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Reporting Summary

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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

Flow cytometry data were collected using a FACSymphony A5 or an LSR Fortessa with FACSDiva Software Version 8.0.1 (BD Pharmingen). Mass cytometry data were collected using a Helios (Fluidigm). Sequencing data were collected using a NovaSeq 6000 or NextSeq 500/550 (Illumina). For single telomere length analysis hybridized fragments were detected using a Typhoon FLA 9500 Phosphorimager (GE Healthcare) and the molecular weights of the DNA fragments were calculated using a Phoretix 1D Quantifier (Nonlinear Dynamics).

Data analysis

FACS: Flow Cytometry Standard (FCS) 3.0 files were analyzed with FlowJo 9 and 10 (FlowJo LLC). In dedicated experiments samples were further analyzed with Python version 3.7.3 using a custom-written script incorporating PhenoGraph retrieved from the scikit-learn package (https://github.com/luglilab/Cytophenograph). UMAP was obtained by UMAP Python package and visualized in FlowJo 10.

scRNA-Seq: Sample demultiplexing, barcode processing, and UMI counting were performed using Cell Ranger version 2.1.1 (10X Genomics). Pooled data were imported into R version 3.5.1 using Seurat version 3.0.1.

RNA-Seq: Raw sequence data were quality-controlled using FastQC version 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Single-end reads were aligned to the human genome (GENCODE Human Release 29; Reference genome sequence: GRCh38/hg38) using STAR version 2.5.1b. Alignments were performed using default parameters. Reads associated with annotated genes were counted using the STAR aligner option "-quantMode geneCounts". Differential gene expression was assessed using the edgeR package version 3.20.9. Benjamini-Hochberg correction was applied to estimate the false discovery rate (FDR). Paired-end reads were processed similarly after removing adapter sequences and poor-quality bases with Trimmomatic version 0.36.

Public data analyses: To identify the signatures "YF_naive vs. effector UP" and "YF_effector vs. naive UP", the expression profiles of effector CD8+ T cells isolated 14 d after vaccination with YF-17D were compared with those of naive CD8+ T cells using the limma algorithm in the same R package version 3.34.9. Public data obtained from Akondy et al., Nature, 2017. Batch effects were eliminated by adjusting gene expression values in the combined data matrix with the empirical Bayes method ComBat coded in the SVA package version 3.26.0. For GSEA, gene sets of interest were retrieved from collections C2 and C7 in the Molecular Signatures Database v6.2. To perform hypergeometric tests between G5 or G10 signatures and TSCM / TCM or TPEX subsets: differentially expressed genes (adjusted

p-value < 0.01) in the pairwise comparison of G5 versus G10 T cell clusters were determined by the "FindAllMarkers" function coded in the Seurat R package (version 3.0.1). Hypergeometric tests were run with the "phyper" R function. Public data obtained from Sade-Feldman et al., Cell, 2018.

ATAC-Seq: Read quality was assessed using FastQC version 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adaptors and poor-quality bases were trimmed using Cutadapt version 1.16. Samples were aligned to the human reference genome GRCh38 using default parameters in BWA-MEM version 0.7.17. Mitochondrial reads were removed using SAMtools version 1.9. PCR duplicates were removed using the "MarkDuplicates" function in Picard Tools version 2.19 (http://broadinstitute.github.io/picard/). Open chromatin was detected using MACS2 version 2.1.2 with an FDR < 0.01. The number of reads in each peak was determined using featureCounts version 1.6.4. Differentially accessible peaks were identified using an FDR cut-off below 0.05 after normalization in DESeq2 version 1.20 (Bioconductor). Peaks were annotated using the "annotatePeaks.pl" function and scanned for motifs using the "findMotifsGenome.pl" function in HOMER version 4.9.1.

TCR-Seq: UMI extraction and consensus assembly were performed using MIGEC software version 1.2.9 with a threshold of at least three reads per UMI. In-frame CDR3β repertoires were extracted using MiXCR software version 3.0.3. Each library contained from 3,000 to 18,000 functional CDR3β clonotypes. Diversity metrics were calculated using VDJtools software version 1.2.1 after normalization to 42,000 randomly selected UMIs per sample. D, R, and F2 metrics were calculated for the top 3,000 clones from each pair of samples using VDJtools software version 1.2.1.

