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Reporting Summary

Nature Research 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 Research 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	1, Flow cytometry: BD FACS, v8.0.1 2, Western Blotting: Image Studio Lite, v5.2 3, qPCR, CFX Manager, v3.1 4, Metabolomics, MultiQuant, v2.1 5, Surface plasmon resonance, Biacore T100 control, v2.0.4 6, Seahorse assay, Wave, v2.6.1
Data analysis	1, Flow cytometry: FlowJo v10 2, Statistics and Data plotting: GraphPad Prism 7 3, NGS and Metabolomic Data analysis and visualization: R v3.60 (with packages: edgeR v3.24.3, gglot2 v3.2.1, pheatmap v1.0.12), Bowtie v2.2.6, Tophat v2.2.1, IGVTools v2.4.13 4, GSEA analysis: GSEA v4.1.0

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 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

All next generation sequencing data have been deposited in Geo/NCBI (accession number GSE138698). All analyzed data are available in the main text or supplementary materials. For Figure 1p and Extended Data Figure 1a and 1b, uncropped gel are available in Supplementary Figure 1. For Figure 2(a-d and h-j), Figure 3(a-b and m-n), Extended Data Figure 3 (a- d and h- k), Extended Data Figure 5 (g-h), Extended Data Figure 6 (e-i), Extended Data Figure 7 (h-o), Extended Data Figure 8 (g-i), raw data are available in Supplementary Tables 1-12.

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	The sample size for each experiment is indicated in the figure legends. Sample sizes were based on our experience and common practice in the related fields, balancing statistic robustness, resource availability and animal welfare. No Statistical methods were used to predetermine sample size.
Data exclusions	No data was excluded from this study.
Replication	Biological replicates are included to ensure the reproducibility and all repeated experiments are successful. For ex vivo experiments, the number of replicates is equal to individual mice used and are independently repeated as reported. For in vivo studies, five mice were used for each single groups and all experiments are independently repeated at least twice with similar results to make the conclusion. For other experiments, at least two biological replicates were included and performed independently to ensure the reproducibility. When representative data are shown, the experimental findings were reproduced independently with similar results.
Randomization	Mice are sex- and age- matched and are randomly assigned to different treatment and control groups. For the KO mice, control WT littermates were used for comparations.
Blinding	For the in vivo tumor experiments, all the mice were coded and the data collection such as tumor measuring and mice survival recording was performed by researcher without the knowledge of the codes. Data analysis was performed by researchers blinded to group allocation. No blinding was involved in other experiments, as machine-based readouts are not subject to investigator bias.

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
☒	☐ Human research participants
☒	☐ 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 flow cytometry: APC-TIM3, dilution: 1:100, clone: RMT3-23, BioLegend, Cat#119706, Lot#B247522

