nature portfolio

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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	BD Fortessa X20 NovoSampler Pro (Agilent) LI-COR Odyssey Biacore T200
Data analysis	GraphPad Prism (v9.5.1) FlowJo v10 (Tree Star) NovoExpress 1.5.0 software (Agilent) ImageJ (v2.9.0) Origin (9.8.0.200) Mestrenova (14.3.1) MOE (2020.0901)

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 data supporting this study's findings can be found in the supplementary information as source data or are available from the corresponding author upon request. Raw and processed characterisation data for compounds, as well as raw data for surface plasmon resonance and sdf files for molecular modelling, are available at the University College London Research Data Repository under DOI 10.5522/04/c.6442970.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	Sample sizes for biological replicates (provided in the figure legends) were determined following pilot experiments to obtain estimates of variance in each assay.
Data exclusions	10 µM CsA datapoints were removed from analysis of %GFP+ cells (U87 and Jurkat datasets) due to small cell numbers as a result of cytotoxicity at this concentration. One dataset testing TWH106 against HIV-1 GFP in U87 cells was removed from analysis due to poor activity as a result of improper storage.
Replication	At least 2 independent experiments were performed for each dataset. Reproducibility confirmed the final conclusions and statistical analysis supported hypotheses reported.
Randomization	Experimental groups are identical except for the specific variable being tested and therefore randomisation is not required.
Blinding	Blinding was not required because all outcomes are measured objectively by automated machines.

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

Methods

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

Antibodies

Antibodies used

Anti-CypA (BML-SA296-0100, Enzo, 1:2000)
Anti-CypB (ab16045, Abcam, 1:1400)
Anti-b-actin (clone AC-15, ab6276, Abcam, 1:10000)
Anti-VHL (sc135657, Santa Cruz Biotechnology, 1:100)
IRDye® 680LT goat anti-mouse (926-68020, LI-COR Biosciences, 1:15000)
IRDye® 680LT goat anti-rabbit (926-68021, LI-COR Biosciences, 1:15000)
IRDye® 800CW goat anti-mouse (926-32210, LI-COR Biosciences, 1:10000)
IRDye® 800CW goat anti-rabbit (926-32211, LI-COR Biosciences, 1:10000)
Anti-CypA (clone 1F4-1B5, ab58144, Abcam, 1:1000-1:2000)
Alexa Fluor® 488 goat anti-mouse (Jackson ImmunoResearch, 115-546-146, 1:400)
Alexa Fluor® 647 goat anti-mouse (405322, BioLegend, 1:400)
Anti-CD3 (clone UCHT1, 300448, BV510, BioLegend, 1:150)
Anti-CD8 (clone SK1, 344742, BV605, BioLegend, 1:250)
PerCp-Cy5.5 anti-CD4 (clone SK3, 344608, BioLegend, 1:150)
PE, FITC anti-HIV-1 Gag (clone FH190-1-1, 6604665, Beckman Coulter, 1:100)

Validation

Anti-CypA (BML-SA296-0100, Enzo, 1:2000): Enzo provides evidence for use of antibody by western blot and cites 4 examples of published use: https://www.enzolifesciences.com/fileadmin/reports/Datasheet-BML-SA296.pdf
Anti-CypB (ab16045, Abcam, 1:1400): Abcam provides evidence for use of antibody by western blot and cites 90 examples of published use: https://www.abcam.com/cyclophilin-b-antibody-ab16045.html
Anti-b-actin (clone AC-15, ab6276, Abcam, 1:10000): Abcam provides evidence for use of antibody by western blot and cites 1949 examples of published use: https://www.abcam.com/beta-actin-antibody-ac-15-ab6276.html
Anti-VHL (sc135657, Santa Cruz Biotechnology, 1:100): Santa Cruz provides evidence for use of antibody by western blot and cites 8 examples of published use: https://datasheets.scbt.com/sc-135657.pdf
IRDye® 680LT goat anti-mouse (926-68020, LI-COR Biosciences, 1:15000): LI-COR provides evidence for use of antibody by western blot: https://www.licor.com/bio/reagents/irdye-680lt-goat-anti-mouse-igg-secondary-antibody
IRDye® 680LT goat anti-rabbit (926-68021, LI-COR Biosciences, 1:15000): LI-COR provides evidence for use of antibody by western blot: https://www.licor.com/bio/reagents/irdye-680lt-goat-anti-rabbit-igg-secondary-antibody
IRDye® 800CW goat anti-mouse (926-32210, LI-COR Biosciences, 1:10000): LI-COR provides evidence for use of antibody by western blot: https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-mouse-igg-secondary-antibody
IRDye® 800CW goat anti-rabbit (926-32211, LI-COR Biosciences, 1:10000): LI-COR provides evidence for use of antibody by western blot: https://www.licor.com/bio/reagents/irdye-800cw-goat-anti-rabbit-igg-secondary-antibody
Anti-CypA (clone 1F4-1B5, ab58144, Abcam, 1:1000-1:2000): Abcam provides evidence for use of antibody by FACS and cites 19 examples of published use: https://www.abcam.com/cyclophilin-a-antibody-1f4-1b5-ab58144.html
Alexa Fluor® 488 goat anti-mouse (Jackson ImmunoResearch, 115-546-146, 1:400): Jackson ImmunoResearch provides evidence for use of antibody by FACS and cites 6 examples of published use: https://www.jacksonimmuno.com/catalog/products/115-546-146
Alexa Fluor® 647 goat anti-mouse (405322, BioLegend, 1:400): BioLegend cites 15 examples of published use: https://www.biolegend.com/en-us/products/alexa-fluor-647-goat-anti-mouse-igg-minimal-x-reactivity-9283?GroupID=BLG2049
Anti-CD3 (clone UCHT1, 300448, BV510, BioLegend, 1:150): BioLegend provides evidence for use of antibody by FACS and cites 25 examples of published use: https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-human-cd3-antibody-9792
Anti-CD8 (clone SK1, 344742, BV605, BioLegend, 1:250): BioLegend provides evidence for use of antibody by FACS and cites 10 examples of published use: https://www.biolegend.com/fr-lu/products/brilliant-violet-605-anti-human-cd8-antibody-12406
PerCp-Cy5.5 anti-CD4 (clone SK3, 344608, BioLegend, 1:150): BioLegend provides evidence for use of antibody by FACS and cites 16 examples of published use: https://www.biolegend.com/it-it/products/percp-cyanine5-5-anti-human-cd4-antibody-6250
PE, FITC anti-HIV-1 Gag (clone FH190-1-1, 6604665, Beckman Coulter, 1:100): Beckman Coulter cites 3 examples of published use: https://www.bc-cytometry.com/PDF/DataSheet/6604665&6604667%20D.S.pdf

