

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
<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 SerialEM 3.8

Data analysis GraphPad Prism 9.0, Phenix 1.19, Coot 0.9.2, MolProbity 4.5, ChimeraX 1.25, PyMOL 2.5, cryoSPARC 3.2, Relion 3.0, MotionCor2, UCSF pyEM v0.5, AlphaFold2, Flowjo v10.8.1, GROMACS 2021, OPM, VMD 1.9.3, Maestro v13.0.135, ClustalX/ClustalW 2.1, Jalview v2.11.1.5, Biostrings 2.66.0, seqinr 4.2-23, ape 5.6-2, ggtree 3.6.2, R studio v 202.07.01

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 propionate OR51E2-Gs have been deposited in the RCSB PDB under accession code 8F76. EM density maps for OR51E2-Gs and the 7TM domain of OR51E2 have been deposited in the Electron Microscopy Data Bank under accession codes EMD-28896, and EMD-28900, respectively. The molecular dynamics simulation trajectories for apo OR51E2, OR51E2 bound to propionate, and OR51E2-Q18145x53D mutant have been deposited in the GPCRmd database under accession codes 1244, 1245, and 1246, respectively. This manuscript makes use of RCSB PDB accession codes 3SN6, 4LDO, and 6FUF.

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 3-4 to enable repeatability and to control for biological variance typical in biochemical assays, with a minimum of two measurements per tested concentration. For molecular dynamics simulations, five independent replicates were performed to control for variance in initial starting conditions.
Data exclusions	No datapoints were excluded from analysis.
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 OR51E2 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		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☐	☒ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Human research participants		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		

Antibodies

Antibodies used	M1-FLAG antibody (made in house using commercially available hybridoma HB-9259 sold via ATCC). Protein C antibody (made in house using commercially available hybridoma HB-9892 sold via ATCC). Anti-rhodopsin 4D2 antibody obtained from Sigma-Aldrich (MABN15) used at a dilution of 1:400 (v/v). Phycoerythrin-conjugated donkey anti-mouse F(ab') ₂ fragment antibody (Jackson Immunologicals: 715-115-150) used at a dilution of 1:200 (v/v).
Validation	Protein C antibody was purified over its respective antigen peptide, which was also used to validate binding. M1-FLAG antibody was purified over its respective antigen peptide, which was also used to validate binding. Anti-rhodopsin 4D2 antibody and Phycoerythrin-conjugated donkey anti-mouse F(ab') ₂ fragment antibody were validated by their respective manufacturers as indicated at these links: https://www.sigmaaldrich.com/US/en/product/mm/mabn15 https://www.jacksonimmuno.com/catalog/products/715-116-150

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Sf9 and Hi5 insect cells (Expression Systems), Expi293F cells (ThermoFisher), BL21 Rosetta Escherichia coli (UC Berkeley QB3 MacroLab), HEK293T cells (ATCC)
Authentication	Sf9, Hi5, and Expi293 cells were not authenticated. Duke DNA Analysis Facility conducted DNA profiling of HEK293T lab stock for polymorphic short tandem repeat (STR) markers using a GenePrint 10 (Promega) and confirmed shared profiles with the reference (ATCC CRL3216).
Mycoplasma contamination	Sf9, Hi5, and Expi293 cells were not tested for mycoplasma contamination. HEK293T cells tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None