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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Single-cell RNA-sequencing data were collected on an Illumina HiSeq4000 sequencer using Hiseq Control Software (version HD 3.4.0.38). Mass cytometry data were collected on a Fluidigm Helios CyTOF System using Fluidigm CyTOF Software (v6.5.358). Flow cytometry data were collected using BD FACSDiva software (v8). Killing assay colorimetric data were collected using SoftmaxPro 5.4.1 software.
Data analysis	scRNA-seq data was aligned using subread v 1.5.1 and reads counted using featureCounts implemented in Rsubread v1.20.6. scRNA-seq and mass cytometry data analyses were performed in R v3.4.3 using the following packages: scater_1.6.3 scran_1.6.9 SingleCellExperiment_1.0.0 dpt_0.6.0 (Haghverdi. 2016. Nat Methods, 13:845.) destiny_2.6.2 sva_3.26.0 edgeR_3.20.9 limma_3.34.9 Rtsne_0.13 pheatmap_1.0.8 classInt_0.1-24 cydar_1.2.1

ncdfFlow_2.24.0
flowCore_1.44.2
Scripts for these analyses will be made available at https://github.com/MarioniLab/SingleCellAPL2018.

Flow cytometry experiments were analyzed using FlowJo (v9) and Graphpad Prism (v7).

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 upon request. 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

scRNA-seq data are available in the ArrayExpress repository, accession number E-MTAB-6051. Mass cytometry data are available in the Cytobank repository, accession number 66456. The remaining data that support the findings of this study are available from the corresponding authors upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed prior to data collection. Our experiments examined the distributions of cell through single-cell analyses. The sample sizes used provided adequate information about these distributions as demonstrated through biological reproducibility.
Data exclusions	Cells were only excluded from single-cell sequencing and mass cytometry experiments if they failed quality control metrics designed to detect poor quality cells. Filtering was performed before analyses. Samples were not excluded from flow cytometry analyses unless a severe anomaly was detected in the cell size gate (which occurred in one sample).
Replication	Findings were reproduced through biological replicates and complementary measurements using second technologies or biological systems. The number of independent experiments are indicated for each figure in the manuscript. Experiments were only deemed failures and thereby excluded if data was not generated or if technical issues made data unreliable. The latter was the case for one protein phenotyping experiment in which a flow cytometry laser failed.
Randomization	Experimental groups consisted of cells treated under different conditions. In all experiments, cells for each condition were taken from a homogeneous pool. For single-cell RNA sequencing, cells from all tested conditions were represented on every plate to avoid confounding from technical effects.
Blinding	For single-cell RNA sequencing, cells from each condition were sorted into different positions on each plate to avoid confounding from technical effects. For mass cytometry experiments, cells from each condition were barcoded and all conditions pooled for staining and analysis to avoid confounding technical effects. All analyses were performed without considering the identity of sample groups except where a control biological group was necessarily used as reference as described in the methods.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used

Antibodies were all commercially available monoclonal antibodies as detailed below. This information is also included in the Methods section and Supplemental Table 4. Antibodies below are listed as target, label (clone, company, catalog number, dilution):

