

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 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 SerialEM 3.8, Schrodinger Prime & Maestro v12.8.117, Dowser, Dabble 2.7.9, AmberTools17

Data analysis GraphPad Prism 9.0, Modeller 9.23, Phenix 1.19, Coot 0.9.2, MolProbity 4.5, Chimera 1.14, ChimeraX v1.25 and v1.3, PyMOL 2.3.2, CTFFIND 4, cryoSPARC 3, Relion 3.1.2, MotionCor2, OPM server, UCSF pyEM 0.5, Skyline, UniDec 4.2.2, Byonic node, Proteome Discoverer 2.5, PropKa 3.0, VMD 1.9.4a12, ISOLDE 1.0b3

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 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

Coordinates for TSH-bound TSHR-Gs, TR1402-bound TSHR-Gs, M22-bound TSHR-Gs, and CS-17-bound TSHR have been deposited in the PDB under accession codes 7T9I, 7UTZ, 7T9N and 7T9M, respectively. Sharpened and unsharpened cryo-EM density maps for TSH-bound TSHR-Gs (composite), TR1402-bound TSHR-Gs (composite), M22-bound TSHR-Gs (composite), CS-17-bound TSHR, and Org 2274179-0-bound TSHR have been deposited in the Electron Microscopy Data Bank under accession codes 25758, 26795, 25763, 25762, and 27640, respectively. Sharpened maps, unsharpened maps, half-maps, and masks for each composite map

component (ligand-bound ECD or 7TM-G protein), for TSH-bound TSHR-Gs, TR1402-bound TSHR-Gs, and M22-bound TSHR-Gs have been deposited in the Electron Microscopy Data Bank under accession codes 27649 (TSH-bound ECD), 27650 (TSH-bound 7TM-Gs), 27647 (TR1402-bound ECD), 27648 (TR1402 7TM-Gs), 27651 (M22-bound ECD) and 27652 (M22-bound 7TM-Gs), respectively. Final particle stacks and .star files for TSH-bound TSHR-Gs, TR1402-bound TSHR-Gs, and M22-bound TSHR-Gs and CS-17-bound TSHR containing particle shift/pose assignments have been uploaded to the Electron Microscopy Public Image Archive under the accession codes EMPIAR-11120. Structure files for OPM-based modeling of inactive and active TSHR are available in the supplementary information. This manuscript makes use of RCSB PDB accession codes 2XWT, 3G04, 3SN6, 7FIH, 7FIJ, 7LJC, 4JQH, and 4AY9 from comparative structural analysis.

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	For cryo-EM studies, the data size was limited by available instrument time and relative particle density on cryo-EM grids. For signaling studies, we used a sample size of 3 to enable repeatability and to control for biological variance typical in biochemical assays, with a minimum of three measurements per tested concentration. For molecular dynamics simulations, 10 independent replicates were performed to control for variance in initial starting conditions.
Data exclusions	No datapoints were excluded from analysis of signaling studies or mass spectrometry experiments.
Replication	Biochemical assays were replicated 2 or 3 times (as indicated), with identical results. The structural biology approaches represent ensemble averages, and the individual experiments were not repeated, as is common and accepted practice in the field. Data processing approaches were replicated and assessed with well-established approaches as outlined in the Methods section.
Randomization	Randomization was not relevant to the experiments in our study as the assays don't have unknown covariates. For example, when we compare wild-type to mutant TSHR in signaling studies, there is no feasible unknown covariate that we can minimize by randomizing experimental units.
Blinding	Blinding was not relevant to the experiments in our study since no subjective allocation was involved.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	M1-FLAG (made with ATCC hybridoma HB-9259), Protein C (made with ATCC hybridoma HB-9892), Alexa647-conjugated anti-M1-FLAG antibody (homemade)
Validation	M1-FLAG and Protein C antibodies were purified over their respective antigen peptides, which was also used to validate binding.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Sf9 and Hi5 insect cells (Expression Systems), Expi293F-TetR cells (ThermoFisher), BL21 Rosetta Escherichia coli (UC Berkeley QB3 MacroLab), CS-17 hybridoma cell line PTA-8174 (ATCC), CHO-DG44-TR1402 cells (Trophogen)
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Authentication

None of the cell lines were authenticated.

Mycoplasma contamination

Sf9, Hi5, Expi293F-TetR, PTA-8174 cell lines were not tested for mycoplasma contamination. CHO-DG44-TR1402 cells were tested and found to be negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

None