

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 EPU (Thermo Fisher Scientific) 3.11.0 for cryo_EM data collection, Patchmaster software v2*73.5 for electrophysiology.

Data analysis MotionCor2 1.1.0; CryoSPARC 4.6.2; Chimera 1.18; ChimeraX 1.8; Coot 0.9.8.7; Phenix 1.20.1; Pymol 2.6; HOLE v2.2.005; LigPlot+ v.2.2; GraphPad Prism v10.1.2; DropletUtils v1.22.0; scuttle/scater v1.12.0/v1.30.1; scDbfFinder v1.16.0; Bio3D v2.4-1.9000; GROMACS version 2019.4 and version 2023.3; Visual Molecular Dynamics version 1.9.2

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 models and maps have been submitted to PDB and EMDB. The data availability statement has been provided in the manuscript as: The atomic coordinates and EM maps for the ligand-free full-length closed state, NA-bound full-length open state, NA-bound and OA-bound extracellular domains have been deposited in the

Protein Data Bank (<http://www.rcsb.org>) and EMDB (<https://www.ebi.ac.uk/pdbe/emdb/>) with the accession codes 43EF (EMD-81812), 43EG (EMD-81813), 43EH (EMD-81814), and 43EI (EMD-81815), respectively. A focused map of the NA-bound full-length LBD has also been deposited under accession code EMD-81816.

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	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](https://www.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	No a priori statistical method was used to determine sample sizes. For cryo-EM, the amount of data collected was governed by microscope availability and sample quality, and proved sufficient to yield high-resolution maps suitable for model building. For electrophysiological experiments, sample sizes correspond to the number of independent whole-cell recordings and are reported as exact values for each experimental group in the corresponding figure legends. These sample sizes were deemed sufficient because the measurements were independently replicated across multiple cells and experiments and yielded consistent, reproducible results.
Data exclusions	For cryo-EM data processing, only high resolution and homogeneous particles were retained. And the details are described in the Materials and Methods.
Replication	We have updated the corresponding figure legends to specify the number of independent replicates for the relevant experiments. For the electrophysiological experiments, each experiment was independently replicated at least four times, with the exact number of recorded cells provided in the corresponding figure legends. All attempts at replication were successful.
Randomization	Experimental groups were predefined by the receptor constructs and experimental conditions. For electrophysiological experiments, cells co-expressing eGFP and mCherry were randomly selected for whole-cell patch-clamp recordings, and WT and mutant receptors were examined under the same experimental conditions. Random allocation was not applicable to the structural, imaging, and computational analyses because the groups were defined by the constructs, ligand conditions, or datasets being analyzed.
Blinding	All the constructs were recorded and analyzed blindly to avoid bias.

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

Antibodies

Antibodies used	Antibodies used for immunology-blotting were: Anti-Flag-Tag Rabbit Monoclonal Antibody (Abcam, Cat# ab205606) Anti-Strep Tag II mouse monoclonal antibody (Sangon Biotech, Cat# D191106) HPR-conjugated Goat Anti-mouse IgG (Cwbio, Cat# D110087-0025) HPR-conjugated Goat Anti-Rabbit IgG (Protein tech, Cat# SA0001-2)
Validation	All the antibodies were commercially obtained and used for immunoblotting/Western blot analysis according to the manufacturers' recommended applications. The corresponding manufacturer-provided validation and application information has been included directly in the experimental protocols. No additional in-house antibody validation was performed.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293F (Invitrogen); HEK293T (ATCC, ACS-4500)
Authentication	No further authentication was performed for commercially available cell lines.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	<i>For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.</i>
Wild animals	<i>Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Reporting on sex	<i>Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not applicable.
Novel plant genotypes	Not applicable.
Authentication	Not applicable.