nature portfolio

Corresponding author(s): Edin Muratspahic, Christoffer Norn

Last updated by author(s): Apr 15, 2026

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	The Rosetta macromolecular modeling suite (https://rosettacommons.org/) is freely available to academic and non-commercial users. Commercial licenses for the suite are available through the University of Washington Technology Transfer Office. RFdiffusion v1.0, ProteinMPNN, AlphaFold2, partial and iterative partial RFdiffusion v1.0, and Rosetta were used for designing and filtering designs. Cryo-EM data were collected on Titan Krios G4, Glacios, Titan Krios, and Talos Arctica microscopes (Thermo Fisher Scientific), using automated acquisition in EPU v3.6.0.6389, v3.9.1.8206 and v3.8.1.7603, and SerialEM v4.2.
Data analysis	Data were analyzed using Python v3.1, GraphPad Prism v8 and v10, NumPy v1.26.4 and pandas v2.2.2 and visualized using seaborn v0.13.2 and matplotlib v3.9.2. Structures were visualized and images were generated using PyMOL v3.1, UCSF Chimera v1.15 and ChimeraX v1.5 and v1.9. FACS data were analyzed using FlowJo v10.4. Cryo-EM data were analyzed using cryoSPARC v4.5.3 and v4.6.0, Coot v0.9.6 and v0.9.8.5 and Phenix v1.19.2, v1.21.2 and v1.20.1. Pharmacokinetic (PK) data were analyzed using Phoenix v8.5.

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

Cryo-EM maps and models have been deposited in the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) under the following accession codes: mM1_068/MRGPRX1, EMD-70205 and 9O7N; mM1_060/MRGPRX1, EMD-70230 and 9O8L; dC2_049/GCRPR, EMD-48385 and 9MM5; dC2_050/CGRPR, EMD-48424 and 9MNI; dCX1_001/CXCR4, EMD-68747 and 22XC. MetaGen-derived scaffolds are available at https://files.ipd.uw.edu/pub/metagen_scaffolds/8920_scaffolds.tar.gz. OPS-RD data are available at https://files.ipd.uw.edu/pub/GPCRs/OPS_RD/MRGPRX1.zip, https://files.ipd.uw.edu/pub/GPCRs/OPS_RD/PAC1R.zip and https://files.ipd.uw.edu/pub/GPCRs/OPS_RD/PTH1R.zip. The NGS data have been deposited in the NCBI database under the accession code PRJNA1451936 with raw sequencing reads available in the Sequence Read Archive. Source data are provided with this publication.

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 research involving human participants was conducted in this study. Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	No research involving human participants was conducted in this study. Not applicable.
Population characteristics	No research involving human participants was conducted in this study. Not applicable.
Recruitment	No research involving human participants was conducted in this study. Not applicable.
Ethics oversight	No research involving human participants was conducted in this study. 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	For cryo-EM studies, the number of micrographs collected was determined by available microscope time and the requirement to obtain sufficient particle numbers for high-resolution 3D reconstruction. For in vitro pharmacological and biophysical binding assays, and OPS-RD, no statistical methods were used to predetermine sample size. Sample sizes varied depending on the assay, with the majority of experiments performed with at least three biological replicates or independent experiments, while a few preliminary assays were performed with fewer replicates. Exact numbers of biological replicates and independent experiments are reported in the figure legends and Supplementary Tables. For in vivo mouse experiments, no statistical methods were used to predetermine sample size. Sample sizes were chosen based on prior experience with similar experiments and established standards in the field and were sufficient to reproduce effects while using the minimum number of animals required to achieve the scientific objective. Exact numbers of mice used for in vivo experiments are provided in the figure legends.
Data exclusions	A small number of data points were excluded from some concentration–response curves due to technical artefacts at specific concentrations. No formal exclusion criteria were pre-established. Excluded data points are indicated as blanks in the Source data files.
Replication	The majority of experiments were performed with at least three biological replicates or independent experiments, while a few preliminary assays were performed with fewer replicates. Exact numbers of biological replicates and independent experiments are reported in the figure legends and Supplementary Tables. All experiments that were independently repeated, including in vitro pharmacological and biophysical binding assays, yielded consistent results. For in vivo mouse experiments, each condition was tested in a single experiment using the indicated number of animals, and results were consistent across animals within the experiment.

