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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	SerialEM (3.5)
Data analysis	FlowJo (10.5.3), EMAN2 (2.2), MotionCor2 (1.0.2), CTFFIND4 (4.1.5), RELION (2.1), I-TASSER, SAMUEL (17.05), Coot (0.8.8), Chimera (1.12), Phenix (1.11.1-2575).

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/reviewers upon request. 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

The atomic structure coordinates are deposited in the Protein Data Bank under the accession number 6MEO and 6MET; and the EM maps in the EMDataBank

under the accession number EMD-9108 and EMD-9109. All other related data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Statistical methods were not needed to predetermine sample size for the biochemical and structural studies in this work. Multiple independent EM data sets were collected for structural analysis. All other experiments were repeated multiple times with the similar results.
Data exclusions	No data were excluded from analyses.
Replication	Multiple EM data sets were collected with very similar quality. All other experiments have been repeated multiple times with excellent reproducibility.
Randomization	Experimental groups are not needed for this work, therefore randomization is not relevant.
Blinding	The investigators were blinded to group allocation during data collection and/or analysis because groups were not necessary for this work.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Unique biological materials
☐	☒	Antibodies
☐	☒	Eukaryotic cell lines
☒	☐	Palaeontology
☒	☐	Animals and other organisms
☒	☐	Human research participants

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☐	☒	Flow cytometry
☒	☐	MRI-based neuroimaging

Antibodies

Antibodies used	Hybridoma cells for production of an anti-V3 antibody 447-52D was kindly provided by Dr. Susan Zolla-Pazner, New York University. PE Mouse antihuman CD195 (Clone 2D7/CCR5 (RUO), Catalog # 550632, Lot # 5219800; BD Biosciences, San Jose, CA). Anti-His tagged PE conjugated Mouse IgG (Catalog # IC050P, Lot # LHN0316101; R&D Systems, Minneapolis, MN). Anti-CCR5 antibody (Catalog # AB1889, Lot # 2816560; EMD Millipore Corp, USA).
Validation	Antibody 447-52D was tested for binding to HIV-1 gp120. The BD Biosciences website states PE Mouse Anti-Human CD195 is routinely tested by flow cytometry. For anti-His tagged PE conjugated Mouse IgG, the R&D Systems website lists the following citations: T Carmenate et al., J. Immunol., 2018;0(0); DX Bu et al., Oncotarget, 2018;9(40):25764-25780; Bozza S et al., J Immunol, 2014;193(5):2340-8; Sun Y et al., J. Biol. Chem., 2012;287(19):15837-50. The EMD Millipore website states that anti-CCR5 antibody (Catalog # AB1889) was confirmed by western blot analysis of CCR5 in THP-1 whole cell lysate with antiCCR5 (NT) at 1:1000 dilution.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK 293T cells were purchased from ATCC; Expi293F from Thermo Fisher Scientific. 293 T or Expi293F stable cell lines were generated either in Bing Chen's lab at Boston Children's Hospital or at Codex Biosolutions.

Authentication	Each cell line was authenticated for protein expression by western blot and/or flow cytometry, and other functional assays, such as cell-cell fusion and chemokine receptor assays.
Mycoplasma contamination	Mycoplasma contamination is routinely tested for our cell culture and no contaminated cells were ever used for our studies.
Commonly misidentified lines (See ICLAC register)	None.

Flow Cytometry

Plots

Confirm that:

- [ ] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- [x] 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	HEK 293T and Expi293F 293T cells stable cell lines was used. For CCR5 expression analysis 1 million of CCR5 expressing cells were washed with PBS and incubated for 30~40 minutes on ice with PE Mouse antihuman CD195 (BD Biosciences, San Jose, CA) in PBS containing 1% BSA. For CCR5-CD4-gp120 complex detection on the cell surface, 1 million of CCR5 expressing cells were washed with PBS and incubated for 30~40 minutes on ice with CD4 and gp120 at concentrations of 4 μg/ml and 10 4 μg/ml respectively in PBS containing 1% BSA. The cells were then washed twice with PBS containing 1% BSA and stained with Anti-His tagged PE conjugated Mouse IgG (R&D Systems, Minneapolis, MN) at 5 μg/ml. All the fluorescently labeled cells were washed twice with PBS containing 1% BSA and analyzed immediately using a BD FACS Canto II instrument and program FACSDIVA (BD Biosciences, San Jose, CA). All data were analyzed by FlowJo (FlowJo, LLC, Ashland, OR).
Instrument	BD FACSCanto II
Software	Flowjo
Cell population abundance	N/A
Gating strategy	Only gating used during analysis was to separate live and single cell populations.

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