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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	Automated data collection on the Titan Krios was performed using serialEM.
Data analysis	The following software was used in this study: MotionCor2, gCTF, RELION 2.1, UCSF Chimera, UCSF ChimeraX, Coot, Cuemol, Phenix, Prime(Schrödinger), Modeller, Dowser, Dabble, AMBER17, AmberTools17 CPPTRAJ package, VMD, FlowJo, Graphpad Prism.

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

All data generated or analyzed during this study are included in this published article. Cryo-EM maps of hNTSR1-Gi1-scFv complex in C and NC states have been deposited in the Electron Microscopy Data Bank under accession codes EMD-20180 and EMD-20181, respectively. The atomic coordinates of hNTSR1-Gi1-scFv complex in C and NC states have been deposited in the Protein Data Bank under accession codes 6OS9 and 6OSA, respectively.

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	Sample sizes were not predetermined by statistical methods. For cryo-EM data, sample sizes were determined by availability of microscope time.
Data exclusions	No data was systematically excluded. The process of generating 3D maps from cryo-EM particles involves sorting for particles that are damaged, have low signal, or are in minority conformations that are unlikely to refine correctly. This is implemented in Relion 2.1.
Replication	All attempts at replication of biochemical and signaling assays succeeded. NanoBiT G-protein dissociation assay shows mean +/- SEM from 3-7 independent experiments (biological replicates, n) performed in duplicate (technical replicates of the biological replicates). Glo assay shows mean +/- SD from single experiment performed in triplicate. There was no attempt to replicate cryo-EM data. This data involves averaging of tens of thousands of particles.
Randomization	No randomization was attempted or needed.
Blinding	No blinding was attempted or needed.

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	scFv16. Anti-FLAG epitope tag monoclonal antibody, Clone 1E6 (Wako Pure Chemicals). Anti-mouse IgG secondary antibody conjugated with Alexa Fluor 647 (ThermoFisher Scientific).
Validation	scFv16 came from an antibody (mAb-16) that binds the Gi heterotrimer. This antibody was generated by Roger Dawson at F Hoffmann-La Roche Ltd (Roche). It is available upon request by MTA. The anti-FLAG epitope tag monoclonal antibody and the anti-mouse IgG secondary antibody conjugated with Alexa Fluor 647 were purchased from manufacturers as indicated above.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Sf9, Expression Systems, Cat 94-001S. Tni Cells (Hi-5), Expression Systems, Cat 94011S. HEK293 Cells (ATCC CRL 1573) HEK293A cells, ThermoFisher Scientific, Cat R70507. ΔGq HEK293 cells, Schrage et al. Nat Commun 2015 (PMID 26658454).

Authentication	ΔGq/11 cells were generated and verified in a previous study by AI (Schrage R et al., Nat Commun, 2015). Other cell lines are maintained by the supplier. No additional authentication was performed by the authors of this study.
Mycoplasma contamination	HEK293A cells and the ΔGq/11 cells were tested for mycoplasma contamination using MycoAlert Mycoplasma detection kit (Lonza). Other cell lines are tested by manufacturer for contamination.
Commonly misidentified lines (See ICLAC register)	None 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	HEK293 cells transiently expressing N-terminally FLAG epitope-tagged GPCR were fluorescently labeled by using the anti-FLAG epitope tag primary antibody and the Alexa Fluor 647-conjugated secondary antibody.
Instrument	EC800 flow cytometer equipped with dual 488 nm and 642 nm lasers, Sony.
Software	Flow cytometry data were collected by using an accessory software (Sony) and analyzed by FlowJo.
Cell population abundance	N/A
Gating strategy	Live cells were gated with a forward scatter (FS-Peak-Lin) cutoff of 390 by setting a gain value of 1.7. Fluorescent signal derived from Alexa Fluor 647 was recorded in a FL3 channel.

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