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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	Chromium Controller (10X Genomics) was used for scRNA-seq library preparation and samples were sequenced using NovaSeq 6000 S4 flow cells (Illumina).
Data analysis	Data analysis was performed using publicly available software and R packages: CellRanger v7.0.1 (10X Genomics, https://www.10xgenomics.com/support/software/cell-ranger) and R v4.1.0 (https://www.r-project.org/) with the R packages: Seurat v4.3.0 (https://satijalab.org/seurat/), DecontX as implemented in Celda v1.10.0 (https://bioconductor.org/packages/release/bioc/vignettes/celda/inst/doc/decontX.html), DoubletFinder v2.0.3 (https://github.com/chris-mcginnis-ucsf/DoubletFinder), limma v3.54.2 (https://bioconductor.org/packages/release/bioc/html/limma.html), edgeR v3.40.2 (https://bioconductor.org/packages/release/bioc/html/edgeR.html), stats v4.2.2 (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/00Index.html), clusterProfiler v4.6.2 (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html), ReactomePA v1.42.0 (https://bioconductor.org/packages/release/bioc/html/ReactomePA.html), decoupleR v2.8 (https://bioconductor.org/packages/devel/bioc/vignettes/decoupleR/inst/doc/decoupleR.html), ComplexHeatmap v2.14 (https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html) and CellChat v1.6.1 (https://github.com/sqjin/CellChat).

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 raw and processed scRNA-seq and CITE-seq data from the 6 resisters and 7 LTBI participants are being deposited to the Gene Expression Omnibus (GEO) and will be available at the time of publication.

Human research participants

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

Reporting on sex and gender	Sex was self-reported by the participants (1 male and 13 females as shown in Table 1). No analysis by sex was performed due to the presence of only one male participant which did not allow for a stratified analysis. As shown by Kroon et al. EBioMedicine, 2020 (doi: 10.1016/j.ebiom.2020.103053), this sex distribution reflects the sex distribution in the study population. A requirement in the definition of resisters was that participants had displayed CD4+ T cell counts of < 200/mm3. This condition could only be met for participants who had become HIV+ in the pre-ART era. A follow-up of these participants (no TB, no conversion, no other AIDS-linked diseases) was only possible for those participants who were followed in formal clinical settings. It is well established that in Cape Town these designated clinics are being avoided by male HIV+ patients. Male patients preferentially seek treatment in more anonymous and informal settings. For this reason, the study population of HIV+ resisters and LTBI in the ResisTB study was shifted towards females (Kroon et al. EBioMedicine, 2020 doi: 10.1016/j.ebiom.2020.103053).
Population characteristics	Demographic characteristics of the participants are presented in Table 1. The participants of this study are part of the ResisTB cohort, described in detail by Kroon et al. EBioMedicine, 2020 (doi: 10.1016/j.ebiom.2020.103053) and Gutierrez et al. Front Immunol, 2021 (doi: 10.3389/fimmu.2021.619988). All participants are people living with HIV (PLWH) with no history of tuberculosis (TB) while living in Cape Town, South Africa, an area of high Mycobacterium tuberculosis (Mtb) transmission. The "resister" group (also "HITTIN") is composed of seven subjects with three consecutive IGRA negative assays and a TST = 0 mm. The "LTBI" group (also "HIT") is composed of seven subjects with IGRA positivity in two consecutive tests and TST ≥ 10 mm. All participants have a history of low peripheral CD4+ T cell count, which was reconstituted after anti-retroviral therapy. Except for one LTBI participant, all participants were female. Participants were from the Xhosa ethnic group, except two LTBI individuals that were from the Sotho ethnic group. The mean (± standard deviation) age was 49±6 years in the resister and 49 ±5 years in the LTBI group. All subjects were non-smokers. Active TB or other lung infections were excluded by chest X-ray, no lung parenchymal abnormalities were observed, and all participants tested negative by sputum GeneXpert Ultra and liquid culture.
Recruitment	Recruitment was done using a prospective design as described by Kroon et al. EBioMedicine, 2020 (doi: 10.1016/j.ebiom.2020.103053). Enrolment criteria into the group of resisters were HIV-positivity, living in an area of high Mtb transmission, no current or previous clinical TB, history of at least one < 200 CD4+ T cells/mm3 count, CD4+ T cells reconstitute to at least 400 cells/mm3 for at least one year, age 35-60 years, three IGRA negative and two TST negative over three years of follow-up. Enrolment criteria for LTBI group were HIV-positivity, living in an area of high Mtb transmission, no current or previous clinical TB, two IGRA positive and one TST > 5 mm. Participants who fulfill the inclusion criteria were identified and re-contacted for this sub-study as allowed by the original informed consent until 7 participants of each classified group (resisters and LTBI) were reached. The priority of invitation for BAL collection of qualifying participants was based on ease of access to subjects and those willing to undergo testing for SARS-Cov-2.
Ethics oversight	Research was performed in accordance with the Declaration of Helsinki and all participants provided written informed consent for the study procedures, which was approved by the Stellenbosch University (SU) Health Research Ethics Committee (N16/03/033), the SU Research Ethics for Biological and Environmental Safety Committee (BES-2023-19406) and the Research Institute of the McGill University Health Centre (MP-CUSM-15-406).