APC-LAG3, dilution: 1:100, clone: C9B7W, BioLegend, Cat#125210, Lot#B243438
FITC-2B4, dilution: 1:100, clone: m2B4(B6)458.1, BioLegend, Cat#133504, Lot#B230000
Bv421-IFNg, dilution: 1:1000, clone: XMG1.2, BioLegend, Cat#505830, Lot#B232596
FITC-TNFa, dilution: 1:1000, clone: MP6-XT22, BioLegend, Cat#506304, Lot#B228111
APC-IL2, dilution: 1:200, clone: JES6-5H4, BioLegend, Cat#503810, Lot#B172029
PE-IL-10, dilution: 1:50, clone: JES5-16E3, Thermo Fisher, Cat#12-7101-81, Lot#E02094-1635
AF488-TCF7, dilution: 1:100, clone: C63D9, Cell Signaling, Cat#6444S, Lot#7
APC-CXCR3, dilution: 1:100, clone: CXCR3-173, BioLegend, Cat#126512, Lot#B233225
PE-BLIMP1, dilution: 1:100, clone: 5E7, BioLegend, Cat#150005, Lot#B202588
PE-TCF1, dilution: 1:100, clone: C63D9, Cell Signaling, Cat#14456S, Lot#6
PE-CD62L, dilution: 1:100, clone: MEL-14, BioLegend, Cat#104407, Lot#B206511
AF647-pSTAT5, dilution: 1:50, clone: 47/Stat5, BD, Cat#612599, Lot#40057
Bv510-CD45.1, dilution: 1:100, clone: A20, BioLegend, Cat#110741, Lot#B253101
PE-TCR vb13, dilution: 1:100, clone: MR12-4, BioLegend, Cat#140704, Lot#B265629
FITC-CD45.1, dilution: 1:100, clone: A20, BioLegend, Cat#110706, Lot#B249479
Bv421-CD90.1, dilution: 1:1000, clone: OX-7, BioLegend, Cat#202529, Lot#B266590
PE-PD1, dilution: 1:100, clone: 29F.1A12, BioLegend, Cat#135206, Lot#B265719
PE-pSTAT5, dilution: 1:50, clone: 47/Stat5, BD, Cat#612567, Lot#296746
Bv421-TIM3, dilution: 1:100, clone: RMT3-23, BioLegend, Cat#119723, Lot#B259713
FITC-CD44, dilution: 1:100, clone: IM7, BioLegend, Cat#103006, Lot#B278352
PECy5.5-CD62L, dilution: 1:100, clone: MEL-14, BioLegend, Cat#104432, Lot#B272105
Bv421-pAKT, dilution: 1:20, clone: M89-61, BD, Cat#562599, Lot#0269802
AF647-pERK, dilution: 1:20, clone: 20A, BD, Cat#612593, Lot#9204589
APC-hTIM3, dilution: 1:100, clone: F38-2E2, BioLegend, Cat#345012, Lot#B262304
APC-hCCR7, dilution: 1:100, clone: G043H7, BioLegend, Cat#353214, Lot#B274551
PE-hGranzyme B, dilution: 1:100, clone: GB12, ThermoFisher, Cat#MHGB04, Lot#349720A
Bv421-hTIM3, dilution: 1:100, clone: F38-2E2, BioLegend, Cat#345008, Lot#B310545
APC7-CD8, dilution: 1:100, clone: 53-6.7, BioLegend, Cat#100766, Lot#B215973
APC-PD1, dilution: 1:100, clone: 29F.1A12, BioLegend, Cat#135210, Lot#B277925
PECy7-CD27, dilution: 1:100, clone: LG.3A10, BioLegend, Cat#124215, Lot#B185456

For T cell stimulation:
CD3, dilution: 1:500, clone: 145-2C11, BioXCell, Cat#BE0001-1
CD28, dilution: 1:1000, clone: 37.51, BioXCell, Cat#BE0015-1

For western blotting:
Perforin, dilution: 1:1000, Cell Signaling, Cat#3693S, Lot#1
Granzyme, dilution: 1:1000, clone: 94E6/GZMB, BioLegend, Cat#674301, Lot#B191505
H3, dilution: 1:1000, clone: 1B1B2, Cell Signaling, Cat#14269, Lot#2
STAT5, dilution: 1:1000, clone: 89/Stat5, BD, Cat#610192,
pERK, dilution: 1:1000, clone: D13.14.4E, Cell Signaling, Cat#4370, Lot#6
ERK, dilution: 1:1000, clone: MAB15761, Novus Bio, Cat#MAB15761-SP, Lot#JGC031708A
STAT5A, dilution: 1:1000, clone: L20, Santa Cruz, Cat#1081, Lot#0205
STAT5B, dilution: 1:1000, clone: G2, Santa Cruz, Cat#1656, Lot#0916
pSTAT5, dilution: 1:1000, Cell Signaling, Cat#9351S, Lot#6
pSTAT5, dilution: 1:1000, clone: 14H2, Cell Signaling, Cat#9356S, Lot#11
DyLight800-Rabbit IgG, dilution: 1:20000, Invitrogen, Cat#35569, Lot#SG2422871A
DyLight680-Mouse IgG, dilution: 1:20000, Invitrogen, Cat#35519, Lot#ESE248218