Other: Microsoft Excel version 15.37, GraphPad Prism version 7.0c and R software version 3.4.4.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Publicly available data were retrieved from the Gene Expression Omnibus: GSE120575 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120575; processed data for single cell RNA sequencing) and GSE26347 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26347; microarray data of YFV-17D specific CD8 T cells in humans). Gene sets of interest were retrieved from the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp). The ATAC-seq data reported in this paper are available on request. The bulk RNA-seq and scRNA-seq data reported in this paper have been deposited in the Gene Expression Omnibus under accession code GSE147398. The TCR-seq data reported in this paper have been deposited at the European Bioinformatic Institute under accession code E-MTAB-8892. All other data that support the findings of this study are available from the corresponding author upon request.

Field-specific reporting

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☒ 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	Sample size was chosen taking into account the means of the target values between the different experimental groups, the standard error and the statistical analyses used. Additionally, the selection of sample size was based on previous studies conducted by this laboratory which allow for statistically valid comparisons.
Data exclusions	Eight-week-old female JAX NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG, #005557, Charles River) mice were infused by retroorbital injection with TSTEM, TPEX or TEM cells (1x10^6 per mouse), freshly sorted from two healthy donors' PBMCs (Fig. 3h-j). An experiment from a third donor led to a poor recovery of cells following transfer in primary recipients. Recovered cells did not pass the pre-established criteria of inclusion which implies to retransfer 1x10^6 cells into secondary recipient mice. Thus, the experiment was excluded. No other data were excluded.
Replication	All data were reliably reproduced in at least two independent experiments with the exception of scRNA-seq (once from four donors), high-dimensional flow cytometry (once from 6 donors per tissue with matched peripheral blood), bulk RNA-seq (ex vivo, once from 5 donors; activated cells, once from 4 donors), ATAC-seq (ex vivo, once from 3 donors; activated cells, once from 3 donors), TCR-seq (once from 6 donors) and STELA assay (once from 6 donors). The experiment evaluating in vivo CAR19-redirected T cell subsets was also performed once.
Randomization	For in vivo tumor experiments, mice were randomized prior to adoptive cell transfer. For all the remaining experiments, donors (healthy and patients) were randomly selected in order to avoid potential biases (see Supplementary Table 1 for more details). No manual randomization was performed on these samples.
Blinding	All the data were collected and analyzed in a non-blind fashion because did not involve subjective measurements.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

