nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio 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	Flow cytometry data were collected by BD FACS Dava; RT-PCR data were collected by Quant studio 3 system; All the deep sequencing data were collected by Yale Center for Genome Analysis (YCGA).
Data analysis	Data analysis was performed using the following software / code: FlowJo v.10.7.; Bowtie 1.1.2; cutadapt 3.2; BWA 0.7.17; Sleuth 0.30.0; GSEA 4.0.3; seq-DeepCpf1 (implementation in CRISPOR 3.1); DAVID (v2019, v2020 and v2021); maSigPro R package; Kallisto v0.48.0; SAMTools v1.11; VarScan v2.4.1; DeepCpf1; Kseq (within CUT&RUNTools v2.0.); MACS2 callpeak v2.2.7; wiggleTools v1.2; deepTools v3.5.1; Integrative Genomics Viewer 2.9.2; gnomAD v3.1.1; Prism 9;

Trimmomatic13;
Gviz20;
MMseqs2;
Array_Analyze_Software_v16.1 supplied from Active Motif.
Codes that support the findings of this research are available from the corresponding author upon reasonable request.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data generated or analyzed during this study are included in this article and its supplementary information files. Specifically, source data and statistics for non-high-throughput experiments such as flow cytometry, qPCR, protein experiments, and other molecular or cellular assays are provided in an excel file of Source data and statistics. Processed data for genomic sequencing (e.g. CLASH, RNA-seq, amplicon sequencing, MIPS, AAV-Off-target) and other forms of high-throughput experiments are provided as processed quantifications in Supplementary Datasets. Genomic sequencing raw data are being deposited to Gene Expression Omnibus (GEO), with accession numbers: GSE207143 for all CLASH screens; GSE219061 for MIPS, Nextera, and AAV integration off-target; GSE207404 for RNA-seq; GSE201997 for Cut-n-Run. CLASH vectors and libraries are available via MTAs. All other data and materials that support the findings of this research are available either via public repositories, or from the corresponding author upon reasonable request to the academic community.

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	Sample size needed for experiments were estimated based on our prior work or similar experiments in the field (Dai et al. 2019; Dong et al. 2019; Eqyuem et al. 2017). For most cases, biological triplicate experiments were performed unless otherwise noted. Details on sample size for experiments were indicated in methods and figure legends. No statistical methods were used to predetermine the sample size. Sample sizes for experiments were estimated based on previous experience with similar setups that showed significance.
Data exclusions	No data were excluded.
Replication	Number of biological replicates (usually n >= 3) are indicated in the figure legends. For all experiments, the findings were replicated in at least two biological replicates.
Randomization	In animal experiments, mice were randomized by sex, cage and littermates. In vitro experiments were not randomized. For in-vitro studies, the allocation was not random, which was not relevant to the experiments due to the experimental settings described in methods and legends.
Blinding	Investigators were blinded to the identity and treatment groups of animals when measuring tumor burden. Investigators were not blinded in in vitro experiments, which did not affect reproducibility, which was not relevant to the experiments due to the experimental settings described in methods and legends. In certain NGS data analysis, such as CLASH, MIPS, AAV-Off-target and RNA-seq, investigators were blinded for initial processing of the original data using key-coded metadata.