For Immunoprecipitation:
STAT5A, clone: A7, Santa Cruz, Cat#166479, Lot#A0816
STAT5B, clone: ST5B-10G1, Invitrogen, Cat#13-5300, Lot#243336

For ChIP seq:
STAT5B, clone: ST5b-10G1, Thermo Fisher, Cat#13-5300, Lot#TF272003
H3K27ac, Diagenode, Cat#C15410196, Lot#A1723-0041D

Validation

Antibodies used in this study are commercially available and are validated by the manufacturers, related information are available from their website:
APC-TIM3, Cat#119706, https://www.biolegend.com/en-us/products/apc-anti-mouse-cd366-tim-3-antibody-8238,
FITC-PD1, Cat#135214, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd279-pd-1-antibody-7004,
APC-LAG3, Cat#125210, https://www.biolegend.com/en-us/products/apc-anti-mouse-cd223-lag-3-antibody-6926,
FITC-2B4, Cat#133504, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd244-2-2b4-b6-alloantigen-antibody-5838,
Bv421-IFNg, Cat#505830, https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-ifn-gamma-antibody-7154,
FITC-TNFa, Cat#506304, https://www.biolegend.com/en-us/products/fitc-anti-mouse-tnf-alpha-antibody-976,
APC-IL2, Cat#503810, https://www.biolegend.com/en-us/products/apc-anti-mouse-il-2-antibody-950,
PE-IL-10, Cat#12-7101-81, https://www.thermofisher.com/antibody/product/IL-10-Antibody-clone-JES5-16E3-Monoclonal/12-7101-81,
AF488-TCF7, Cat#6444S, https://www.cellsignal.com/products/antibody-conjugates/tcf1-tcf7-c63d9-rabbit-mab-alexa-fluor-488-conjugate/6444,
APC-CXCR3, Cat#126512, https://www.biolegend.com/en-us/products/apc-anti-mouse-cd183-cxcr3-antibody-4683,
PE-BLIMP1, Cat#150005, https://www.biolegend.com/en-us/products/pe-anti-mouse-blimp-1-antibody-12328,
PE-TCF1, Cat#14456S, https://www.cellsignal.com/products/antibody-conjugates/tcf1-tcf7-c63d9-rabbit-mab-pe-conjugate/14456,
PE-CD62L, Cat#104407, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd62l-antibody-386,
AF647-pSTAT5, Cat#612599, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-647-mouse-anti-stat5-py694.612599,

Bv510-CD45.1, Cat#110741, https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd45-1-antibody-9609,
PE-TCR vb13, Cat#140704, https://www.biolegend.com/en-us/products/pe-anti-mouse-tcr-vbeta13-antibody-6886,
FITC-CD45.1, Cat#110706, https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-1-antibody-198,
Bv421-CD90.1, Cat#202529, https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-rat-cd90-mouse-cd90-1-thy-1-1-antibody-7307,
PE-PD1, Cat#135206, https://www.biolegend.com/en-us/products/pe-anti-mouse-cd279-pd-1-antibody-6170,
PE-pSTAT5, Cat#612567, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-stat5-py694.612567,
Bv421-TIM3, Cat#119723, https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd366-tim-3-antibody-13392,
FITC-CD44, Cat#103006, https://www.biolegend.com/en-us/products/fitc-anti-mouse-human-cd44-antibody-314,
PECy5.5-CD62L, Cat#104432, https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd62l-antibody-4272,
Bv421-pAKT, Cat#562599, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-mouse-anti-akt-ps473.562599,
AF647-pERK, Cat#612593, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-647-mouse-anti-erk1-2-pt202-py204.612593,
APC-hTIM3, Cat#345012, https://www.biolegend.com/en-us/products/apc-anti-human-cd366-tim-3-antibody-8302,
APC-hCCR7, Cat#353214, https://www.biolegend.com/en-us/products/apc-anti-human-cd197-ccr7-antibody-7536,
PE-hGranzyme B, Cat#MHGB04, https://www.thermofisher.com/antibody/product/Granzyme-B-Antibody-clone-GB12-Monoclonal/MHGB04,
Bv421-hTIM3, Cat#345008, https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-cd366-tim-3-antibody-7401,
APC7-CD8, Cat#100766, https://www.biolegend.com/en-us/products/apc-fire-750-anti-mouse-cd8a-antibody-13048,
APC-PD1, Cat#135210, https://www.biolegend.com/en-us/products/apc-anti-mouse-cd279-pd-1-antibody-6497,
PE7-hCD27, Cat#124215, https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-rat-human-cd27-antibody-6925,
STAT5A, Cat#166479, https://www.scbt.com/p/stat5a-antibody-a-7,
STAT5B, Cat#13-5300, https://www.thermofisher.com/antibody/product/STAT5-beta-Antibody-clone-ST5b-10G1-Monoclonal/13-5300,
CD3, Cat#BE0001-1, https://bxcell.com/product/m-cd3e/,
CD28, Cat#BE0015-1, https://bxcell.com/product/m-cd28/,
Perforin, Cat#3693S, https://www.cellsignal.com/products/primary-antibodies/perforin-antibody-mouse-specific/3693,
Granzyme, Cat#674301, https://www.biolegend.com/en-us/products/purified-anti-granzyme-b-antibody-10619,
H3, Cat#14269, https://www.cellsignal.com//products/primary-antibodies/histone-h3-1b1b2-mouse-mab/14269,
STAT5, Cat#610192, https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-stat5.610192,
pERK, Cat#4370, https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370,
ERK, Cat#MAB15761-SP, https://www.rndsystems.com/products/human-mouse-rat-erk1-erk2-antibody-631122_mab15761,
STAT5A, Cat#1081, https://www.scbt.com/p/stat5a-antibody-l-20,
STAT5B, Cat#1656, https://www.scbt.com/p/stat5b-antibody-g-2,
pSTAT5, Cat#9351S, https://www.cellsignal.com/products/primary-antibodies/phospho-stat5-tyr694-antibody/9351,
pSTAT5, Cat#9356S, https://www.cellsignal.com/products/primary-antibodies/phospho-stat5-tyr694-14h2-mouse-mab/9356,
STAT5B, Cat#13-5300, https://www.thermofisher.com/antibody/product/STAT5-beta-Antibody-clone-ST5b-10G1-Monoclonal/13-5300
H3K27ac, Cat#C15410196, https://www.diagenode.com/en/p/h3k27ac-polyclonal-antibody-premium-50-mg-18-ml
DyLight800-Rabbit IgG, Cat#35569, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/35569,
DyLight680-Mouse IgG, Cat#35519, https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/35519

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Platinum-E Retroviral Packaging Cell Line was purchased from Cell BioLabs Inc.
Authentication	Platinum-E cell lines were not authenticated by us.
Mycoplasma contamination	Platinum-E cells were free of mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

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

Laboratory animals	All animal experiments were performed with the approval of the NHLBI and NCI Animal Care and Use Committees and housed in pathogen-free mouse facilities. Female and male C57BL/6 mice (#000664), B6.SJL-PtprcaPepcb/BoyJ (#002014) , B6.Cg-Thy1a/Cy Tg(TcraTcrb)8Rest/J(#005023), B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ(#022071) and B6(Cg)-Tcf7tm1Hhx/J (030909) were obtained from Jackson Laboratory. Female and male Stat5a KO and Stat5b KO mice were provided by Drs. Alejandro Villarino and John O'Shea, NIAMS. pmel-1 CD45.1 mice were obtained by crossing pmel-1 mice with CD45.1 mice. Female and male mice between the age of 8-12 weeks of age were used for experiments. Mice were housed at 72+/- 3 F with humidity at an average of 50%. The dark/light cycles was 12/12.

