

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	No software was used for data collection.
Data analysis	GraphPad Prism (Version 9.3.1) was used for the data analysis. Adobe Photoshop 2024 was used to analyze western blot images. Adobe Illustrator 2024 was used to create figures. SnapGene 6.0.2 was used to create vector maps. Confocal images were analyzed using ZEISS Zen Software version 2.1. Cell surface proteins from IP-MS were identified from UniProt (https://www.uniprot.org). FlowJo was used to analyze flow cytometry data. Fiji/ImageJ was used to quantify fluorescent images and Western blotting bands. ChatGPT was used to generate the Nef-ADAM17 model in HIV infection.

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](#)

A source data file is available with this paper.

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 N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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](https://www.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 sizes. Sample sizes were selected based on established practice for cell-based virology and molecular biology experiments, prior experience with the variability of these assays, and the feasibility of obtaining reproducible measurements across biologically independent experiments. Unless otherwise indicated in the figure legends, experiments were performed with at least three biologically independent replicates; four independent replicates were used for the experiment presented in Fig. 3D. For microscopy analyses, at least 10 images from three biologically independent experiments were evaluated. Reproducibility was further assessed using independent knockout clones, genetic reconstitution or rescue experiments, and complementary experimental readouts where applicable. These sample sizes were considered sufficient because the principal findings were consistently reproduced across independent experiments and experimental approaches. Statistical tests were applied after data collection and were not used to determine sample size. Exact sample sizes are reported in the corresponding figure legends.

Data exclusions No data were excluded.

Replication Experiments were repeated three times. All attempts at replication were successful.

Randomization Proper controls were included in each experiments. No randomization design was necessary.

Blinding Proper controls were included in each experiments. No blinding design was necessary.

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

Antibodies for Western blotting include mouse monoclonal anti-FLAG (clone M2, 1:5000 dilution) and anti-HA (clone HA-7, 1:5000 dilution) (Sigma-Aldrich, F3165, H3663), HRP-conjugated mouse monoclonal anti-GAPDH (clone 1E6D9, 1:5000 dilution) (Proteintech, 60004-1-Ig), HRP-conjugated goat anti-rabbit IgG antibody (1:10000 dilution), goat anti-mouse IgG antibody (1:10000 dilution), and rabbit anti-goat IgG antibody (1:10000 dilution) (Sigma-Aldrich, 12-348, 12-349, AP106), mouse monoclonal anti-ADAM17 antibody (clone 2F4C2, 1:1000 dilution) (Proteintech, 68725-1-Ig), rabbit polyclonal anti-ADAM17 antibody (1:1000 dilution) (Proteintech, 29948-1-AP), rabbit polyclonal anti-ADAM15 antibody (1:500 dilution) (Proteintech, 27124-1-AP), goat polyclonal anti-ADAM9 antibody (1:1000 dilution) (Bio-Techne R&D, AF949), rabbit polyclonal anti-ADAM10 antibody (1:1000 dilution) (Abcam, ab1997), rabbit polyclonal anti-GFP antibody (1:2000 dilution) (Proteintech, 50430-2-AP).

Antibodies for flow cytometry include mouse monoclonal anti-human ADAM17 PE-conjugated antibody (clone #111633, 1:100 dilution) (Bio-Techne R&D, FAB9301P); mouse monoclonal anti-human ADAM10 PE-conjugated antibody (clone SHM14, 1:100 dilution) (Biolegend, 352704).

Antibodies for immunofluorescence were rabbit polyclonal anti-HA (1:200 dilution) (Proteintech, 51064-2-AP), goat anti-rabbit secondary antibody conjugated with Alexa Fluor 647 (1:500 dilution) (ThermoFisher, A-21245), mouse monoclonal 750-conjugated anti-MYC (clone 1A5A2, 1:200 dilution) (Proteintech, CL750-60003), rabbit polyclonal 647-conjugated anti-HA (1:200 dilution) (Proteintech, CL647-51064-2).

Antibodies obtained from BEI include mouse monoclonal anti-HIV-1 gp120 (clone 902, 1:1000 dilution) (ARP-522), mouse monoclonal anti-HIV-1 gp41 (Chessie 8, 1:200 dilution) (ARP-13049), mouse monoclonal anti-HIV-1 gp120 (clone ID6, 1:1000 dilution) (ARP-2343), recombinant monoclonal anti-HIV-1 gp41 (clone 246-D, 1:1000 dilution) (ARP-1245), rabbit polyclonal anti-HIV-1 Nef (1:1000 dilution) (ARP-2949), mouse monoclonal anti-HIV-1 Nef (clone AE6, 1:500 dilution) (ARP-709), and mouse monoclonal anti-HIV-1 p24Gag (clone #24-3, 1:3000 dilution) (ARP-6458).

Validation

All antibodies used in this study were validated by the manufactures and BEI. Data sheets for these antibodies are provided on their websites with antibody profiles and citations.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

HEK293T cells (Cat No CRL-3216), HeLa cells (CRM-CCL-2), and A549 cells (CCL-185) were purchased from American Type Culture Collection. MOLT-3 cells (RT-12187), TZM-bl cells (HRP-8129), A549-ACE2-TMPRSS2 cells (NR-55293), GHOST (3)-CXCR4-CCR5 cells (ARP-3942) were obtained from BEI. CEM Rev-A3R5-GFP reporter cells were provided by Yuntao Wu.

Authentication

Because all these cell lines have clear track-records and commonly used, they were not recently authenticated.

Mycoplasma contamination

All these cell lines were negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Human PBMCs nucleofected with Cas9-GFP RNPs were washed twice with PBS, and GFP+ cells were sorted out similarly using the 488 nm excitation channel. HIV-1 infected Rev-A3R5-GFP reporter cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min. GFP+ cells were analyzed by Beckman MOFLO ASTRIOS. To detect ADAM10 and ADAM17 expression, MOLT-3 cells or human PBMCs were washed twice with PBS containing 2% FBS. Cells were incubated with PE-conjugated anti-human ADAM17 or ADAM10 antibodies for 30 min at 4°C in the dark. After being washed, cells were resuspended in PBS with 2% FBS and analyzed similarly.
Instrument	Beckman MOFLO ASTRIOS
Software	FlowJo v10.8.
Cell population abundance	The positive cell populations were over 50% in our analyzed cells.
Gating strategy	Forward versus side scatter (FSC vs SSC) gating was used to identify cells of interest based on size and granularity (complexity).

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