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

Antibodies used
APC/Cyanine7 anti-human CD8a, clone: HIT8a,Biolegend Catalog Number: 300926
FITC anti-human CD197 (CCR7) Antibody,clone: G043H7 , Biolegend Catalog Number: 353216
FITC anti-human CD3 Antibody, clone: HIT3a , Biolegend Catalog Number: 300306A
PE anti-human IgG Fc, clone: HP6017, Biolegend Catalog Number: 409304
Brilliant Violet 510™ anti-human CD8, clone: SK1 , Biolegend Catalog Number: 344732
Brilliant Violet 421™ anti-human CD62L, clone: DREG-56, Biolegend Catalog Number: 304828
PerCP/Cyanine5.5 anti-human/mouse Granzyme B, clone: QA16A02, Biolegend Catalog Number: 372212
Brilliant Violet 421™ anti-human CD366 (Tim-3), clone: F38-2E2, Biolegend Catalog Number: 345008
FITC anti-human TNF, clone: MAb11, Biolegend Catalog Number: 502906
PE anti-DYKDDDDK Tag, clone: L5, Biolegend Catalog Number: 637310
PE/Cy7 anti-human CD197 (CCR7), clone: G043H7, Biolegend Catalog Number: 353225
APC anti-human CD45RO, clone: UCHL1, Biolegend Catalog Number: 304210
APC anti-human IFN, clone: B27, Biolegend Catalog Number: 506510
PerCP/Cyanine5.5 anti-human CD223 (LAG-3), clone: 11C3C65, Biolegend Catalog Number: 369312
APC anti-human CD127 (IL-7Rα) [Clone: A019D5] Biolegend Catalog Number: 351315
PE/Cyanine7 anti-human CD244 (2B4), clone: C1.7, Biolegend Catalog Number: 329519
FITC anti-human CD39, clone: A1, Biolegend Catalog Number: 328205
FITC anti-human CD19, clone: HIB19, Biolegend Catalog Number: 302256
PE anti-human CD22, clone: S-HCL-1, Biolegend Catalog Number:
PE anti-human HER2, clone: , Biolegend Catalog Number: 363504
FITC anti-human PD-1, clone: A17188A, Biolegend Catalog Number: 379206
APC anti-human PD-1, clone: A17188A, Biolegend Catalog Number: 379208
APC anti-human perforin, clone: dG9, Biolegend Catalog Number: 308111
FITC anti-human CD69 [Clone: FN50] Biolegend Catalog Number: 310903
Human Monoclonal BLIMP1/PRDM1 Antibody, clone: 646702, R&D Catalog Number: MAB36081
Blimp-1/PRDI-BF1 (C14A4) Rabbit mAb, clone: C14A4, CST Catalog Number: 9115
Recombinant Human Siglec-2/CD22 Fc Chimera Protein R&D Catalog Number : 1968-SL-050
Pierce™ Recombinant Biotinylated Protein L ThermoFisher Catalog Number : 21189
CellTrace™ Violet Cell Proliferation Kit ThermoFisher Catalog Number : C34571
APC anti-human CD226 (DNAM-1) Antibody, clone: 11A8, Biolegend Catalog Number: 338311
APC anti-human CD5 Antibody, clone: UCHT2, Biolegend Catalog Number: 300611
Histone H4 (L64C1), Mouse mAb, CST #2935
anti-mouse IgG HRP antibodies, santa cruz: sc-2005
anti-rabbit IgG HRP antibodies, Rockland, 611-1302
PRMT5 (D5P2T) Rabbit mAb CST #79998
HDAC1 (D5C6U) Rabbit mAb CST #34589
HDAC2 (D6S5P) Rabbit mAb CST #57156S
anti-GST(91G1) Mouse mAb CST#2624
anti-GAPDH, clone: 6C5, santa cruz: sc-32233
anti-FLAG, clone: D6W5B, CST Catalog Number: 14793

Antibody dilutions
Antibody dilutions were made according to the manufactuers' suggestions in various assays:
Flow: 1:200
Western: primary Ab 1: 1000
Goat anti-Rabbit IgG HRP conjugate, 1:10000
Co-IP: 1:1000
CutnRUn: 1:50

Antibody validation
All antibodies were validated for the specific application by the manufacturers and validation data are available on the manufacturer's website.
APC/Cyanine7 anti-human CD8a, clone: HIT8a,Biolegend Catalog Number: 300926
https://www.biolegend.com/fr-lu/antibodies-and-more/apc-cyanine7-anti-human-cd8a-antibody-6658
FITC anti-human CD197 (CCR7) Antibody, clone: G043H7 , Biolegend Catalog Number: 353216
https://www.biolegend.com/fr-lu/products/fitc-anti-human-cd197-ccr7-antibody-7537
FITC anti-human CD3 Antibody, clone: HIT3a , Biolegend Catalog Number: 300306A