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	For the present study, we recruited 14 participants (7 resisters and 7 LTBI) for BAL cell collection, and we aimed for obtaining four scRNA-seq samples per subject (non-infected vs in-vitro Mtb challenged samples from two time-points, 6h and 24h). In addition, we generated two non-infected samples with scRNA-seq plus cell-surface antigen capture (CITE-seq) from one resister and one LTBI participant. We successfully obtained data from 55 scRNA-seq from 6 resisters and 7 LTBI participants and from the 2 CITE-seq samples (Supplementary Table 1), totalizing 257,671 high-quality cells for the data analysis (105,142 and 152,529 cells from the resister and LTBI participants, respectively). No sample-size calculation was performed.
Data exclusions	AT RECRUITMENT AND BAL COLLECTION: During the patient follow-up for the ResisTB cohort, participants that did not reach all the enrolment criteria for the resister and LTBI phenotype groups (see recruitment section above) were excluded. Prior to bronchoscopy, the IGRA status of all subjects was validated and a sputum GeneExpert was done to exclude undetected tuberculosis infection. Specifically, participants were excluded from the bronchoscopy procedure if they presented one of the following exclusion criteria: i) could not offer a permanent residential address or planned to relocate for the duration of the study, ii) participated in a drug/vaccine trial, iii) their hemoglobin levels were measured to be <9g/L, iv) were pregnant (current, during the study or within the last 6 months) or breastfeeding, v) had any severe systemic condition/co-morbidity that could affect their safety or performance of the assays, including uncontrolled diabetes, cancer, uncontrolled hypertension or ischemic heart disease, vi) suffered from any other major medical conditions (apart from HIV),vii) used immunosuppressive medications such as TNF-alpha inhibitors or systemic/inhaled corticosteroid within the past 2 weeks, viii) had a history of taking specific antibiotics in the past 4 weeks (INH, Rifampin, Rifabutin, Rifapentine, Ethambutol, PZA, Kanamycin, Amikacin, Streptomycin, Capreomycin, Moxifloxacin, Levofloxacin, Gatifloxacin, Ofloxacin, Cprofloxacin, other Floroquinolones, Ethionamide, Prothionamide, Cycloserine, Terizidone, Para-aminosalicylic acid, linezolid, Clofazamine,Delamanid or Bedaquiline), ix) had any known allergy or intolerance to components of or drugs used in procedural sedation, x) had a known history or family history of malignant hyperthermia, xi) presented with symptoms suggestive of active TB disease (e.g. cough of any duration, fever for more than two weeks, unexplained weight loss, drenching night sweats), xii) tested positive for SARS-CoV-2 by PCR, or xiii) presented any other condition the physician/study nurse considered not appropriate for the study, or that the study is not in the person's best interest (e.g. if substance or alcohol abuse may interfere with the participant's adherence to study procedures). AT scRNA-SEQ LIBRARY PREPARATION: One sample was excluded during the library preparation process as it failed during Reverse-Transcription step and did not yield cDNA (6h non-infected sample from 2RTB0205). AT scRNA-SEQ DATA ANALYSIS: Low quality, doublets and dead cells were excluded during the pre-processing quality control steps as described in the Methods section. Dead cells were defined by >20% mitochondrial genes per cell. Four scRNA-seq samples obtained from the BAL of one participant (2RTB0224) were excluded due to high proportion of dead cells in the scRNA data (>70%). During the different steps of data analysis, inclusion/exclusion of scRNA-seq samples were related to their infection status, time-point or number of cells in the clusters, according to the aim of the analysis. This is explained in more detail in the Methods section and the exact list of samples used in each analysis is presented in Supplementary Table 1.
Replication	By splitting the BAL cells into four scRNA-seq samples with specific experimental conditions per participant, we observed an experimental replication of the lymphocyte populations abundance (Extended figures 1 and 3). No replication in an independent population sample was performed as this is an observational study from participants carefully selected based on their phenotype and high exposure to Mtb.
Randomization	Not applicable since this is an observational study.
Blinding	Not applicable since this is an observational study.

