**Supplementary Methods**

*Clinical data collection*

Clinical data were retrospectively collected from health records following institutional approval (EA2/142/20) and patients consent.

*Spectral flow cytometry*

Sequentially collected PBMCs were profiled over four consecutive days on a Cytek Aurora 5L using a 45-marker full-spectrum panel (see below). Thawed cells were washed and resuspended in PBS supplemented with 2% FCS. Extracellular staining was performed in three sequential steps at 37 °C: first, CD19 antibody; second, CAR detection antibody; and finally, the remaining surface markers. Following surface labeling, cells were fixed with the BD Fixation Kit, then incubated overnight at 4 °C with intracellular antibodies. For the analysis of cellular frequencies raw .fcs files were pre-processed using PeacoQC to remove artefact events and anomalies. Populations of interest (live cells) were exported using channel values as csv files.1 Further analyses were performed in R (v. 4.3.0) using the Seurat package.2

*TCR-ß sequencing*

TCR-ß profiling was carried out using the TCRsafeTM analysis pipeline (HS Diagnomics) using a two-step PCR approach and subsequent paired-end next generation sequencing as described previously.3 TCR sequences were cross-referenced to a curated epitope database (VDJdb).4 Further downstream analysis was performed in R (v.4.4.1) using the tidyverse and immunarch package (v.0.9.1).5,6

*Cytokine detection assay*

Cytokine levels were measured using a bead-based multiplex assay (LEGENDplex™ Human CD8/NK Panel; BioLegend) following the manufacturer’s instructions. Briefly, serially collected plasma samples were thawed and incubated with capture beads for 2 hours. After washing, a biotinylated detection antibody was applied for 1 hour, followed by a 30-minute incubation with streptavidin-phycoerythrin. All steps were performed according to the manufacturer’s protocol. Samples were washed and analyzed on a CytoFLEX flow cytometer (Beckman Coulter). Each experiment was performed in duplicate, and data analysis was conducted using the LEGENDplex™ Data Analysis Software Suite.

*Antibody panel*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fluorochrome** | **Marker** | **Company** | **Catalog number** | **Clone** |
| Spark UV387 | CD45RA | Biolegend | 304180 | HI100 |
| BUV395 | CD33 | BD | 568374 | WM53 |
| BUV496 | CD1c | BD | 750182 | L161 |
| BUV563 | CD3 | BD | 741448 | SK7 |
| BUV615 | PRDX2 | BD | 570740 | EPR5154 |
| BUV661 | CD27 | BD | 741609 | M-T271 |
| BUV737 | CD123 | BD | 741769 | 7G3 |
| BUV805 | CD45RO | BD | 748367 | UCHL1 |
| BV421 | CD141 | BD | 565321 | 1A4 |
| AF405 | GLUT1 | abcam | ab210438 | EPR3915 |
| Pacific Blue | CD11c | Biolegend | 301626 | 3,9 |
| BV480 | CD223/LAG3 | BD | 746609 | T47-530 |
| BV510 | CD71 | BD | 743305 | M-A712 |
| cfluorV547 | CD8 | Cytek | R7-20063 | SK1 |
| BV605 | CD15 | Biolegend | 323032 | W6D3 |
| BV650 | CD279 | BD | 564104 | EH12.1 |
| BV711 | CD56 | Biolegend | 318336 | HCD56 |
| BV750 | IgD | BD | 747484 | IA6-2 |
| BV786 | CD86 | BD | 747526 | BU63 |
| BB515 | CD57 | BD | 565945 | NK-1 |
| AF488 | Foxo1 | Cell signaling | 58223S | C29H4 |
| RB545 | CD197 | BD | 569271 | 2-L1-A |
| Spark Blue 574 | CD20 | Biolegend | 285019 | 2H7 |
| Novafluor Blue 610 70S | CD44 | ThermoFisher | M010T02B06 | IM7 |
| NovaFluor Blue 660-120S | CD200 | ThermoFisher | H038T03B08-A | OX104 |
| PerCP | CD38 | Biolegend | 303520 | HIT2 |
| BB700 | CD16 | BD | 742286 | B73.1 |
| RB705 | TCF1 | BD | 570635 | S33-966 |
| RB744 | CD137/4-1BB | BD | 757878 | 4B4-1 |
| RB780 | CD4 | BD | 568675 | SK3 |
| PE | anti-biotin CD19/BCMA CAR detection reagent | Miltenyi | CD19: 130-129-550, BCMA: 130-126-090, anti-biotin: 130-113-291 | CD19: AB\_2811310 (RRID); BCMA: no clone number provided: Biotin: Bio3-18E7 |
| RY586 | CD94 | BD | 753479 | HP-3D9 |
| AF594 | ATP5A | abcam | ab216385 | EPR13030(B) |
| PE-Fire 640 | CD19 | Biolegend | 302274 | HIB19 |
| PE-Cy5 | CD69 | BD | 555532 | FN50 |
| PE-Fire700 | CD25 | Biolegend | 356146 | M-A251 |
| PE Cy7 | TCRgd | BD | 655410 | 11F2 |
| PE-Fire810 | CD39 | Biolegend | 328245 | A1 |
| APC | FOXP3 | Miltenyi | 130-125-580 | REA1253 |
| AF647 | HK1 | BD | 569319 | EPR10134(B) |
| SPARK-NIR | CD14 | Biolegend | 399210 | S18004B |
| AF700 | FcER1A | Biolegend | 334630 | AER-37 |
| Zombie NIR | LD | Biolegend | 423105 | N/A |
| APC-Vio770 | CD45 | Miltenyi | 130-110-635 | REA747 |
| APC-Fire810 | HLA-DR | Biolegend | 307674 | L243 |

**Supplementary References**

1 Emmaneel A, Quintelier K, Sichien D, Rybakowska P, Marañón C, Alarcón-Riquelme ME *et al.* PeacoQC: Peak-based selection of high quality cytometry data. *Cytometry A* 2022; **101**: 325–338.

2 Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A *et al.* Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol* 2024; **42**: 293–304.

3 Seitz V, Kleo K, Dröge A, Schaper S, Elezkurtaj S, Bedjaoui N *et al.* Evidence for a role of RUNX1 as recombinase cofactor for TCRβ rearrangements and pathological deletions in ETV6-RUNX1 ALL. *Sci Rep* 2020; **10**: 10024.

4 Goncharov M, Bagaev D, Shcherbinin D, Zvyagin I, Bolotin D, Thomas PG *et al.* VDJdb in the pandemic era: a compendium of T cell receptors specific for SARS-CoV-2. *Nat Methods* 2022; **19**: 1017–1019.

5 Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R *et al.* Welcome to the Tidyverse. *JOSS* 2019; **4**: 1686.

6 Nazarov VI, Tsvetkov VO, Fiadziushchanka S, Rumynskiy E, Popov AA, Balashov I *et al.* *immunarch: Bioinformatics Analysis of T-Cell and B-Cell Immune Repertoires*. ImmunoMind, 2023https://immunarch.com/.