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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 Authors & Referees and the Editorial Policy Checklist.

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	Confocal microscopy images were collected using Micro-Manager 1.4.22.
Data analysis	Confocal microscopy images were analyzed using ImageJ/FIJI. Flow cytometric data was analyzed using FlowJo v.9. Amplicon sequencing was analyzed using CRISPRESSO package. CUT&RUN reads were aligned using bowtie2-2.3.2. Output .sam files were converted to .bam files using samtools v1.5. Bam files were converted to bedgraphs using a python script (py_sam_2_avenormbg) published in (Skene and Henikoff, eLife, 2017), and available at https://github.com/peteskene. Reads were visualized using IGV v 2.3.93. Peaks were called using a python script (py_peak_calling) and heatmaps were generated using another python script (py_meta_analysis), both available at https://github.com/peteskene.

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

CUT&RUN data has been deposited in GEO as record GSE108600. TLA and amplicon sequencing data is available upon request. Source data for animal experiments (Fig. 4g, h and Extended Data Fig. 10) is provided. Plasmids containing the HDR template sequences used in the study are available through AddGene.

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 ☐ Ecological, evolutionary & environmental 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	For mouse experiments, at least 5 mice per condition were used to ensure experimental robustness. Some experiments used 7 mice per condition. No power analysis were performed prior to experiments to determine sample size.
Data exclusions	No data was excluded.
Replication	For all editing experiments findings were replicated in at least two independent healthy human donors.
Randomization	For mouse experiments, littermates were randomized to treatment condition (specific type of modified T cells transfered) at the time of adoptive transfer (day 7).
Blinding	For tumour sizing experiments, the researcher recording tumor sizes was not aware of the treatment condition of the mice being sized.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Antibodies

Antibodies used

Target- Clone- Fluorophore- Vendor- Catolog Number
CD3- SK7- APC-eFluor 780- eBiosciences- 47-0036-42
CD4- SK3- PerCP- Tonbo- 67-0047-T500
CD4- OKT-4- BV510- Biolegend- 317444
CD8- RPA-T8- BV605- Biolegend- 301040
CD8- SK1- PE-Cy7- BD- 335787
IL2RA/CD25- BC96- APC- Tonbo- 20-0259-T100
TCR αβ- IP26- BV421- Biolegend- 306722
CD107a- H7- APC- BD- 561343
CD45- HI30- FITC- BD- 555482
CD62L- 145/15- PE- Miltenyi- 130-099-717

CD45- HI30- APC- BD- 561864
PD1- EH12.1- BV-510- BD- 563076
TIM3- F38-2E2- FITC- Biolegend- 345022
LAG3- 3DS223H- PerCP-eFluor 710- Invitrogen- 46-2239-42
pStat5(Y694)- clone 47- PacBlue- BD- 562077
FoxP3- 206D- AF488- Biolegend- 320112
IFNɣ- PE-Cy7- 4S.B3- BD- 559326
TNFα- Mab11- PerCP-Cy7- Biolegend- 502930
Viability- GhostDye 780- - Tonbo- 13-0865-T500
Viability- Zombie UV- - Biolegend- 423107
1G4 TCR (NY-ESO-1 Specific)- HLA-A*0201/SLLMWITQV- PE- Immudex- WB3247-PE

Validation

Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Melanoma cell lines (M202, M257, M407) were established from the biopsies of melanoma patients under the UCLA IRB approval #11-003254. A375 cells were acquired from ATCC (ATCC CRL-1619).

Authentication

Cell lines (M202, M257, M407) were periodically authenticated using GenePrint® 10 System (Promega, Madison, WI), and were matched with the earliest passage cell lines. A375 cells acquired from ATCC and used within 3 months of thawing. No additional authentication was performed on A375 cells.

Mycoplasma contamination

Cell lines (M202, M257, M407) were periodically tested for mycoplasma contamination and tested negative. A375 cells were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified 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

We used 8 to 12 week old NOD/SCID/IL-2Rɣ-null (NSG) male mice (Jackson Laboratory) for all animal experiments

Wild animals

N/A

Field-collected samples

N/A

Human research participants

Policy information about studies involving human research participants

Population characteristics

Healthy human blood donors were male or female and between the ages of 21 and 50.

Recruitment

Fresh blood was taken from healthy human donors under a protocol approved by the UCSF Committee on Human Research (CHR #13-11950). Patient samples used for gene editing were obtained under a protocol approved by the Yale Human Investigation Committee (HIC). Additional leukapheresis products from healthy donors were collected either under UCLA Institutional Review Board (IRB) approval #10-001598 or purchased from AllCells, LLC. All patients and healthy donors provided informed consent.