FLOW CYTOMETRY
Anti-human CD45 - PE-Cy7 - 1:666 - HI30 - BioLegend - Cat # 304016 - RRID:AB_314404
Anti-human CD8 - BUV 805 - 1:166 - SK1 - BD Biosciences - Cat # 564912 - RRID:AB_2744465
Anti-human CD8 - BV786 - 1:322 - RPA-T8 - BD Biosciences - Cat # 563823 - RRID:AB_2687487
Anti-human CD4 - FITC - 1:80 - M-T477 - BD Biosciences - Cat # 556615 - RRID:AB_396487
Anti-human CCR7 - BV711 - 1:40 - G043H7 - BioLegend - Cat # 353228 - RRID:AB_2563865
Anti-human CD45RA - BV480 - 1:166 - HI100 - BD Biosciences - Cat # 566114 - RRID:AB_2739516
Anti-human CD45RO - APC-H7 - 1:80 - UCHL1 - BD Biosciences - Cat # 561137 - RRID:AB_10562194
Anti-human CCR7 - BB660 - 1:100 - 150503 - BD Biosciences - Cat # 625454 - N/A
Anti-human CD95 - BV421 - 1:166 - DX2 - BioLegend - Cat # 305624 - RRID:AB_2561830
Anti-human CD95 - BUV563 - 1:40 - DX2 - BD Biosciences - Cat # 624284 - N/A
Anti-human CD95 - APC - 1:333 - DX2 - BD Biosciences - Cat # 558814 - RRID:AB_398659
Anti-human CD45RO - BUV395 - 1:80 - UCHL1 - BD Biosciences - Cat # 562491 - N/A
Anti-human CD3 - BV650 - 1:40 - OKT3 - BioLegend - Cat # 317324 - RRID:AB_2563352
Anti-human CD4 - BV570 - 1:80 - RPA-T4 - BioLegend - Cat # 300534 - RRID:AB_2563791
Anti-human CD4 - BUV615 - 1:3,333 - SK3 - BD Biosciences - Cat # 624297 - N/A
Anti-human CD3 - BUV496 - 1:40 - UCHT1 - BD Biosciences - Cat # 564809 - RRID:AB_2744388
Anti-human CD27 - BV570 - 1:40 - O323 - BioLegend - Cat # 302825 - RRID:AB_11149686
Anti-human TNF-α - APC-Cy7 - 1:80 - mAB11 - BioLegend - Cat # 502944 - RRID:AB_2562870
Anti-human IFN-γ - PE-Cy7 - 1:666 - B27 - BioLegend - Cat # 506518 - RRID:AB_2123321
Anti-human IL-2 - APC - 1:322 - MQ1-17H12 - BD Biosciences - Cat # 554567 - RRID:AB_398571
Anti-human CD45 - Pacific Blue - 1:166 - HI30 - BioLegend - Cat # 304022 - RRID:AB_493655
Anti-human CD3 - PE-Cy5 - 1:20 - HIT-3A - BD Biosciences - Cat # 555341 - RRID:AB_395747
Anti-human CCR7 - PE-CF594 - 1:40 - 150503 - BD Biosciences - Cat # 562381 - RRID:AB_11153301
Anti-human CD27 - PE - 1:10 - M-T271 - BD Biosciences - Cat # 560985 - RRID:AB_10563213
Anti-human CD28 - BV785 - 1:166 - CD28.2 - BioLegend - Cat # 302950 - RRID:AB_2632607
Anti-human CD25 - APC-R700 - 1:80 - 2A3 - BD Biosciences - Cat # 565106 - RRID:AB_2744339
Anti-human CD69 - BUV737 - 1:322 - FN50 - BD Biosciences - Cat # 564439 - RRID:AB_2722502
Anti-human CD107a - PE-Cy5 - 1:80 - H4A3 - BD Biosciences - Cat # 555802 - RRID:AB_396136
Anti-human CD107a - BB630 - 1:2,500 - H4A3 - BD Biosciences - Cat # 624294 - N/A
Anti-human CD127 - PE-Cy5 - 1:40 - eBioRDR5 - eBioscience - Cat # 15-1278-42 - RRID:AB_2043801
Anti-human PD-1 - BV480 - 1:27 - EH12.1 - BD Biosciences - Cat # 566112 - RRID:AB_2739514
Anti-human PD-1 - PE-Cy7 - 1:166 - EH12.2H7 - BioLegend - Cat # 329918 - RRID:AB_2159324
Anti-human TIGIT - PerCP-eFluor 710 - 1:40 - MBSA43 - eBioscience - Cat # 46-9500-42 - RRID:AB_10853679
Anti-human TIGIT - FITC - 1:40 - MBSA43 - eBioscience - Cat # 11-9500-41 - RRID:AB_2572529
Anti-human TIGIT - BV421 - 1:80 - A15153G - BioLegend - Cat # 372710 - RRID:AB_2632925
Anti-human HLA-DR - BUV661 - 1:166 - G46-6 - BD Biosciences - Cat # 565073 - RRID:AB_2722500
Anti-human CD38 - BV711 - 1:166 - HIT2 - BioLegend - Cat # 303528 - RRID:AB_2563811
Anti-human CD103 - BV421 - 1:166 - Ber-ACT8 - BioLegend - Cat # 350213 - RRID:AB_2563513
Anti-human CD161 - BV605 - 1:20 - HP-3G10 - BioLegend - Cat # 339916 - RRID:AB_2563607
Anti-human CD14 - BV510 - 1:20 - M5E2 - BioLegend - Cat # 301842 - RRID:AB_2561946
Anti-human Granulysin - Alexa Fluor 488 - 1:20 - RB1 - BD Biosciences - Cat # 558254 - N/A
Anti-human EOMES - PE-eFluor 610 - 1:80 - WD1928 - eBioscience - Cat # 61-4877-41 - RRID:AB_2574615
Anti-human Granzyme B - APC-R700 - 1:80 - GB11 - BD Biosciences - Cat # 561016 - RRID:AB_2033973
Anti-human Granzyme K - PE - 1:166 - GM6C3 - Santa Cruz - Cat # sc-56125 PE - RRID:AB_2263772
Anti-human Granzyme K - Alexa Fluor 647 - 1:322 - GM6C3 - Santa Cruz - Cat # sc-56125 AF647 - RRID:AB_2263772
Anti-human IRF4 - Alexa Fluor 488 - 1:322 - IRF4.3E4 - BioLegend - Cat # 646406 - RRID:AB_256326
Anti-human IRF8 - APC - 1:666 - V3GYWCH - eBioscience - Cat # 17-9852-80 - RRID:AB_2573317
Anti-human T-bet - PE-Cy7 - 1:666 - 4-B10 - eBioscience - Cat # 25-5825-82 - RRID:AB_11042699
Anti-human LEF1 - PE - 1:111 - C12A5 - Cell Signaling - Cat # 14440 - N/A
Anti-mouse CD45.1 - PE-Cy7 - 1:100 - A20 - BD Biosciences - Cat # 560578 - RRID:AB_1727488
Anti-mouse CD45 - PerCP-Cy5.5 - 1:1,250 - 30-F11 - BioLegend - Cat # 103132 - RRID:AB_893340