Wild animals	No wild animal were used in this study.
Field-collected samples	No field-collected samples were used in the studies
Ethics oversight	All animal experiments were performed with the approval of the NHLBI and NCI Animal Care and Use Committees.

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

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

Data were deposited to GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE138698)

Files in database submission

GSE138698 An engineered IL-2 partial agonist promotes CD8+ T cell stemness and anti-tumor efficacy Apr 01, 2020 approved None
GSM4116400 RNA-Seq preA d0 rep1 Apr 01, 2020 approved RPKM
GSM4116401 RNA-Seq preA IL-2 d2 rep1 Apr 01, 2020 approved RPKM
GSM4116402 RNA-Seq preA H9 d2 rep1 Apr 01, 2020 approved RPKM
GSM4116403 RNA-Seq preA H9T d2 rep1 Apr 01, 2020 approved RPKM
GSM4116404 RNA-Seq preA IL-2 d4 rep1 Apr 01, 2020 approved RPKM
GSM4116405 RNA-Seq preA H9 d4 rep1 Apr 01, 2020 approved RPKM
GSM4116406 RNA-Seq preA H9T d4 rep1 Apr 01, 2020 approved RPKM
GSM4116407 RNA-Seq preA IL-2 d6 rep1 Apr 01, 2020 approved RPKM
GSM4116408 RNA-Seq preA H9 d6 rep1 Apr 01, 2020 approved RPKM
GSM4116409 RNA-Seq preA H9T d6 rep1 Apr 01, 2020 approved RPKM
GSM4116410 RNA-Seq preA d0 rep2 Apr 01, 2020 approved RPKM
GSM4116411 RNA-Seq preA IL-2 d2 rep2 Apr 01, 2020 approved RPKM
GSM4116412 RNA-Seq preA H9 d2 rep2 Apr 01, 2020 approved RPKM
GSM4116413 RNA-Seq preA H9T d2 rep2 Apr 01, 2020 approved RPKM
GSM4116414 RNA-Seq preA IL-2 d4 rep2 Apr 01, 2020 approved RPKM
GSM4116415 RNA-Seq preA H9 d4 rep2 Apr 01, 2020 approved RPKM
GSM4116416 RNA-Seq preA H9T d4 rep2 Apr 01, 2020 approved RPKM
GSM4116417 RNA-Seq preA IL-2 d6 rep2 Apr 01, 2020 approved RPKM
GSM4116418 RNA-Seq preA H9 d6 rep2 Apr 01, 2020 approved RPKM
GSM4116419 RNA-Seq preA H9T d6 rep2 Apr 01, 2020 approved RPKM
GSM4116420 ChIP-Seq H3K27ac d0 Apr 01, 2020 approved BED
GSM4116421 ChIP-Seq H3K27ac IL2 d6 Apr 01, 2020 approved BED
GSM4116422 ChIP-Seq H3K27ac H9 d6 Apr 01, 2020 approved BED
GSM4116423 ChIP-Seq H3K27ac H9T d6 Apr 01, 2020 approved BED
GSM4116424 ATAC-Seq d0 Apr 01, 2020 approved BED
GSM4116425 ATAC-Seq IL2 d6 Apr 01, 2020 approved BED
GSM4116426 ATAC-Seq H9 d6 Apr 01, 2020 approved BED
GSM4116427 ATAC-Seq H9T d6 Apr 01, 2020 approved BED

Genome browser session
(e.g. UCSC)

No longer applicable.

Methodology

Replicates

For STAT5 ChIP-seq, data showing were from two replicates. For the H3K27ac ChIP-seq, there are no technical replicates.