Randomization

Cryo-EM studies did not involve allocation of samples to experimental groups; therefore, randomization was not applicable. For in vitro pharmacological and biophysical binding assays, no randomization was performed because no group allocation was used. A subset of miniproteins was independently reproduced in in vitro pharmacological assays in a second laboratory, yielding consistent results.

For in vivo mouse experiments, two independent studies were performed: a pharmacokinetic (PK) study targeting CGRPR and an HSPC mobilization study targeting CXCR4. In the PK study, animals were allocated based on experimental logistics. In the HSPC study, animals were randomly assigned to experimental groups in a blinded manner by a technician.

Blinding

For cryo-EM and biophysical binding studies, blinding was not performed because data acquisition and analysis were based on automated, instrument-derived readouts without subjective assessment. For in vitro pharmacological assays, blinding was not performed because experiments did not involve group allocation.

In mouse experiments, blinding was not performed during experimental procedures in the HSPC mobilization study (CXCR4). In the pharmacokinetic (PK) study (CGRPR), experimenters were blinded to group allocation during experiments and analysis.

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

Anti-c-Myc antibodies (Immunology Consultants Laboratory, CMYC-45F; BioLegend, clone 9E10, 626810; and Cell Signaling Technology, clone 9B11, 2233; conjugated to FITC or Alexa Fluor 647), PE-conjugated anti-FLAG antibody (BioLegend, 637310), HRP-conjugated anti-FLAG M2 antibody (Sigma-Aldrich, A8592), PE-conjugated anti-baculovirus gp64 antibody (Thermo Fisher Scientific, clone AcV1, 12-6991-82), anti-human IgG (Fc) monoclonal antibody (Cytiva, Human Antibody Capture Kit, BR100839), biotin-conjugated lineage (Lin) antibody cocktail (Miltenyi Biotec, 130-092-613), BV711-conjugated anti-mouse c-Kit antibody (BioLegend, clone 2B8, 105835), BV421-conjugated anti-mouse CD45 antibody (Thermo Fisher Scientific, clone 30-F11, 404-0451-82), APC-conjugated anti-mouse CD3 antibody (BioLegend, clone 17A2, 100236), PE-Cy7-conjugated anti-mouse CD19 antibody (Thermo Fisher Scientific, eBio1D3, 25-0193-82), BV711-conjugated anti-mouse Gr-1 antibody (BioLegend, clone RB6-8C5, 108443)

Validation

Anti-c-Myc antibodies recognize the EQKLISEEDL epitope tag and are reported by the manufacturers to be validated for immunocytochemistry, ELISA, and immunoelectrophoresis, and suitable for Western blot, ELISA, and immunofluorescence applications. These reagents were used for detection of epitope-tagged miniproteins displayed on yeast cells. The PE-conjugated anti-FLAG antibody detects FLAG-tagged proteins and is validated for flow cytometry and intracellular flow cytometry. This reagent was used for detection of FLAG-tagged GPCR nanodisc targets. The HRP-conjugated anti-FLAG M2 antibody is validated by the manufacturer for Western blot, ELISA, and immunoprecipitation. It was used to confirm cell-surface expression of FLAG-tagged CXCR4, CXCR7 and CCR5 receptors by ELISA.
The PE-conjugated anti-baculovirus gp64 antibody is validated by the manufacturer for flow cytometry–based detection of infected insect cells and viral titration. In this study, it was used to determine baculovirus titers by flow cytometric analysis of gp64-positive Sf9 cells. An anti-human IgG (Fc) monoclonal antibody is validated by the manufacturer for capture of human or humanized IgG in immunoassay and biosensor-based interaction formats, including ELISA and surface plasmon resonance applications. In this study, it was used to capture GLP1R–Fc on the sensor chip surface for subsequent SPR measurement of miniprotein binding.
A biotin-conjugated lineage (Lin) antibody cocktail is validated by the manufacturer for flow cytometric analysis of mouse hematopoietic lineage populations. The BV711-conjugated anti-mouse c-Kit antibody and BV421-conjugated anti-mouse CD45 antibody are validated for flow cytometric detection of mouse hematopoietic stem and immune cell populations. The APC-conjugated anti-mouse CD3 antibody, PE-Cy7-conjugated anti-mouse CD19 antibody, and BV711-conjugated anti-mouse Gr-1 antibody are validated by the manufacturers for flow cytometric detection of mouse T cells, B cells, and myeloid cells, respectively. These antibodies were used for flow cytometric assessment of the in vivo efficacy of the dCX1_001 antagonist in mice.