Tetramer CMV-pp65 (wt) - PE - 1:50 - Sequence NLVPMVATV
Tetramer CMV-pp65 (D227K/T228A [KA]) - PE - 1:50 - Sequence NLVPMVATV

MASS CYTOMETRY
Anti-human CD45 - 89 - 1:200 - Hi30 - Fluidigm - Cat # 3089003B
Anti-human CD14 - 112/114 - 1:200 - TüK4 - Invitrogen - Cat # Q10064
Anti-human CD57 - 115 - 1:400 - HCD57 - BioLegend - Cat # 322325 (discontinued)
Anti-human Granzyme B - 141 - 1:300 - 2C5/F5 - BD Biosciences - Cat # 550558
Anti-human HLA-DR - 142 - 1:100 - L243 - BioLegend - Cat # 307651
Anti-human ITB7 - 143 - 1:200 - FIB504 - BioLegend - Cat # 321202
Anti-human TIGIT - 144 - 1:50 - MAB7898 - R&D Systems - Cat # MAB7898
Anti-human Granzyme K - 145 - 1:50 - GM6C3 - Life Technologies - Cat # MA1-17755
Anti-human CD8a - 146 - 1:400 - SK1 - BioLegend - Cat # 344727
Anti-human CD4 - 147 - 1:400 - SK3 - BioLegend - Cat # 344625
Anti-human CD45RO - 148 - 1:200 - UCHL1 - BioLegend - Cat # 304239
Anti-human CD161 - 149 - 1:100 - HP-3G10 - BioLegend - Cat # 339919
Anti-human KLRG1 - 150 - 1:200 - 13F2F12 - eBioscience - Cat # 16-9488-85
Anti-human CD27 - 151 - 1:200 - LG.7F9 - eBioscience - Cat # 5012495
Anti-human 2B4 - 152 - 1:100 - C1.7 - BioLegend - Cat # 329502
Anti-human CD103 - 153 - 1:400 - B-Ly7 - eBioscience - Cat # 5012794
Anti-human TCRgd-PE - 154 - 1:100 - 5A6.E9 - Invitrogen - Cat # MHGD04
Anti-PE - 1:100 - PE001 - BioLegend - Cat # 408105
Anti-human CD95 - 155 - 1:100 - DX2 - BioLegend - Cat # 305631
Anti-human CD3 - 156 - 1:200 - UCHT1 - BioLegend - Cat # 300443
Anti-human Granzyme A - 157 - 1:200 - CB9 - BioLegend - Cat # 507202
Anti-human CD56 - 158 - 1:200 - NCAM16.2 - BD Biosciences - Cat # 559043
Streptavidin - 159 - in house
Anti-human PD-1 - 160 - 1:50 - eBioJ105 - eBioscience - Cat # 14-2799-80
Streptavidin - 161 - in house
Anti-human CD19 - 162 - 1:200 - HIB19 - BioLegend - Cat # 302247
Streptavidin - 163 - in house
Streptavidin - 164 - in house
Anti-human CXCR5 - 165 - 1:100 - RF8B2 - BD Biosciences - Cat # 552032
Anti-human CTLA4 - 166 - 1:200 - BNI3 - BD Biosciences - Cat # 555851
Streptavidin - 167 - in house
Anti-human CCR7 - 168 - 1:100 - MAB197-100 - R&D Systems - Cat # MAB197-100
Anti-human CD45RA - 169 - 1:100 - HI100 - BioLegend - Cat # 304143
Streptavidin - 170 - in house
Anti-human CCR5 - 171 - 1:100 - NP-6G4 - Fluidigm - Cat # 3171017A
Anti-human CD39 - 172 - 1:100 - A1 - BioLegend - Cat # 328221
Streptavidin - 173 - in house
Anti-human CD127 - 174 - 1:100 - A019D5 - BioLegend - Cat # 351337
Anti-human Perforin - 175 - 1:200 - B-D48 - Abcam - Cat # ab47225
Anti-human CD38 - 176 - 1:100 - HIT2 - BioLegend - Cat # 303535
DNA - 191/193 - 1:2000 - Fluidigm - Cat # 201192B
Cisplatin - 194 - 1:4000 - Sigma-Aldrich - Cat # 479306-1G
Anti-human CD16 - 209 - 1:200 - 3G8 - Fluidigm - Cat # 3209002B
Tetramer CMV-IE1 - Sequence VLEETSVML
Tetramer CMV-pp65 - Sequence NLVPMVATV
Tetramer CMV-pp65-2 - Sequence QMWQARLTV
Tetramer EBV-BRLF1 - Sequence YVLDHLIVV
Tetramer EBV-BLMF1 - Sequence GLCTLVAML
Tetramer EBV-LMP1-1 - Sequence YLLEMLWRL
Tetramer EBV-LMP1-2 - Sequence YLQQNWWTL
Tetramer EBV-LMP2A - Sequence CLGGLLTMV
Tetramer Flu-M1 - Sequence GILGFVFTL
Tetramer Flu-PB1 - Sequence NMLSTVLGV
Tetramer Rota-VP6 - Sequence TLLANVTAV