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.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108600

Files in database submission

D1-NE-RaM.2.rpm.aligned.bedgraph
D1-NE-aBATF.2.rpm.aligned.bedgraph
D1-NE-aGFP.rpm.aligned.bedgraph
D1-GFPBATF-aBATF.2.rpm.aligned.bedgraph
D1-GFPBATF-aGFP.2.rpm.aligned.bedgraph
D2-NE-RaM.rpm.bedgraph
D2-NE-aBATF.2.rpm.aligned.bedgraph
D2-NE-aGFP.2.rpm.aligned.bedgraph
D2-GFP-BATF-aBATF.rpm.bedgraph

D2-GFPBATF-aGFP.rpm.bedgraph
D1-NE-RaM__N443Barcode701-501_S1_R1_001.fastq
D1-NE-aBATF__N443Barcode701-502_S2_R1_001.fastq
D1-NE-aGFP__N442Barcode701-503_S3_R1_001.fastq
D1-GFPBATF-aBATF__N443Barcode702-502_S5_R1_001.fastq
D1-GFPBATF-aGFP__N443Barcode702-501_S4_R1_001.fastq
D2-NE-RaM__N442Barcode701-505_S9_R1_001.fastq
D2-NE-aBATF__N443Barcode701-506_S7_R1_001.fastq
D2-NE-aGFP__N443Barcode701-507_S8_R1_001.fastq
D2-GFPBATF-aBATF__N442Barcode702-506_S14_R1_001.fastq
D2-GFPBATF-aGFP__N442Barcode702-505_S13_R1_001.fastq
D1-NE-RaM__N443Barcode701-501_S1_R2_001.fastq
D1-NE-aBATF__N443Barcode701-502_S2_R2_001.fastq
D1-NE-aGFP__N442Barcode701-503_S3_R2_001.fastq
D1-GFPBATF-aBATF__N443Barcode702-502_S5_R2_001.fastq
D1-GFPBATF-aGFP__N443Barcode702-501_S4_R2_001.fastq
D2-NE-RaM__N442Barcode701-505_S9_R2_001.fastq
D2-NE-aBATF__N443Barcode701-506_S7_R2_001.fastq
D2-NE-aGFP__N443Barcode701-507_S8_R2_001.fastq
D2-GFPBATF-aBATF__N442Barcode702-506_S14_R2_001.fastq
D2-GFPBATF-aGFP__N442Barcode702-505_S13_R2_001.fastq

Genome browser session
(e.g. UCSC)

No longer applicable.

Methodology

Replicates

Experiment was performed independently in two separate healthy human donors.

Sequencing depth

Each library was sequenced with 50bp paired end reads.
uniquely mapped total mapped reads
D1-GFPBATF-antiBATF 24,765,389 33,930,192
D1-GFPBATF-antiGFP 25,108,930 34,484,760
D1-NE-antiBATF 21,602,222 29,557,725
D1-NE-antiGFP 20,009,078 27,196,115
D1-NE-RbaMs 22,194,728 29,960,380
D2-GFPBATF-antiBATF 12,851,714 17,631,088
D2-GFPBATF-antiGFP 26,194,681 36,735,170
D2-NE-antiBATF 26,368,039 35,627,148
D2-NE-antiGFP 29,710,628 40,529,412
D2-NE-RbaMs 20,057,688 26,797,942

Antibodies

rabbit anti-mouse: abcam, ab46540-1mg
anti-BATF: Santa Cruz, sc-100974
anti-GFP: abcam, ab290-50ul

Peak calling parameters

Peaks were called as described in (Skene and Henikoff, eLife, 2017), using a script available at https://github.com/peteskene. An example command line argument used: py_peak_calling.py_peak_calling(bedgraph='D2-NE-aBATF.2.rpm.bedgraph', threshold=1, min_length=20, inter_peak_distance=5000), using the untagged anti-BATF CUT&RUN samples from each donor. Reads were normalized to reads per million, and the peak threshold was set at 1 rpm.

Data quality

The purpose of the experiment was to show that CUT&RUN using a epitope-tagged transcription factor produced similar results since antibodies to the transcription factor or the tag. The heatmaps shown in ED Fig 2h, generated as described above and below, showed comparable results.

Software

CUT&RUN reads were aligned using bowtie2-2.3.2. Output .sam files were converted to .bam files using samtools v1.5. Bam files were converted to bedgraphs using a python script (py_sam_2_avenormbg) published in (Skene and Henikoff, eLife, 2017), and available at https://github.com/peteskene. Reads were visualized using IGV v 2.3.93. Peaks were called using a python script (py_peak_calling) and heatmaps were generated using another python script (py_meta_analysis), both available at https://github.com/peteskene.

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	Surface staining for flow cytometry and cell sorting was performed by pelleting cells and resuspending in 25 µL of FACS Buffer (2% FBS in PBS) with antibodies at the indicated concentrations (Supplementary Table 2) for 20 minutes at 4C in the dark. Cells were washed once in FACS buffer before resuspension.
Instrument	Flow cytometric analysis was performed on an Attune NxT Acoustic Focusing Cytometer (ThermoFisher) or an LSRII flow cytometer (BD).
Software	FlowJo v.9 was used for flow ctyometry data analysis.
Cell population abundance	Abundance of sorted regulatory T cells are displayed in Extended Data Fig. 6.
Gating strategy	A lymphocyte gate was defined first from FSC-A v SSC-A. Singlet gates were then defined on FSC-A v FSC-W and SSC-A v SSC-W. Finally, a live cell gate was defined with a Cell Viability Dye. Additional gating was performed as described in figure and extended data legends for individual experiments.

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