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

FOR CITE-SEQ OF BAL:
TotalSeq™-B Human TBNK Cocktail for surface antigens CD19 (clone HIB19), CD3 (clone UCHT1), CD16 (clone 3G8) , CD4 (clone RPA-T4), CD11c (S-HCL-3), CD56 (clone 5.1H11), CD14 (clone M5E2), CD8 (clone SK1) and CD45 (clone 2D1) (BioLegend, Cat # 399902, RRID AB_2832784).

FOR FLOW CYTOMETRY OF PBMC:
B cell/monocyte panel:
BD CD45 (clone 2D1) PerCP (BD Biosciences, Cat # 345809)
BD CD19 (clone SJ25C1) APC (BD Biosciences, Cat # 345791)
BD CD14 (clone M0P9) FITC (BD Biosciences, Cat # 345784)
T cell panel:
BD CD8 (clone SK1) FITC (BD Biosciences, Cat # 345772)
BD CD4 (clone SK3) PE (BD Biosciences, Cat # 345769)
BD CD3 (clone SK7) APC (BD Biosciences, Cat # 345767)
BD CD45 (clone 2D1) PerCP (BD Biosciences, Cat # 345809)

Validation

FOR CITE-SEQ OF BAL:
The commercially available antibodies from TotalSeq™-B Human TBNK Cocktail used in this study were acquired from BioLegend and validated by the vendor by flow cytometry.

FOR FLOW CYTOMETRY OF PBMC:
The commercially available antibodies from Becton, Dickinson and Company (BD) were validated by the vendor for flow cytometry. Experienced staff at the haematology laboratory of the National Health Laboratory Service (NHLS, South Africa) performed additional validation through internal quality controls (Beckman/Coulter Immunotrol) and external quality assurance (UKNEQAS).

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

Blood was collected by phlebotomy in a heparinized vacutainers and PBMC isolated according to the standard Ficoll isolation method. PBMC were cryopreserved in 10% dimethyl sulfoxide (MilliporeSigma, Massachusetts, USA) and 90% fetal bovine serum (Cytiva, Massachusetts, USA).

Instrument

Becton, Dickinson and Company (BD) FACSLyric S/N R659180000361

Software

BD FACSuite vs1.5.0.925

Cell population abundance

Percent of parent population.

Gating strategy

Two antibody panels were gated independently. For the B cell/monocyte panel, the first gate was placed on all cells, to exclude debris, followed by a gate on single cells, to exclude doublets, whereafter lymphocytes, monocytes and granulocytes were gated based on FSC-SSC. Thereafter CD45+CD14+ and CD45+CD19+ populations were gated from the monocyte and lymphocyte population respectively. For the T cell panel, the first gate was placed on all cells, to exclude debris, followed by a gate on single cells, to exclude doublets, whereafter lymphocytes, monocytes and granulocytes were gated based on FSC-SSC. Thereafter CD3+CD8+ and CD3+CD8+ populations were gated from the lymphocyte population.

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