Validation

All the reagents for flow cytometry and mass cytometry used in this manuscript went through stringent validation steps as previously described (Simoni et al., Nature, 2018; Mazza et al., Cytometry A., 2018; Brummelman et al., Nat. Protoc., 2019). Antibodies were all titrated to determine the optimal concentration. Flow cytometry antibodies were serially diluted as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1,280. Mass cytometry antibodies were serially diluted as follows: 1:50, 1:100, 1:200, and 1:400. Titration stainings included additional markers in order to validate the expression patterns on known cell subsets (e.g. T cells). Optimal concentration was defined by comparing the expression with other previously validated clones of a given antibody or with other previously validated lots of the same antibody clone. Any further information on the validation performed by the manufacturer can be retrieved from the manufacturers' websites.

Flow cytometry:
Fluorochrome-conjugated monoclonal antibodies were purchased from commercial vendors. High-dimensional flow cytometry was performed as previously described (Brummelman et al., Nat. Protoc., 2019). Biotinylated wildtype and D227K/T228A (KA) HLA-A*0201 complexes refolded with CMV pp65495-503 NLVPMVATV (NV9) were multimerized with streptavidin-PE (Sigma-Aldrich) as described previously (Roberto et al., Blood, 2015). Cells were stained with each tetramer at a concentration of 5 µg/ml for 15 min at 37°C.

