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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.

Sample sizes were chosen to provide sufficient confidence to validate methodoligical conclusions of the applicability of T-ATAC-seq. In general, sample sizes for primary cells were calculated in order to capture at least 20-50 single cells per aggregate cell state. The rationale for obtaining 20-50 cells per state is based on the ability of aggregated single-cell ATAC-seq data to accurately replicate ensemble profiles. This is described in detail in Supplementary Figure 2d-e. Single cells needed to obtain this number were estimated from flow cytometry of healthy T cell samples or clinical phenotyping of Sezary samples. Sample sizes for cell line data were calculated based on generating matched single cell data for comparison to previously published cell line scATAC-seq data.

2. Data exclusions

Describe any data exclusions.

No inclusion or exclusion criteria were used for human studies. No data were excluded from the manuscript.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All results presented in manuscript were reliably reproduced.

4. Randomization

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

No randomization of human participants was used. The experiments were designed to demonstrate the applicability of T-ATAC-seq to human T cell samples and not to determine treatment or clinical outcome.

5. Blinding

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

No blinding was used. The experiments were designed to demonstrate the applicability of T-ATAC-seq to human T cell samples and not the effect of treatments or perturbations on phenotypes.

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.

GraphPad Prism (Version 7) was used for statistical tests on ensemble population data. For single-cell ATAC-seq analysis, we used chromVAR, which is described in the methods, the original publication, and available on GitHub. Further downstream analysis of TF matrices obtained from chromVAR was performed using MATLAB (Version 8), Cluster 3.0, and Java Treeview (Version 3.0), and RStuido (Version 1.0.136). Visualization of scATAC-seq data was performed scHemeR (described in the original publication) and viewed on tcr.buenrostrolab.com.

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.

There are no restrictions on data availability.

9. Antibodies

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

We used anti-human CD45RA-PERCPCy5.5 (Clone HI100, Lot# B213966, Cat# 304107, Biolegend), anti-human CD127-Brilliant Violet 510 (Clone A019D5, Lot# B197159, Cat# 351331, Biolegend), anti-human CD4-APC-Cy7 (Clone OKT4, Lot# B207751, Cat# 317417, Biolegend), anti-human CCR6-PE (Clone G034E3, Lot# B203239, Cat# 353409, Biolegend), anti-human CD25-FITC (Clone BC96, Lot# B168869, Cat# 302603, Biolegend), anti-human CXCR3-Brilliant Violet 421 (Clone G025H7, Lot# B206003, Cat# 353715, Biolegend), anti-human CXCR5-AlexaFluor647 (Clone RF8B2, Lot# 5302868, Cat# 558113, BD Pharmingen), anti-human CD26-PE (Clone 2A6, Lot# 4301881, Cat# 12-0269-42, Thermo Fisher), and anti-human CD3E-Pacific Blue (Clone UCHT1, Lot# 4341657, Cat# 558117, BD Biosciences). All antibodies were validated by the manufacturer in human peripheral blood samples, used at a 1:200 dilution, and compared to isotype and no staining control samples.

10. Eukaryotic cell lines

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

Jurkat cells were obtained from ATCC (Clone E6-1).

b. Describe the method of cell line authentication used.

Jurkat cells were used immediately used for experiments after acquisition from the commercial source. Jurkat cells were further authenticated by FACS for CD3 and CD4 prior to use in experiments.

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

All cell lines tested negative for mycoplasma contamination prior to use in experiments.

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.

None of the cell lines used in this study are listed in this database.

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.

N/A

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.

This study was approved by the Stanford University Administrative Panels on Human Subjects in Medical Research, and written informed consent was obtained from all participants. Healthy human subjects were male, ages 30-50. Leukemic patients were female, ages 40-70.

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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.	CD4+ T cells from healthy volunteers or Sezary syndrome patients were enriched from peripheral blood using the RosetteSep Human CD4+ T Cell Enrichment Cocktail (StemCell Technology). Jurkat cells were obtained ATCC (Clone E6-1) and cultured in RPMI- 1640 Medium with 10% FBS and Penicillin/Streptomycin.
6. Identify the instrument used for data collection.	BD FACSAria II
7. Describe the software used to collect and analyze the flow cytometry data.	Flowjo v10
8. Describe the abundance of the relevant cell populations within post-sort fractions.	>95%. Examples of post-sort purities for each population are provided in the supplementary information.
9. Describe the gating strategy used.	For single cell experiments, CD4+ T helper cells were sorted as Naïve T cells (CD4+CD25-CD45RA+), Memory T cells (CD4+CD25-CD45RA-), or TH17 cells (CD4+CD25-CD45RA-CCR6+CXCR5-). For ensemble ATAC-seq experiments, CD4+ T helper cells were sorted as Naïve T cells (CD4+CD25-CD45RA+), Treg (CD4+CD25+IL7Rlo), TH1 (CD4 +CD25-,IL7Rhi,CD45RA-,CXCR3+,CCR6-), TH2 (CD4 +CD25-,IL7Rhi,CD45RA-,CXCR3-,CCR6-), TH17 (CD4 +CD25-,IL7Rhi,CD45RA-,CXCR3-,CCR6+), and TH1-17 (CD4 +CD25-,IL7Rhi,CD45RA-,CXCR3+,CCR6+). For single cell Sezary cell experiments, cells were sorted as CD4+CD26+ or CD4+CD26- populations. Example gating strategies for each population are provided in Supplementary Figures 3 and 7.

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