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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

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 v1.06, RELION 2.1, FREALIGN, cisTEM, BSoft, UCSF Chimera, UCSF ChimeraX, Coot, Pymol, Phenix, Rosetta, PRODRG Server, AMBER17, VIPER

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 upon request. 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 and its Supplementary Information. Sequences of constructs used in this study

are listed in Supplementary Figure 1. The cryo-EM density maps for the mOR-Gi complex with, and without scFv16 have been deposited in the Electron Microscopy Data Bank under accession codes EMD-XXXX and EMD-YYYY respectively. The coordinates for the models of mOR-Gi with, and without scFv16 have been deposited in the Protein Data Bank under accession codes XXXX and YYYY, respectively.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not pre-determined by any statistical metrics. Sample size was limited by time allocation on microscopes, subsequent processing of images confirmed we had enough data to reach high resolution.
Data exclusions	No data was systematically excluded. Over the course of refinement of our maps, particles with low signal, or particles that did not align well to the consensus map were excluded from final map calculations as implemented in RELION 2.1 and FREALIGN.
Replication	No replication studies were attempted, nor were necessary. Our primary data is a cryo-EM structure that was calculated according to standard procedures and does not need replicates.
Randomization	No randomization was necessary for this study. Our primary data is a calculated cryo-EM structure that was calculated according to standard procedures with freely available software and does not need randomization.
Blinding	No blinding was used or necessary during data collection or analysis. As above, Our primary data is a calculated cryo-EM structure that was calculated according to standard procedures with freely available software and did not require blinding. We calculated our initial model ab-initio to avoid bringing model bias into our map calculation, and in a sense, blinded the software.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	scFv16 is the property of F. Hoffmann-La Roche Ltd (Roche) and is available upon request by MTA.

Antibodies

Antibodies used	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). As above, it is available upon request by MTA.
Validation	Binding was confirmed by size-exclusion chromatography on purified protein, as well as by ELISA. Sequencing was performed on mouse hybridoma cells producing mAb16 and the scFv fragment was cloned. Binding of this scFv fragment was confirmed by size exclusion chromatography and electron microscopy. This antibody was generated for the purposes of this study. Most of the scFv could be built into density and register was confirmed.

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. PAI mouse myeloma cell lines (a variant of the P3-x63-AG8 myeloma) as : PAI (RRID:CVCL_J288). The myeloma cells were obtained from the Basel Institute of Immunology.

Authentication	Cell lines are maintained by the supplier. No additional authentication was performed by the authors of this study.
Mycoplasma contamination	Cell lines are tested by manufacturer for contamination, but not were not further tested by the authors of this study. The mouse myeloma cells are tested by manufacturer for ectromelia virus (mousepox).
Commonly misidentified lines (See ICLAC register)	None used.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	Initially, 4 female BALB/c mice (mus musculus) at 6-8 weeks of age were immunized with the Rhodopsin-Gi complex, and two showed good responses. These two were sacrificed for splenocyte isolation.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging