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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.

   n.a. / single cell expression profiling datasets with >8000 single cells.

2. **Data exclusions**

   Describe any data exclusions.

   All data were included in the paper.

3. **Replication**

   Describe whether the experimental findings were reliably reproduced.

   Experiments were reproduced at smaller scale using 2500 cells and 5 antibodies. ADT levels were independently validated by flow cytometry.

4. **Randomization**

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

   n.a.

5. **Blinding**

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

   n.a.

   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

7. Software

Describe the software used to analyze the data in this study.	10x Genomics Pipelines, Dropseq Tools (available online)

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

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.	All materials used in this study can be aquired from commercial vendors listed in the methods section of the manuscript.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Highly optimized flow cytometry antibodies were used for this study that have been shown to be specific in previous studies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	All cell lines used in this study were obtained from ATCC.
b. Describe the method of cell line authentication used.	n.a.
c. Report whether the cell lines were tested for mycoplasma contamination.	n.a.
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.	n.a.

▶ Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	n.a.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	n.a.

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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.	Peripheral blood mononuclear cells were obtained from Allcells (USA). After washing cells in PBS and fixing in 0.5% paraformaldehyde, samples were acquired on a BD Symphony A5 flow cytometer and data was analyzed using FlowJo v9 (Eugene, OR).
6.	Identify the instrument used for data collection.	BD Symphony A5 flow cytometer and Sony SH800
7.	Describe the software used to collect and analyze the flow cytometry data.	FlowJo v9.
8.	Describe the abundance of the relevant cell populations within post-sort fractions.	Abundance of cell populations was verified with CITE-seq. Sort fractions were reanalyzed by flow to verify sort efficiency.
9.	Describe the gating strategy used.	Cells were first Live/Dead selected (using DAPI). Cells were sorted subsequently based on CD8 expression levels. (see Figure 3c)

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