CD16/32 unconjugated (clone 93, Biolegend, 101302, used for FcR blocking at 1 ug / million cells)
CD3e unconjugated (clone 145-2C11, BD Biosciences, 553058, used for coating plate for activation 1 ug / mL)
CD8a eVolve 655 (clone 53-6.7, eBioscience, 86-0081-41, 1:100)
CD8a PE-Cy7 (clone 53-6.7, Biolegend, 100722, 1:500)
CD8a APC (clone 53-6.7, Biolegend, 100712, 1:500)
CD44 APC-eFluor780 (clone IM7, eBioscience, 47-0441-82, 1:400)
CD44 APC-FIRE750 (clone IM7, Biolegend, 103061, 1:400)
CD44 APC (clone IM7, Biolegend, 103011, 1:400)
CD62L PE (clone MEL-14, eBioscience, 12-0621-81, 1:400)
CD62L APC (clone MEL-14, Biolegend, 104411, 1:500)
CD25 Alexa Fluor 488 (clone PC61.5, eBioscience, 53-0251-80, 1:400)
CD69 APC (clone H1.2F3, eBioscience, 17-0691-80, 1:400)
LAMP1 PE (clone eBio1D4B, eBioscience, 12-1071-83, used for degranulation 1:100 and for cytometry 1:200)
Granzyme B FITC (clone GB11, Biolegend, 515403, 1:100)
CTLA4 (CD152) Brilliant Violet 605 (clone UC1-4B9, Biolegend, 106323, 1:100)
IFNg (clone XMG1.2, Biolegend, 505807, 1:200)
CD11a FITC (clone M17/4, eBioscience, 11-0111-81, 1:400)
PE unconjugated (clone PE001, Biolegend, 408105, used for conjugation to 160Gd)
CD44 unconjugated (clone IM7, Biolegend, 103051, used for conjugation to 155Gd)
CD127 (IL-7Ra) 174Yb (clone A7R34, Fluidigm, 3174013B, 1:100)
CD272 (BTLA) 156Gd (clone 6F7, Fluidigm, 3156028B, 1:100)
CD27 150Nd (clone LG.3A10, Fluidigm, 3150017B, 1:100)
TCRb 169Tm (clone H57-597, Fluidigm, 3169002B, 1:50)
CD161 (NK1.1) 170Er (clone PK136, Fluidigm, 3170002B, 1:50)
CD279 (PD-1) 159Tb (clone 29F.1A12, Fluidigm, 3159024B, 1:100)
CD8a 168Er (clone 53-6.7, Fluidigm, 3168003B, 1:100)
CD25 (IL-2R) 151Eu (clone 3C7, Fluidigm, 3151007B, 1:100)
CD45 89Y (clone 30-F11, Fluidigm, 3089005B, 1:200)
FITC 144Nd (clone FIT-22, Fluidigm, 3144006B, 1:100)
APC 163Dy (clone APC003, Fluidigm, 3163001B, 1:100)
CTLA-4 (CD152) 154Sm (clone UC10-4B9, Fluidigm, 3154008B, 1:100)
Perforin 172Yb (clone OMAK-D, Fluidigm, 3172018B, 1:100)
Granzyme B 173Yb (clone GB11, Fluidigm, 3173006B, 1:50)
Foxp3 158Gd (clone FJK-16s, Fluidigm, 3158003A, 1:50)
T-bet 161Dy (clone 4B10, Fluidigm, 3161014B, 1:50)
IFNg 165Ho (clone XMG1.2, Fluidigm, 3161014B, 1:100)

Validation

Monoclonal antibodies listed above are validated in the literature as cited on the manufacturers' websites, as well as by the manufacturers themselves. In addition, we validated the anti-PD1 and anti-CTLA4 clones listed above by transfecting HEK 293T cells with murine gene constructs before running flow cytometry.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

EL4 murine lymphoma cells were from the Sir William Dunn School of Pathology Cell Bank, Oxford.

Authentication

Immediately before study, we validated EL4 expression of the class I MHC allele H2Kb to ensure they would present SIINFEKL peptide and variants to OT-I T cells.

Mycoplasma contamination

These cells tested negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Research animals

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

Animals/animal-derived materials

C57BL/6-Tg(TcraTcrb)1100Mjb Rag1<tm1Bal> and C57BL/6N mice were used in this study. Mice were a mixture of male and female adults, aged 8-25 weeks.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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	T cells isolated from the spleens of OT-I Rag1-deficient mice were stained immediately or cultured for the indicated times before staining.
Instrument	BD LSRFortessa for flow cytometry BD Influx for FACS
Software	Flow cytometry data was collected using BD FACSDIVA software and analyzed using FlowJo v9 software.
Cell population abundance	For all cell populations analyzed, abundances are indicated in the Figure plots and gating strategies.
Gating strategy	All gate strategies captured cells by FSC v SSC area, single cells by FSC height versus area, and live cells by low binding of Zombie Aqua cell viability dye. Gating strategies beyond this differed by experiment and are included in the methods and Supplementary Figures.

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