https://www.biolegend.com/en-us/products/fitc-anti-human-cd3-antibody-751
PE anti-human IgG Fc, clone: M1310G05, Biolegend Catalog Number: 410708
https://www.biolegend.com/fr-ch/products/pe-anti-human-igg-fc-11933
Brilliant Violet 510™ anti-human CD8, clone: SK1 , Biolegend Catalog Number: 344732
https://www.biolegend.com/fr-ch/products/brilliant-violet-510-anti-human-cd8-antibody-10739
Brilliant Violet 421™ anti-human CD62L, clone: DREG-56, Biolegend Catalog Number: 304828
https://www.biolegend.com/fr-ch/products/brilliant-violet-421-anti-human-cd62l-antibody-7278
PerCP/Cyanine5.5 anti-human/mouse Granzyme B, clone: QA16A02, Biolegend Catalog Number: 372212
https://www.biolegend.com/fr-ch/products/percp-cyanine5-5-anti-humanmouse-granzyme-b-recombinant-antibody-15597
Brilliant Violet 421™ anti-human CD366 (Tim-3), clone: F38-2E2, Biolegend Catalog Number: 345008
https://www.biolegend.com/fr-ch/products/brilliant-violet-421-anti-human-cd366-tim-3-antibody-7401
FITC anti-human TNF, clone: MAb11, Biolegend Catalog Number: 502906
https://www.biolegend.com/fr-ch/products/fitc-anti-human-tnf-alpha-antibody-1345
PE anti-DYKDDDDK Tag, clone: L5, Biolegend Catalog Number: 637310
https://www.biolegend.com/fr-ch/products/pe-anti-dykddddk-tag-antibody-9383
PE/Cy7 anti-human CD197 (CCR7), clone: G043H7, Biolegend Catalog Number: 353225
https://www.biolegend.com/fr-ch/products/pecyanine7-anti-human-cd197-ccr7-antibody-7694
APC anti-human CD45RO, clone: UCHL1, Biolegend Catalog Number: 304210
https://www.biolegend.com/fr-ch/products/apc-anti-human-cd45ro-antibody-856
APC anti-human IFN, clone: B27, Biolegend Catalog Number: 506510
https://www.biolegend.com/fr-ch/products/apc-anti-human-ifn-gamma-antibody-1533
PerCP/Cyanine5.5 anti-human CD223 (LAG-3), clone: 11C3C65, Biolegend Catalog Number: 369312
https://www.biolegend.com/fr-ch/products/percp-cyanine5-5-anti-human-cd223-lag-3-antibody-13552
APC anti-human CD127 (IL-7Rα) [Clone: A019D5] Biolegend Catalog Number: 351315
https://www.biolegend.com/fr-ch/products/apc-anti-human-cd127-il-7ralpha-antibody-7172
PE/Cyanine7 anti-human CD244 (2B4), clone: C1.7, Biolegend Catalog Number: 329519
https://www.biolegend.com/fr-ch/products/pe-cyanine7-anti-human-cd244-2b4-antibody-12929
FITC anti-human CD39, clone: A1, Biolegend Catalog Number: 328205
https://www.biolegend.com/fr-ch/products/fitc-anti-human-cd39-antibody-4363
FITC anti-human CD19, clone: HIB19, Biolegend Catalog Number: 302256
https://www.biolegend.com/fr-ch/products/fitc-anti-human-cd19-antibody-717
PE anti-human CD22, clone: S-HCL-1, Biolegend Catalog Number: 363504
https://www.biolegend.com/fr-ch/products/pe-anti-human-cd22-antibody-10725
PE anti-human HER2, clone: 24D2, Biolegend Catalog Number: 324406
https://www.biolegend.com/fr-ch/products/pe-anti-human-cd340-erbb2-her-2-antibody-3766
FITC anti-human PD-1, clone: A17188A, Biolegend Catalog Number: 379206
https://www.biolegend.com/fr-ch/products/fitc-anti-human-cd279-pd-1-antibody-21956
APC anti-human PD-1, clone: A17188A, Biolegend Catalog Number: 379208
https://www.biolegend.com/fr-ch/products/apc-anti-human-cd279-pd-1-antibody-21957
APC anti-human perforin, clone: dG9, Biolegend Catalog Number: 308111
https://www.biolegend.com/fr-ch/products/apc-anti-human-perforin-antibody-4001
FITC anti-human CD69 [Clone: FN50] Biolegend Catalog Number: 310903
https://www.biolegend.com/fr-ch/products/fitc-anti-human-cd69-antibody-1671
APC anti-human CD226 (DNAM-1) Antibody, clone: 11A8, Biolegend Catalog Number: 338311
https://www.biolegend.com/fr-ch/products/apc-anti-human-cd226-dnam-1-antibody-8465
APC anti-human CD5 Antibody, clone: UCHT2, Biolegend Catalog Number: 300611
https://www.biolegend.com/fr-ch/products/apc-anti-human-cd5-antibody-868
Human Monoclonal BLIMP1/PRDM1 Antibody, clone: 646702, R&D Catalog Number: MAB36081
https://www.rndsystems.com/products/human-blimp1-prdm1-antibody-646702_mab36081
Blimp-1/PRDI-BF1 (C14A4) Rabbit mAb, clone: C14A4, CST Catalog Number: 9115
https://www.cellsignal.com/products/primary-antibodies/blimp-1-prdi-bf1-c14a4-rabbit-mab/9115
Recombinant Human Siglec-2/CD22 Fc Chimera Protein R&D Catalog Number : 1968-SL-050
https://www.rndsystems.com/products/recombinant-human-siglec-2-cd22-fc-chimera-protein-cf_1968-sl
Histone H4 (L64C1), Mouse mAb, CST #2935
https://www.cellsignal.com/products/primary-antibodies/histone-h4-l64c1-mouse-mab/2935
anti-mouse IgG HRP antibodies, santa cruz: sc-2005
https://www.scbt.com/p/goat-anti-mouse-igg-hrp
anti-rabbit IgG HRP antibodies, Rockland, 611-1302
https://www.rockland.com/categories/secondary-antibodies/rabbit-igg-hl-secondary-antibody-peroxidase-conjugated-611-1302/
PRMT5 (D5P2T) Rabbit mAb CST #79998
https://www.cellsignal.com/products/primary-antibodies/prmt5-d5p2t-rabbit-mab/79998
HDAC1 (D5C6U) Rabbit mAb CST #34589
https://www.cellsignal.com/products/primary-antibodies/hdac1-d5c6u-xp-rabbit-mab/34589
HDAC2 (D6S5P) Rabbit mAb CST #57156S
https://www.cellsignal.com/products/primary-antibodies/hdac2-d6s5p-rabbit-mab/57156
anti-GST(91G1) Mouse mAb CST#2624
https://www.cellsignal.com/products/primary-antibodies/gst-26h1-mouse-mab/2624
anti-GAPDH, clone: 6C5, santa cruz: sc-32233
https://www.scbt.com/p/gapdh-antibody-6c5
anti-FLAG, clone: D6W5B, CST Catalog Number: 14793
https://www.cellsignal.com/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/14793

