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Reporting Summary

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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	Cryo-EM data was collected using the SerialEM sv3 oftware package at the UT Southwestern Cryo-EM Facility.
Data analysis	Cryo-EM data was analyzed using the Relion Version 3.0 software package. The structure described in the paper was built using Buccaneer and real space refinement in Coot, as well as refinement in the Phenix suite. Ligand binding data was analyzed using Graphpad Prism Version 8 (Mac). Protein design was carried out using the iPHoLD software package in addition to RosettaMembrane.

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

Structural data has been deposited in the Protein Data Bank (PDB) with coordinate accession number 6vms, and maps have been deposited in the Electron Microscopy Data Bank (EMDB) with accession numbers EMD-21243, EMD-21244, and EMD-21245. All other data generated or analyzed during this study are included in this published article or are available from the corresponding authors on reasonable request.

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	Ligand binding and receptor activation studies were replicated as 3-4 biologically independent experiments (n=3-4). The ligand concentrations and number of replicates were selected to allow fitting to binding/activation models in Graphpad Prism Version 8, such that accurate S.E.M. values could be calculated. For cryo-EM studies, >2 million single particle images were used as initial input in order to produce the 3D reconstructions in Relion Version 3.0. Particle numbers for different reconstructions, as described in Extended Data Fig. 5c and Extended Data Fig. 6a&b, were selected to produce the resolutions (gold-standard FSC) reported in Extended Data Table 1.
Data exclusions	No data was excluded from the analyses.
Replication	All of the experiments in this study (ligand binding, receptor activation, cryo-EM imaging) were replicated successfully multiple times. Ligand binding and activation studies in Extended Data Figures 2 & 3 were performed as 3-4 biologically independent experiments. Cryo-EM analysis was performed on >2 million particles picked in an automated fashion from >9000 high-resolution images in Relion Version 3.0.
Randomization	Randomization is not applicable. The biological experiments in this study were carried out on purified protein or membrane samples that were validated in multiple ways (sequencing of constructs, PAGE analysis of molecular weights, and cryo-EM structure determination). Neither animal or human experiments were carried out.
Blinding	For cryo-EM studies, >2 million particle images were derived from >9000 images using unbiased (automated) particle picking in Relion Version 3.0. 2D and 3D classification and refinement were carried out without any prior reference information, as described in Methods. The 3D reference used for 3D classification and refinement was made de novo in Relion Version 3.0, using data collected in this study (on the Talos Arctica).

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	For protein purification purposes, Anti-FLAG M1 Agarose Affinity Gel was used (Millipore Sigma catalog # A4596, undiluted, multiple lot #s). This resin was used for protein purification purposes only. For structure determination, the single chain antibody scFv16 was used, as originally described by Koehl et al., Nature 558: 547 (2018). The scFv16 used in this study was produced in Sf9 cells using a synthetic gene, as described in Methods.
Validation	The scFv16 sequence was validated by DNA sequencing of the expression vector and resulting baculovirus construct, as well as SDS PAGE analysis of the purified protein.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The Sf9 cell line used in this study was obtained from Expression Systems Inc. (catalog # 94-001S). The HEK293T cell line was obtained from ATCC (catalog # CRL-3216).

Authentication	None of the cell lines used were authenticated, beyond what was provided by the supplier (above).
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination. Sf9 and HEK293T cells were used solely for the purpose of producing recombinant proteins and/or membranes.
Commonly misidentified lines (See ICLAC register)	n/a