Sequencing depth

All reads from RNASeq and ChIPSeq were single end with sequencing lengths of 50bp. All reads from ATACSeq were paired-end with sequencing lengths of 2x50bp. Sample names were followed by Raw reads and mapped reads, separated by semicolon.
Samples;Raw_Reads;Mapped_Reads;
RNASeq_preA_d0_rep1;69076728;65593781;
RNASeq_preA_IL2_d2_rep1;78596659;74360558;
RNASeq_preA_H9_d2_rep1;72061961;68513612;
RNASeq_preA_H9T_d2_rep1;90837355;86385005;
RNASeq_preA_IL2_d4_rep1;78027610;74015158;
RNASeq_preA_H9_d4_rep1;83446491;79173021;
RNASeq_preA_H9T_d4_rep1;74517076;70532637;
RNASeq_preA_IL2_d6_rep1;75527884;72317415;
RNASeq_preA_H9_d6_rep1;77267456;73654504;
RNASeq_preA_H9T_d6_rep1;80937770;77078668;
RNASeq_preA_d0_rep2;86514704;76101849;
RNASeq_preA_IL2_d2_rep2;71048947;61238180;
RNASeq_preA_H9_d2_rep2;73781571;59883792;

RNASeq_preA_H9T_d2_rep2;83410113;71753753;
RNASeq_preA_IL2_d4_rep2;80579613;66899742;
RNASeq_preA_H9_d4_rep2;93258208;74037085;
RNASeq_preA_H9T_d4_rep2;90104607;72450266;
RNASeq_preA_IL2_d6_rep2;79910388;64746466;
RNASeq_preA_H9_d6_rep2;100311391;83703968;
RNASeq_preA_H9T_d6_rep2;75796417;61058704;
ATACSeq_d0;95778170;39402102;26616790
ATACSeq_IL2_d6;94075527;30650884;22187848
ATACSeq_H9_d6;102890457;27796997;19282314
ATACSeq_H9T_d6;101841696;33239231;23685315
ChIPSeq_H3K27ac_d0;31024209;30073991;25188075
ChIPSeq_H3K27ac_IL2_d6;24875572;24005818;
ChIPSeq_H3K27ac_H9_d6;36917017;35435613;
ChIPSeq_H3K27ac_H9T_d6;38691579;37112827;

Antibodies	STAT5a antibodies and STAT5b antibodies from R&D were previously reported. H3K27ac antibody was purchased from diagenode (Cat# C15410196, Lot# A1723-0041D)
Peak calling parameters	--pvalue=1e-5, --gsize=1.87e9, --shiftsize=100
Data quality	Mapping quality is provided in GEO data deposited
Software	Mapping (Bowtie v2.2.6), BAM and BED processing (bedtools v2.25.0), peak calling (MACS v1.4.2)

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	Mouse CD8+ T lymphocytes were isolated using the EasySep Mouse CD8+ T cell isolation kit (STEMCELL #19853) and cultured in RPMI-1640 medium (ATCC) supplemented with 10% FBS and 50 uM b-mercaptoethanol. Purified cells were activated using plate-bound anti-CD3 (2 ug/ml) plus soluble anti-CD28 (1 ug/ml) monoclonal antibodies for two days. For human CD8+ T cells related assays, naïve CD8+ T cells were isolated with EasySep™ Human Naïve CD8+ T Cell Isolation Kit II (STEMCELL, #17968), and activated with Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher Scientific, #111.31D) for two days, and cells were isolated by a magnet per the manufacturer's protocol.
Instrument	BD LSRFortessa and BD FACSCanto II
Software	BD FACS Diva was used to collect data. FlowJo v10 was used to analyze the flow cytometry data.
Cell population abundance	Expanded CD8+ T cell abundance is higher than 90% with flow cytometry validation.
Gating strategy	Cells were identified with FSC-A/SSC-A gating and followed by FSC-H/FSC-A for singlets. Live cells were distinguished based on the staining of Live/Dead. CD8+ T cells were distinguished based on positive and negative populations after staining.

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