Validation

Antibodies were validated based on manufacturing instructions. Primary antibodies:

Validation

https://www.rndsystems.com/products/human-blimp1-prdm1-antibody-646702_mab36081

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293FT ThermoFisher Catalog Number : R70007
Human Peripheral Blood CD8+ T Cells STEMCELL Catalog Number : 70027
Human Peripheral Blood CD4+ T Cells STEMCELL Catalog Number : 200-0165
Human Peripheral Blood Mononuclear Cells STEMCELL Catalog Number : 70025.1
HT29 ATCC Catalog Number : HTB-38
NALM6 ATCC Catalog Number : CRL-3273

Authentication

All cell lines used have been authenticated by the original vendors, and were examined in lab morphologically by microscope and/or by flow cytometry for key surface antigen(s). Human T cells were validated by surface expression of CD3, CD4 and/or CD8 by flow.

Mycoplasma contamination

All cell lines used here tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified line was used in the study.

Animals and other organisms

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

Laboratory animals

NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from the Jackson Laboratory and bred in-house. Both male and female NSG mice between 6 to 12 weeks old were used. Mice were housed in standard vivarium condition at YARC, with free access to water and food, ambient room temperature (approximately 22 degree Celsius) and humidity-controlled. Mice were maintained on a 14h:10h light/dark cycle (07:00 to 21:00 light on). Mouse health checks were performed regularly. The general health of for all the mice in this study is in good condition (BAR: bright, alert and responsive) before the related experiments started.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All recombinant DNA work was performed under the guidelines of Yale RBC and EHS with approved protocols (Chen-15-45 and Chen-20-18). All animal work was performed under the guidelines of Yale University Institutional Animal Care and Use Committee (IACUC) with approved protocols (Chen-2015-20068; Chen-2018-20068; Chen-2021-20068).

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.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE201997
Reviewer code: ohutqycafdantqp

Files in database submission

XDCut_Run01-12_S12_L004_R1_001.fastq.gz
XDCut_Run01-12_S12_L004_R2_001.fastq.gz
XDCut_Run01-13_S13_L004_R1_001.fastq.gz
XDCut_Run01-13_S13_L004_R2_001.fastq.gz
XDCut_Run01-1_S1_L004_R1_001.fastq.gz
XDCut_Run01-1_S1_L004_R2_001.fastq.gz
XDCut_Run01-2_S2_L004_R1_001.fastq.gz
XDCut_Run01-2_S2_L004_R2_001.fastq.gz
XDCut_Run01-3_S3_L004_R1_001.fastq.gz
XDCut_Run01-3_S3_L004_R2_001.fastq.gz
XDCut_Run01-4_S4_L004_R1_001.fastq.gz
XDCut_Run01-4_S4_L004_R2_001.fastq.gz
XDCut_Run01-5_S5_L004_R1_001.fastq.gz
XDCut_Run01-5_S5_L004_R2_001.fastq.gz
XDCut_Run01-7_S7_L004_R1_001.fastq.gz
XDCut_Run01-7_S7_L004_R2_001.fastq.gz

