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Corresponding author(s): Renjie Chai, Yan Zhang, Chunyou Mao, Jianfeng Liu

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# 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<br>*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<br>*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 collection was performed on EPU 2.14. MD simulation data were collected using Gromacs 2019.4. The images from immunofluorescence staining were obtained on the different microscopes (Zeiss LSM 900 and Lecia TCS SP8X STED). The data from ABR testing were collected using TDT system III workstation (Tucker-Davis Technologies, RZ6). |
| Data analysis | The following softwares were used in this study: CryoSPARC (v4.4), RELION (v4.0), Gctf (v1.18), UCSF Chimera (v1.17.3), UCSF ChimeraX (v1.8), Coot (V0.9.8.1), Phenix (V1.19.2-4158), Graphpad Prism (v9), MSDIAL (v.4.9), GROMACS-2019.4. |

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

All data generated in this study are included in the main text or Supplementary Information. The cryo-EM density maps and the atomic coordinates have been

deposited in the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) databases under accession codes EMD-39345 and 8YJP for apo-GPR156; and EMD-39356 and 8YK0 for the GPR156–Gi3 complex. The LC-MS/MS data of phospholipid ligands for GPR156 have been deposited on the Figshare server (https://doi.org/10.6084/m9.figshare.25838170.v1). The MD simulation data (cleaned trajectories, start structure, simulation parameters) generated in this study have been deposited in the github (https://github.com/Yanzhang-ZJU/GPR156.git) and Zenodo (https://doi.org/10.5281/zenodo.13208133). Materials are available from the corresponding authors upon request. Source data are provided with this paper.

## 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 | None. |
| Reporting on race, ethnicity, or other socially relevant groupings | None. |
| Population characteristics | None. |
| Recruitment | None. |
| Ethics oversight | None. |

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

# 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. Sample size in all experiments were estimated based in previous published experiments (PMID: 33911284 and PMID: 36917323). For both in vitro and in vivo data, the sample sizes were determined based on the reproducibility of experiments, which were usually more than 3. For cryo-EM data, sample sizes were determined/limited by time availability of the microscope. |
| Data exclusions | No data was excluded. Generation of maps from cryo-EM particles involves use of CryoSPARC and Relion to sort particles, and remove damaged or poor quality particles to achieve a high-resolution final reconstruction. |
| Replication | For both in vitro and in vivo data, at least three biologically independent experiments were performed to demonstrate reproducibility as described in relevant figure legends. |
| Randomization | No randomization was attempted or needed. Randomization was not necessary as the independent variables to be tested were sufficient for the functional interpretation within this study. i.e. WT vs mutant vs control conditions. |
| Blinding | For cryo-EM study, no blinding was used or necessary during data collection or analysis, because no grouping was needed for this study. And no blinding was performed in the in vivo experiments as mice from the same groups were cultured in the same cape with visible identification labels according to the guidelines of the animal facility. For functional analysis, blinding was not necessary due to the quantitative nature of the experiment. All experimental data acquired or analyzed in this study are included in this published article, and subjected to statistical analysis whenever necessary. |

# 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: Myosin7a (Proteus Bioscience, 25-6790, 1:1000 dilution), Ctbp2 (BD Biosciences, 612044.0, 1:400 dilution), Phalloidin (Invitrogen, A22287, 1:1000 dilution), Anti-Strep-tag II primary antibody (Abcam, ab76950, 1:2000 dilution), Goat anti-rabbit secondary antibody (Abclonal, AS014, 1:5000 dilution), HRP-labeled Goat Anti-Rabbit IgG (H+L) antibody (Beyotime, A0208, 1:1000 dilution)

Validation: All antibodies used are commercially purchased and have been validated by the vendors, All antibodies are well characterized.
Myosin7a (Proteus Bioscience, 25-6790): https://www.proteus-biosciences.com/product/antibodies/organellemarkers/myosin-viia.html
Ctbp2 (BD Biosciences, 612044.0): https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-ctbp2.612044
Phalloidin (Invitrogen, A22287): https://www.thermofisher.com/order/catalog/product/A22287
Anti-Strep-tag II primary antibody (Abcam, ab76950): https://www.abcam.cn/products/primary-antibodies/strep-tag-ii-antibody-ab76950.html
Goat anti-rabbit secondary antibody (Abclonal, AS014): https://ap.abclonal.com/catalog-antibodies/HRPGoatAntiRabbitIgGHL/AS014
HRP-labeled Goat Anti-Rabbit IgG (H+L) antibody (Beyotime, A0208):https://www.beyotime.com/product/A0208.htm

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK-293T cells (ATCC, CRL-3216). HEK293 GnTI- cells (ATCC, CRL-3022). Sf9 insect cells (Expression Systems, 94-001S).

Authentication: All of the cell lines are maintained by the supplier. No additional authentication was performed by the authors of this study.

Mycoplasma contamination: All cell lines tested negative for mycoplasma contamination by the manufacturer for contamination.

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell 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: C57BL/6J wild-type mice were purchased from GemPharmatech (Nanjing, China).(Stock No.N000013). The mice were raised at room temperature of 22 ± 1°C under a 12h light-dark cycle with food and water available ad libitum.

Wild animals: This study did not involved wild animals.

Reporting on sex: Both sexes C57BL/6J Wild-type mice were used at 1:1 ratio in all animal experiments.

Field-collected samples: This study did not involved samples collected from the field.

Ethics oversight: All experiments were approved by the Institutional Animal Care and Use Committee of Southeast University, China, and all efforts were made to minimize the number of mice used (no.20210606001).

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

## Plants

| | |
|---|---|
| Seed stocks | None |
| Novel plant genotypes | None |
| Authentication | None |