nature portfolio

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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 *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	As described in the methods: CryoEM data collected using EPU 2.12, FSEC data collected using Shimadzu LabSolutions v5.111, Cre-luciferase assay data collected using EnVision Manager v1.14. No custom code used.
Data analysis	As described in the methods: CryoEM data analyzed using Relion 3, cryoSPARC v3.3.2, TOPAZ v0.2.5, Coot 0.8.9, Phenix 1.19 (including MolProbity), CCP4i2 1.1.0 (including PISA), Chimera 1.16, PyMOL 2.5.4. FSEC data analyzed using Shimadzu LabSolutions v5.111, Cre-luciferase assay data analyzed using Prism 6. No custom code used.

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

Regeneron materials described in this manuscript may be made available to qualified, academic, noncommercial researchers through a materials transfer

agreement upon request at https://regeneron.envisionpharma.com/vt_regeneron/. For questions about how Regeneron shares materials, use the email address preclinical.collaborations@regeneron.com. Structural coordinates have been deposited to the Protein Data Bank (PDB) and cryoEM maps have been deposited to the Electron Microscopy Data Bank (EMDB) with accession numbers 8U4N [http://doi.org/10.2210/pdb8U4N/pdb] and EMD-41888 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41888] (Apo CXCR4/Gi complex), 8U4O [http://doi.org/10.2210/pdb8U4O/pdb] and EMD-41889 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41889] (CXCL12-bound CXCR4/Gi complex), 8U4P [http://doi.org/10.2210/pdb8U4P/pdb] and EMD-41890 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41890] (AMD3100-bound CXCR4/Gi complex), 8U4Q [http://doi.org/10.2210/pdb8U4Q/pdb] and EMD-41891 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41891] (REGN7663 Fab-bound CXCR4/Gi complex), 8U4R [http://doi.org/10.2210/pdb8U4R/pdb] and EMD-41892 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41892] (REGN7663 Fab-bound CXCR4), 8U4S [http://doi.org/10.2210/pdb8U4S/pdb] and EMD-41893 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41893] (trimeric CXCR4 in complex with REGN7663 Fab), 8U4T [http://doi.org/10.2210/pdb8U4T/pdb] and EMD-41894 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-41894] (tetrameric CXCR4 in complex with REGN7663 Fab).

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	No human research is reported in this manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	Numbers of particles in the cryoEM samples are described in the methods. FSEC data was analyzed qualitatively, not statistically/numerically, based on single chromatograms. Cre-luciferase data reported is from 2 or 4 data points, with three independent replicates averaged to produce the reported mean and SD for IC50/EC50.
Data exclusions	CryoEM data processing, including which particles are included or excluded from the final map, is described in the methods. No data were excluded from the FSEC or Cre-luciferase analyses.
Replication	CryoEM structure determinations were not repeated, as is standard in the field due to the large amount of time and effort involved in each structure determination. FSEC experiments were repeated 3 or 4 times showing similar results. Cre-luciferase assays were repeated 3 times independently, as shown in Figure 2a/2b and noted in the figure legend.
Randomization	CryoEM particles were randomly assigned to two half-sets, following the "gold standard" FSC protocol. Randomization is not relevant to the FESC and Cre-luciferase experiments, as all data were used in a single analysis procedure.
Blinding	Blinding is not relevant to the cryoEM, FSEC, or Cre-luciferase assay techniques used in this study; none of the information collected and processed is subject to experimenter bias in a way that blinding would address.

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: Some cryoEM experiments used REGN7663 antibody, a Regeneron proprietary antibody described further in the Methods.

Validation: Regeneron antibodies are validated by DNA sequencing of the expression plasmids, and intact mass spectrometry of the purified proteins. No additional validation was done by the authors of this manuscript.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK293 and ExpiSf9 cells for cryoEM protein production came from Thermo Fisher.

Authentication: HEK293 and ExpiSf9 cells were not authenticated by the authors of this manuscript.

Mycoplasma contamination: HEK293 and ExpiSf9 cells were not tested for mycoplasma.

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used in this study.