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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	The CCR6/SQA1/OXM1 dataset was collected using EPU v3.2/v2.12.1.2782REL (Thermo Scientific) on a Titan Krios operating at 300 KeV and equipped with a Selectris energy filter (Thermo Scientific). The CCR6/SQA1/OXM2 dataset was collected using EPU v3.2 (Thermo Scientific) on a Titan Krios G3i operating at 300 KeV and equipped with a Bioquantum imaging filter. Protein thermal shift data were collected on Tycho NT.6 instrument (NanoTemper) and UNit (Unchained Labs). Rotating-frame nuclear Overhauser enhancements (ROEs) or nuclear Overhauser effect (NOE) experiments and all NMR experiments were collected on a Bruker AVANCE III spectrometer equipped with Bruker Topspin software 3.2 patch level 7. SPR experiments were performed on Biacore T200 instrument (Cytiva) equipped with either a Biacore Series S NTA sensor chip (Cytiva cat no BR100532) or a NiHC 200M sensor chip (Xantec). Protein surface expression FACS data was collected on a BD LSRFortessa cytometer (BD Biosciences). Radioligand binding data were collected using a TriLux MicroBeta2 plate reader (PerkinElmer).
Data analysis	We used CryoSPARC v3.3.1, COOT v.0.9.8.1, Phenix v1.20, Molprobity v 4.4, UCSF Chimera v1.16, ChimeraX v1.4, PyMol v2.5.4, and MOE v2022.02 for cryo-EM data processing, modeling, refinement, and analysis. GraphPad Prism v9.5.1 was used for assay data analysis. MestreNova software v11.0 was used for all NMR spectral assignments. Conformer generation was done using Macromodel software v10.5 with the OPLS3e forcefield. Virtual screening using field-based 3D similarity search was performed in the Blaze module within the Cresset software (v2.0). Mestre MSpin/Sterofitter software v2.3.2 were used for RDC data analysis. SPR data was processed using either Biacore Biaeval and Scrubber 2.0 software or Biacore T200 Evaluation Software (Version 2.0, Cytiva). Protein surface expression was analyzed using FlowJo™ Software (Becton, Dickinson and Company).

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

The cryo-EM density maps for the complexes have been deposited into the Electron Microscopy Data Bank (EMDB) under accession codes EMD-46534 for CCR6/SQA1/OXM1 and EMD-46533 for CCR6/SQA1/OXM2. The coordinates for the models have been deposited into the Worldwide Protein Data Bank (wwPDB) under accession codes 9D3G for CCR6/SQA1/OXM1 and 9D3E for CCR6/SQA1/OXM2. Source data are provided with this paper. Solution conformer library files of OXM1-4 in .sdf format and the corresponding input file to allow RDC data fit using Mestre MSpin/Stereofitter software are provided as Source Data files with this paper. All the other data supporting the findings of this study are provided with the article and the Supplementary Information file.

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	Not applicable
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	No statistical methods were used to predetermine sample size. A sample size of three or greater was used for all statistical analysis to conform with the standard. For cryo-EM study, sufficient data were collected to achieve adequate single-particle EM analysis and 3D cryo-EM reconstructions.
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 CryoSPARC.
Replication	Each experiment was repeated at least three times in independent experiments. Experimental findings were reproduced reliably.
Randomization	No randomization was necessary for this study.
Blinding	No blinding was used nor necessary, because no grouping was needed for this study. The investigators were not blinded to allocation during experiments and outcome assessment.

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

Sequence of the anti-BRIL Fab was acquired from the published paper [http://doi:10.1038/s41467-020-15363-0]. Sequence of the anti-BRIL-Fab Nb was acquired from the published paper [https://doi:10.1016/j.jmb.2017.12.010]. Fab and Nb proteins were recombinantly produced and purified for cryo-EM studies. For receptor-Fab-Nb complex formation, purified Fab and Nb proteins were added in a molar ratio of 1 (CCR6-BRIL) : 1.2 (anti-BRIL Fab) : 1.5 (Fab-Nb). Commercial anti-human CCR6 antibody (BD Biosciences, 562515, 1:30 dilution, 1.25 ug/ml), BV421 isotype (BD Biosciences, 562438, 1:312 dilution, 1.25 ug/ml), mouse IgG (Sigma, I-5381, 1:40 dilution, 0.1 mg/ml) were used to measure surface expression.

Validation

For anti-BRIL Fab and anti-BRIL-Fab Nb, validation of antigen binding was described in the published papers, [http://doi:10.1038/s41467-020-15363-0] and [https://doi:10.1016/j.jmb.2017.12.010]. Additional validation was provided by observation of binding to purified CCR6-BRIL protein and by the high-resolution cryo-EM reconstruction where antibodies were unambiguously confirmed forming complexes with CCR6-BRIL as shown in Supplementary Figs. 3 - 4. Validation of the commercial anti-human CCR6 antibody and the control antibody BV421 isotype were performed by the manufacturers and no further validation was performed by the authors of this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Spodoptera frugiperda (Sf9) insect cells used for protein expression were originally purchased from ATCC (catalog number no longer available) and adapted internally for recombinant protein production. HEK293T cells were purchased from ATCC (CRL-3216). HEK293 cells were purchased from Charles River (CTN6199). CHO-K1 cells were purchased from Eurofins (CYL3038). Human CD4+CCR6+CXCR3- T cells were isolated from human donor leukopaks using EasySep™ Human Th17 Cell Enrichment Kit (StemCell Technologies, 18162).

Authentication

Authentication of cell lines was performed by the manufacturers and no further validation was performed by the authors of this study.

Mycoplasma contamination

Cell lines were tested for mycoplasma contamination by the manufacturers, with the exception of Sf9 cells used for recombinant protein production. All Sf9 cells were tested for mycoplasma contamination upon receipt from the manufacturer, with negative results. No further mycoplasma testing was performed by the authors of this study.

Commonly misidentified lines
(See ICLAC register)

None of the cell lines used are listed in the ICLAC database.

Plants

Seed stocks

Not applicable

Novel plant genotypes

Not applicable

Authentication

Not applicable