevant - No commonly misidentified cell lines were used.