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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.

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	Beam line at Advanced Photon Source beamline 24-ID-E is controlled by in house developed "Console 6.2.0" suite of programs. Automated data processing is enabled by locally developed software suite called RAPD.
Data analysis	HKL2000, CCP4 7.0, PHENIX-1.17.1, PyMol 2.0, LigPlot+ program and PDBePISA web server Ver.1.48

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. 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 and structure factors have been deposited to the Protein Data Bank with accession number 6VW1.

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

Life sciences study design

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

Sample size	No sample-size calculation was performed. For the protein expressions in insect cells, 2 liters cell culture (about 2-3x10^6 cells/ml) were used each time.
Data exclusions	No data were excluded from the analyses
Replication	We have successfully repeated the crystallization condition more than 20 times. Pull-down assay and pseudovirus assay were each repeated 3 times.
Randomization	Randomization was not relevant to our study. Because there's no allocation of samples/organisms/participants involved in our study.
Blinding	Investigators were not blinded to group allocation during data collection and/or analysis. Because there's no group allocation involved in this study.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Primary antibody for C9 tag detection: rhodopsin (1D4). Its supplier: Santa Cruz Biotechnology. Its catalog number: sc-57432. Its clone name: 1D4. Its lot #: E0819. Primary antibody for HIV-1 p24 detection: HIV-1 p24 (24-4). Its supplier: Santa Cruz Biotechnology. Its catalog number: sc-69728. Its clone name: 24-4. Its lot #: F1417. Peroxidase-conjugated secondary antibody was also used for Western blotting (WB). Its supplier: Jackson ImmunoResearch. Its catalog number: 115-035-062. Its lot #: 139773
Validation	Anti-rhodopsin Antibody (1D4) is a mouse monoclonal IgG1, which is recommended for detection of rhodopsin of mouse, rat and human origins by WB, IP, IF, IHC(P) and ELISA; also reactive with additional species, including bovine. The dilution ratio is 1:1,000 for WB. Anti-HIV-1 p24 Antibody (24-4) is a mouse monoclonal IgG2b which is recommended for detection of Gag p24 of HIV-1 origin by WB, IP, IF and FCM. The dilution ration is 1:1,000 for WB. Peroxidase-conjugated secondary antibody is a goat anti-mouse IgG (H+L) which is recommended for WB with a dilution ratio of 1:10,000 - 1:20,000.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	sf9 insect cells were purchased from ATCC (ATCC® CRL-1711™). HEK293T cells were purchased from ATCC (ATCC® CRL-3216™). ESF 921 Insect Cell Culture Medium were purchased from Thermofisher Scientific (catalog #: 96-001-01). DMEM (Dulbecco's Modified Eagle Medium) were purchased from Gibco (catalog #: 11965092).
Authentication	Cell lines used were not authenticated
Mycoplasma contamination	Cell lines used were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used