Cytometry by Time of Flight (CyTOF):
Purified monoclonal antibodies were purchased from commercial vendors (Supplementary Table 7) and labeled according to the Maxpar Antibody Labeling Kit Protocol (Fluidigm). Streptavidin was produced and labeled as described previously (Ramachandiran et al., J. Immunol. Methods, 2007; Newell et al., Nat. Biotechnol., 2013). Myc-tagged peptide-HLA class I monomers were synthesized and biotinylated as described previously (Toebes et al., Nat. Med., 2006; Bakker et al., Proc. Natl. Acad. Sci. U. S. A., 2008). Peptide-HLA class I tetramers were generated via the addition of heavy metal-labeled streptavidins in a triple coding scheme and used to stain cells in a cocktail format as described previously (Ramachandiran et al., J. Immunol. Methods, 2007; Newell et al., Nat. Biotechnol., 2013). Antibody staining, live/dead discrimination, and DNA staining were performed as described previously (Simoni et al., Nature, 2018).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The human NALM-6 cell line was purchased from DSMZ, Germany.
Authentication	NALM-6 cell line was validated by the vendor, and by the authors' assessment of cell morphology and of CD19 expression by FACS.
Mycoplasma contamination	NALM-6 cell line was confirmed mycoplasma negative (Mycoplasmacheck, Eurofins Genomics).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Eight-week-old female JAX NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG, #005557, Charles River) mice were used. Mice were housed and bred in a specific pathogen-free animal facility, treated in accordance with the European Union guidelines.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were employed in this study.
Ethics oversight	All experiments using mice were conducted upon the approval of the institutional IACUCs (Humanitas Clinical and Research Center and IRCCS San Raffaele Scientific Institute) and the Italian Ministry of Health.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Samples from healthy donors, HIV infected individuals, patients with head and neck cancer and non-small cell lung cancer patients were utilized in this study. A total of 124 individuals (95 males, 28 females and 1 unknown gender) were included in the study. Age range: 22-77 years old (3 unknown age). Two individuals were HIV infected patients. Twelve individuals were cancer patients: six with head and neck cancer and six with non-small cell lung cancer. All cancer patients were treatment-naive. Please, refer to Supplementary Table 1 for further details.
Recruitment	Healthy donors were obtained from Humanitas Clinical and Research Center and Fred Hutchinson Cancer Research Center. HIV infected individuals were obtained from Fred Hutchinson Cancer Research Center. Patients with head and neck cancer and non-small cell lung cancer patients were obtained from Humanitas Clinical and Research Center. We randomly selected samples from healthy donors or HIV infected individuals or treatment-free cancer patients that underwent surgical resection of the adjacent tumor-free lung tissue or lymph node. All the samples were anonymized. No other self-selection biases are present.
Ethics oversight	Humanitas Clinical and Research Center IRB and Fred Hutchinson Cancer Research Center HTVN approved sample and data collection.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	In all assays, cells were stained for 15 min at room temperature with Zombie Aqua fixable viability dye (BioLegend) to exclude dead cells. Fluorochrome-conjugated monoclonal antibodies were purchased from BD Biosciences, BioLegend, eBioscience, Santa Cruz Biotechnology and Cell Signaling and titrated to determine optimal concentrations. Chemokine receptor expression was measured by incubating cells at 37°C for 20 min. Surface markers were evaluated by incubating cells at RT for 20 min. The Cytofix/Cytoperm kit (BD Biosciences) was used to detect intracellular cytokine expression on sorted T cell subsets. Measurement of transcription factors and intranuclear molecules was performed with FoxP3 Transcription Buffer Set (Invitrogen) or Transcription Factors Buffer Kit (BD) according to manufacturers' instructions. Cell proliferation was determined by the analysis of 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution (final concentration: 2 uM; used according to the manufacturer's protocol; Life Technologies). The proliferation index (PI) was calculated as: MFI non-proliferating fraction / MFI proliferating fraction × % cells with diluted CFSE, as previously reported.
Instrument	T cell subsets were FACS-sorted to purity by using a FACSAria III (BD Biosciences). All samples were acquired on FACSymphony A5 (equipped to detect 30 parameters) or LSR Fortessa flow cytometers.
Software	Flow cytometry and cell sorting: Flow cytometry data were collected with FACSDIVA software and analyzed and compensated with FlowJo 9 and 10 (FlowJo LLC) by using single-stained controls prepared with antibody-capture beads (BD Biosciences). High-dimensional flow cytometry data analysis: Flow Cytometry Standard (FCS) 3.0 files were imported into FlowJo software version 9, analyzed by standard gating to remove aggregates and dead cells, and CD95+ bulk memory CD8+ T cells were identified. 5,000 CD95+ T cells per sample were subsequently imported in FlowJo version 10, biexponentially transformed and exported for further analysis in Python (version 3.7.3) by a custom-made script that makes use of PhenoGraph (originally retrieved from the scikit-learn package; full script available at https://github.com/luglilab/Cytophenograph). Lymph nodes, bone marrow and adjacent cancer-free lung tissues were labeled with a unique computational barcode for further identification and converted in comma separated (CSV) files and concatenated in a single matrix by using the merge function of pandas package. K value, indicating the number of nearest neighbors identified in the first iteration of the algorithm, was set equal to 1000 for clustering. UMAP was obtained by UMAP Python package and visualized in FlowJo 10.
Cell population abundance	Post-sort fractions were considered pure when the subset of interest was >93% of the sample. Purity was evaluated by FACS acquisition immediately after sort.
Gating strategy	Flow cytometric gating strategy for the isolation of CD8+ naive, TSTEM, TSCM PD-1− TIGIT−, TCM PD-1− TIGIT−, TPEX, and TEM cells is provided in Supplementary Figure 1. Briefly, lymphocytes were first selected on the basis of physical parameters and doublets and dead cells were excluded. Then, CD8+ naive and memory T cells were selected on the basis of CD95 expression. Early memory compartment was further identified as CD27+CCR7+ while TEM were defined as CD27+CCR7- cells. TSTEM and TPEX were then distinguished as PD1-TIGIT- and PD1+TIGIT+, respectively. In dedicated experiments, TSTEM were further subdivided into TSCM PD-1− TIGIT− and TCM PD-1− TIGIT− according to preferential expression of CD45RA and CD45RO, respectively.

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