XDCut_Run01-1.bw
XDCut_Run01-2.bw

XDCut_Run01-3.bw
XDCut_Run01-4.bw
XDCut_Run01-5.bw
XDCut_Run01-7.bw
XDCut_Run01-12.bw
XDCut_Run01-13.bw
XDCut_Run01.mergedControl.bw

Sample5.MACSsummits.200flank.bed
Sample7.MACSsummits.200flank.bed
Sample12.MACSsummits.200flank.bed
Sample13.MACSsummits.200flank.bed
Sample5_7.MACS.merged.bed
Sample12_13.MACS.merged.bed

Genome browser session (e.g. UCSC)

N/A

Methodology

Replicates

3 replicates of IgG negative control, 2 replicates each of anti-PRDM1 for wildtype and mutant cells. Statistically significant overlaps of peaks between replicates, and the majority of peaks are shared between replicates.

Sequencing depth

Paired end sequencing at 2x150bp; average of 17.1 ± 2.1 million mapped reads per sample; 12.2 ± 1.2 million uniquely mapped reads per sample.

Antibodies

Blimp-1/PRDI-BF1 (C14A4) Rabbit mAb, catalog number #9115 ; antibody concentration 1:100

Peak calling parameters

Raw fastq files were first trimmed and cleaned using Trimmomatic2 and Kseq3. Cleaned fastq reads were then separately aligned to the human genome (hg38.p13) and the E. coli genome (GCF_000005845.2) using BWA mem with settings -M -t 8 -K 10000000. Peaks were called from the hg38 BAM alignment files using MACS2 callpeak, with default settings, comparing each experimental sample to each IgG control. The resulting peaks for a given sample were then merged with BEDTools. These peaks were then further merged, combining the peaks from each biological condition (vector or PRDM1-cr1).

Data quality

Alignments, signal tracks, and peaks were manually inspected in IGV. Mapping %s were similar across samples. Peak numbers passing MACS2 criteria were as follows (vs rep 1, rep 2, and rep3 of IgG controls):
-WT PRDM1 rep 1: 6851 ± 778
-WT PRDM1 rep 2: 2310 ± 555
-Mutant PRDM1 rep 1: 31,493 ± 2053
-Mutant PRDM1 rep 2: 42,413 ± 1148

Software

Raw fastq files were first trimmed and cleaned using Trimmomatic2 and Kseq3. Cleaned fastq reads were then separately aligned to the human genome (hg38.p13) and the E. coli genome (GCF_000005845.2) using BWA mem with settings -M -t 8 -K 10000000. Peaks were called from the hg38 BAM alignment files using MACS2 callpeak, with default settings, comparing each experimental sample to each IgG control. The resulting peaks for a given sample were then merged with BEDTools. These peaks were then further merged, combining the peaks from each biological condition (vector or PRDM1-cr1). To define genes that are bound by PRDM1, we annotated the peak calls from the vector-treated samples using HOMER, assigning each peak to the closest TSS. Overlaps between PRDM1-bound genes and differentially expressed genes were assessed by hypergeometric test, assuming 20,465 total human genes.

The number of aligned reads to each genome were then determined in order to calculate library scaling factors, as described in the EpiCypher CUTANA user manual. Specifically, the hg38-aligned read counts were scaled such that the reads aligned to the spike-in E. coli genome would be normalized across samples. The scaling factors were used to generate normalized bedgraph files, which were then converted into bigWig files. Replicate IgG control bigWig files were averaged by wiggleTools to create a single IgG control sample. Finally, signal visualization was performed using deepTools and Gviz.

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 50 µL of FACS Buffer

Sample preparation	(2% FBS in PBS) with antibodies (1:200 dilution) for 30 minutes at 4C in the dark. Cells were washed once in FACS buffer before resuspension. For intracellular staining, cells were fixed and permeabilized by fixation/permeabilization solution (BD) for 20 min. and resuspending in 50 µL of permeabilization/wash Bufferwith antibodies (1:200 dilution) for 30 minutes at 4C in the dark. Cells were washed once in FACS buffer before resuspension.
Instrument	Flow cytometric analysis was performed on an BD FACSAria II or thermo Attune™ NxT.
Software	FlowJo v.10.7.1 was used for flow cyometry data analysis.
Cell population abundance	CAR-T cells were sorted. The sorted cells were re-measured by FACS to confirm the purity (>90%).
Gating strategy	A lymphocyte gate was defined first from FSC-A v SSC-A. Singlet gates were then defined on FSC-H v FSC-W. Additional gating was performed as described in figure and supplementary figure legends for individual experiments.

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