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Calu-3 (ATCC HTB-55)
HEK 293T (ATCC CRL-3216)
Huh7 (ATCC)

	U87 CCR5 expressing cells (ATCC) Jurkat T cells (Clone E6-1; ATCC TIB-152) THP-1 Dual reporter cells (Invivogen)
Authentication	All cell lines were originally purchased from ATCC or Invivogen, both companies possesses rigorous standards for cell line authentication using short tandem repeat profiling. This confirms the identify of cells and detects misidentified, cross-contaminated, or genetically drifted cells.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	For HIV-1 GFP lentiviral infection of U87 and Jurkat cells, cells were fixed in 4 % formaldehyde in PBS. The % GFP positive (infected) cells was determined using a NovoSampler Pro (Agilent) flow cytometer. For primary T cell experiments, cells were washed in PBS and stained with fixable Zombie UV Live/Dead dye (423108, Biolegend) for 5 mins at 37℃. Excess stain was quenched with FBS-complemented RPMI. Where appropriate, cell surface staining for CD3, CD8 and CD4 was performed in PBS, complemented with 20 % Super Bright Staining Buffer (ThermoFisher), at 4℃ for 30 mins. Unbound antibody was washed off thoroughly and cells were fixed with 4 % formaldehyde before intracellular staining. Permeabilisation for intracellular staining of primary T cells and Jurkat cells was performed with IC perm buffer (Biolegend) according to the manufacturer's instructions. Where appropriate, intracellular staining for HIV-1 Gag was performed for 30 mins at room temperature. Intracellular CypA was detected by incubation of permeabilised cells with CypA antibody (clone 1F4-1B5, ab58144, Abcam, 1:1000-1:2000) for 1 hr, followed by 20 min incubation with secondary anti-mouse AlexaFluor488 or AlexaFluor647-tagged antibody. Excess antibody was removed through several wash steps performed at 1600 rpm for 5 min at 4℃. Data were acquired on a BD Fortessa X20 or NovoSampler Pro (Agilent).
Instrument	BD Fortessa X20 NovoSampler Pro (Agilent)
Software	FlowJo v10 (Tree Star) NovoExpress 1.5.0 software (Agilent)
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Cells were first gated to exclude cell debris (SSC-H vs FSC-H) and gated on single cells (FSC-H vs FSC-A). For Jurkats and U87s, cells were then gated on FITC-H (GFP+, threshold set using uninfected cells), and for Jurkat cells, APC-A (CypA, threshold set using cells without secondary antibody staining). For T cell CypA level experiments, after single cell gating, cells were then gated on FITC-H (CypA, threshold set using cells without secondary antibody staining). For T cell infection experiments, after single cell gating, cells were regated (FSC-W vs FSC-A), then gated as follows: Live cells (ZombieUV Live/Dead vs FSC-A) > CD3, CD8- T cells (CD3-BV510 vs CD8-BV605) > Gag+ (infected cells) (Gag-FITC vs FSC-A). Cells were also gated on APC-A (CypA). Examples of the gating strategies are given in Supplementary Figure 11.

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