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.

- 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, χ²) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values wherever 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

- Zeiss ELVRA1 super-resolution microscope (Zeiss), 120kV Talos L120CTEM (Thermo Fisher Scientific), Ceta 16M CCD camera, Model 2020 advanced tomography holder (Fischione), Keyence inverted fluorescence phase contrast microscope BZ-X710, chemiluminescence imager (ChemiDoc™ Imaging Systems, Bio-Rad Laboratories), Cytiva AKTA pure chromatography system (Cytiva), Refeyn TwoMP mass spectrometer (Refeyn Ltd.), BD LSRFortessa flow cytometer

Data analysis

- Zeiss Zen2.1 software (Zeiss), GraphPad Prism8, SerialEM 82 software, Etomo in the MDT software package, version 4.11, Etomo, Quicktime, 3D Slicer Version 5.0.2, ImageJ/Fiji (NIH), Image Lab (Bio-Rad), Cytiva Unicorn 7.6 (Cytiva), DiscoverMP program (Refeyn Ltd.), Flowjo (BD Biosciences), FlowJo software

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.

All the raw data used for quantification and statistical analyses are available from the corresponding author upon request.

**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.

<table>
<thead>
<tr>
<th>Policy</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting on sex and gender</td>
<td>N/A</td>
</tr>
<tr>
<td>Reporting on race, ethnicity, or other socially relevant groupings</td>
<td>N/A</td>
</tr>
<tr>
<td>Population characteristics</td>
<td>N/A</td>
</tr>
<tr>
<td>Recruitment</td>
<td>N/A</td>
</tr>
<tr>
<td>Ethics oversight</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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](http://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.

<table>
<thead>
<tr>
<th>Policy</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>The sample size was determined based on the size of the standard deviation and the reproducibility of the data. All the sample numbers are provided. Statistical analyses were performed with data obtained from at least three independent experiments.</td>
</tr>
<tr>
<td>Data exclusions</td>
<td>No data points were excluded from quantifications.</td>
</tr>
<tr>
<td>Replication</td>
<td>All experiments were successfully reproduced at least three times. In all cases, the results reliably support conclusions stated in the manuscript.</td>
</tr>
<tr>
<td>Randomization</td>
<td>For quantifying immunostained samples and chromosome spreads, images were taken randomly from undefined areas. All the samples acquired were quantified to generate the data shown in the manuscript.</td>
</tr>
<tr>
<td>Blinding</td>
<td>Acquisition of images and quantifications were conducted in a blinded manner.</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>☒</td>
<td>Antibodies</td>
</tr>
<tr>
<td>☒</td>
<td>Eukaryotic cell lines</td>
</tr>
<tr>
<td>☒</td>
<td>Palaeontology and archaeology</td>
</tr>
<tr>
<td>☒</td>
<td>Animals and other organisms</td>
</tr>
<tr>
<td>☒</td>
<td>Clinical data</td>
</tr>
<tr>
<td>☒</td>
<td>Dual use research of concern</td>
</tr>
<tr>
<td>☒</td>
<td>Plants</td>
</tr>
</tbody>
</table>

Antibodies

Antibodies used: All the antibodies used in this study are listed in the Supplementary Table 3 with Source information and catalog numbers. All antibodies are available upon request.

Validation: All the antibodies used in this study were either internally controlled, validated by siRNA, or previously published. For the VprBP antibody, an antibody blocking was performed to confirm the validity of the antibody.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): U2OS and HEK293T cells were purchased from American Type Culture Collection (ATCC) and cultured as recommended by ATCC. CEM-SS and TZM-bl cells were obtained through the NIH HIV Reagent Program. Human CD4+ T cells were purified and validated by flow cytometry. PBMCs from HIV-positive patients were obtained through the NIH clinical center.

Authentication: Cells have been authenticated by the vendor and used as low passage cell lines for experiments. No further authentication was performed for cell lines from ATCC.

Mycoplasma contamination: No mycoplasma contamination was found.

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration: N/A

Study protocol: N/A

Data collection: N/A

Outcomes: 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: CD4+ T cells were purified using the EasySep human CD4+ T-cell isolation kit (STEMCELL Technologies)

Instrument: BD LSRFortessa
<table>
<thead>
<tr>
<th>Software</th>
<th>FlowJo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell population abundance</td>
<td>10,000–20,000 cells were acquired before and after purifying PBMCs from healthy individuals. After purification, CD4+ T cells were more than 90% abundant as determined by flow cytometry.</td>
</tr>
<tr>
<td>Gating strategy</td>
<td>Cells were analyzed by FlowJo software with the gating strategy: FSC vs SSC &gt;&gt; FSC-H vs FSC-A &gt;&gt; SSC-H vs SSC-A &gt;&gt; TCR-b+CD4+</td>
</tr>
</tbody>
</table>

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