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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Based on previous findings of B-cell pathogenesis in HIV infection, a minimum of six individuals per category is sufficient to achieve meaningful conclusions. We included 108 study subjects, as many as we could from our study protocol while taking into account specimen availability, stable disease or treatment status, as well as gender/race/age proportionality for each category.

2. Data exclusions

Describe any data exclusions.

No data were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The findings were highly reproducible and given that our protocol participants have many return visits, we were able to establish reproducibility by repeating the assays on the same participant at follow-up visits.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

There was no randomization; however, race was found to be a factor and black/non-black participants happened to be evenly split among our main group of chronic viremic individuals.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The HIV infection status and antiretroviral treatment category were known at the time of specimen collection, consistent with the study protocol. Given that cell-based assays were performed on day of visit, blinding for these assays was not possible. For serum profiling, assays were performed from batched sources that were coded and unblinded only after determination of IgG3 transfer status.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Statistical analyses were performed with Prism v7 for Mac OS X (Graphpad Software Inc) TIRF images were analyzed with ImageJ (NIH, available at https://imagej.nih.gov/ij/docs/guide/146-2.html), Metamorph v7.7 (Molecular Devices), and Matlab v2017a (Mathworks). Flow cytometry was analyzed with FlowJo v9.9.6 (BD Biosciences). ImageStream data were analyzed with IDEAS v6.2 (EMD Millipore). Serologic IgG data were analyzed with FCAP Array v1.01 (BD Biosciences.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Please find attached appendix.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	HIV-infected individuals were enrolled under NIAID protocol 02-I-0202. Characteristics of study participants have been described in Table 1. There were three groups of HIV-infected individuals: early infected HIV-viremic individuals, defined as having been infected within 6 months and not receiving ART; chronically infected HIV-viremic individuals, defined as having been infected at least 6 months and not receiving ART; HIV-infected individuals on ART with suppressed HIV plasma viremia (< 40 or < 50 copies HIV RNA per ml). Protocol participation was contingent on HIV-infected study subjects maintaining a primary care physician and were encouraged to initiate ART, per US Department of Health and Human services current HIV treatment guidelines (http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf). HIV-negative individuals were enrolled under NIH clinical center blood donor protocols.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Ficolled cells were resuspended in ~200 µl of staining buffer, antibodies were added and incubated at 4C for 15 min, washed, fixed and acquired on instrument.
6. Identify the instrument used for data collection.	BD LSR-Fortessa for standard flow cytometry and BD FACSAria II for cell sorting assays.
7. Describe the software used to collect and analyze the flow cytometry data.	Software used for collection was FACS Diva and latest version of FlowJo for analysis.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	For standard flow cytometry, we typically collect 100,000 events. For cell sorting, a minimum of 50,000 cells were collect per fraction.
9. Describe the gating strategy used.	To establish FSC/SSC gates, viable single cells were identified and any debris, dead cells and doublets were excluded. To define positive and negative staining cell populations, negative and positive controls were used and real positive signals were determined. All antibodies were titrated to determine optimal amounts to use.

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