No additional antibody validation was performed beyond that provided by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T, SK-N-MC, COS-7, BHK-21, and CHO-K1 cells were obtained from American Type Culture Collection (ATCC). HEK293A cells were obtained from Thermo Fisher Scientific. CHO-K1/CXCR4 and CHO-K1/Cre-Luc/CGRPR stable cell lines, as well as CHO-S and TurboCHO-Express 2.0 cells, were obtained from GenScript. Lenti-X 293T cells were obtained from Takara, and HeLa cells were obtained from the laboratory of Iain Cheeseman. CHO-K1/PTH1R and CHO-K1/MRGPRX1 cells were obtained from DiscoverX. HTLA and HEK293/OXTR cells were obtained from the laboratories of Bryan L. Roth and Princess I. Imoukhuede, respectively. Spodoptera frugiperda (Sf9) and Trichoplusia ni cells were obtained from Expression Systems. Trichoplusia ni High Five cells were obtained from Thermo Fisher Scientific.
Authentication	Cell lines were not authenticated, but were routinely monitored for normal morphology and growth characteristics.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination, but were routinely monitored for contamination.
Commonly misidentified lines (See ICLAC register)	SK-N-MC cells (listed in the ICLAC register of misidentified cell lines) were used for CGRPR binder screening due to their endogenous expression of CGRPR. Key antagonists identified in this system were further pharmacologically validated in COS-7 cells transiently expressing human CGRPR.

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	For the pharmacokinetic (PK) study (CGRPR), C57BL/6J mice (approximately 12 weeks of age) were used. For the HSPC mobilization study (CXCR4), C57BL/6-based transgenic mice aged 6–8 weeks were used.
Wild animals	This study did not involve wild animals.
Reporting on sex	For the pharmacokinetic (PK) study (CGRPR), female mice were used. For the HSPC mobilization study (CXCR4), male and female mice were used.
Field-collected samples	This study did not involve animals collected from the field.
Ethics oversight	For the pharmacokinetic (PK) study (CGRPR), all animal procedures were reviewed and approved by the Lundbeck veterinarians and conducted in accordance with applicable institutional and national guidelines for the care and use of laboratory animals. For the HSPC mobilization study (CXCR4), all animal experiments were reviewed and approved by the University of Washington Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with institutional guidelines and relevant national regulations.

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

Plants

Seed stocks	No research involving plants was conducted in this study. Not applicable.
Novel plant genotypes	No research involving plants was conducted in this study. Not applicable.
Authentication	No research involving plants was conducted in this study. Not applicable.

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	Sample preparation was performed as described in the manuscript.
Instrument	Flow cytometry was performed using a Sony SH800, a BD FACSymphony A3, or a CytoFLEX flow cytometer.
Software	Sorting was performed as described in the manuscript.
Cell population abundance	The percentage of sequences that match the designed binder in each NGS sample was analyzed.
Gating strategy	The FSC/SSC gate was used to select single living yeast cells. The positive gate for flow cytometry was used to select cells which express both the binder protein and show binding signal above the negative sort. For peripheral blood mononuclear cells (PBMNCs), cells were first gated based on FSC and SSC (P1), followed by selection of $CD45^+$ cells. Within this population, $CD3^+$ T cells, $CD19^+$ B cells, and $Gr\text{-}1^+$ cells were identified using fluorescence gates.

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