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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	SH800S Cell Sorter Software version 2.1.5 (Sony); Incucyte SX1 version 2021A (Sartorius)
Data analysis	FlowJo version 10.7.1 (BD); Prism version 9.1.2 (GraphPad); star version 2.7.3a; cutadapt version 1.13; bowtie version 1.1.2; RStudio version 1.1.419; R version 4.0.0.2 or 4.1.1; 10X Genomics Cell Ranger version 3.1.0; kallisto version 0.46.0; bustools version 0.39.3; Seurat version 4.0.1; ImageJ v1.52; MaGeCK version 0.5.9.5; DESeq2 version 1.32.0; topGO version 2.46.0; flowfit version 1.25.0; flowcore version 2.6.0; tximport version 1.22.0; bowtie2 version 2.4.4; Samtools version 1.9; Picard version 4.1.8.1; MACS3 version 3.0.0; bedtools version 2.29.0; ChromVAR version 1.14.0; deeptools version 3.4.2; pyGenomeTracks version 3.6

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

Data from the ORF screen, OverCITE-seq, bulk RNA-seq, and ATAC-seq have been deposited in GEO (accession number: GSE193736). The following publicly available datasets have also been used in the study: Database of Immune Cell eQTLs, Expression, Epigenomics (https://dice-database.org/), Genotype-Tissue Expression Project v8 (https://www.gtexportal.org/), and Single Cell Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP424/single-cell-comparison-pbmc-data).

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	No sample size calculations were performed. To ensure reproducibility, three healthy donors were used for conducting the screen and a minimum of two donors were used for screen validation. Single cell sequencing allowed for analysing thousands of individual cells, similar to other functional single cell studies.
Data exclusions	No experimental replicates were excluded. Single cell filtering was performed to capture only true cells without high mitochondrial contamination that could have confidently assigned ORF perturbation and transcriptome.
Replication	To ensure replication and mitigate individual donor variability, at least two donors were tested for screen validation experiments. Several orthogonal phenotypes were used to ensure robustness of the perturbation. The top-ranked validation gene was tested in CD4+ and CD8+ T cells from n=9 donors in total, resulting in the same phenotypes.
Randomization	There were no variables to be randomised in this study.
Blinding	Blinding was not possible for data collection or analysis due to the exploratory nature of this study.

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

Flow cytometry:
BATF - Biolegend cat#654803 lot#B293009 clone 9B5A13
CCR7 - Biolegend cat#353204 lot#B326032 clone G043H7
CD14 - Biolegend cat#325608 lot#B313146 clone HCD14
CD154 - Biolegend cat#310832 lot#B331444 clone 24-31
CD25 - Biolegend cat#302606 lot#B296102 clone BC96
CD39 - Biolegend cat#328206 lot#B327168 clone A1
CD4 - Biolegend cat#300538 lot#B293940 clone RPA-T4
CD45RO - Biolegend cat#304210 lot#B328883 clone UCHL1
CD54 - Biolegend cat#322708 lot#B274189 clone HCD54
CD69 - Biolegend cat#310910 lot#B320835 clone FN50
CD70 - Biolegend cat#355104 lot#B324455 clone 113-16
CD74 - Biolegend cat#326812 lot#B314438 clone LN2
CD8 - Biolegend cat#301050 lot#B279939 clone RPA-T8
F(ab')2 - Jackson Immunoresearch cat#115-066-072 lot#152750 clone polyclonal
FLAG - Biolegend cat#637323 lot#B303098 clone L5
HLA-ABC - Biolegend cat#311404 lot#B223038 clone W6/32
HLA-DR, DP, DQ - Biolegend cat#361716 lot#B331813 clone Tu39
IL12B - Biolegend cat#501806 lot#B307376 clone C11.5
Ki-67 - Biolegend cat#350504 lot#B272259 clone Ki-67

LAG3 - Biolegend cat#369212 lot#B311711 clone 7H2C65
LIGHT - Biolegend cat#318709 lot#B282606 clone T5-39
LTA - Biolegend cat#503105 lot#B290366 clone 359-81-11
LTBR - Biolegend cat#322008 lot#B327120 clone 31G4D8
MS4A3 - Miltenyi Biotec cat#130-110-854 lot#5210806427 clone REA756
NGFR - Biolegend cat#345110 lot#B289922 clone ME20.4
PD1 - Biolegend cat#329906 lot#B335320 clone EH12.2H7
phospho-RelA - BD Biosciences cat#558423 lot#1096212 clone K10-895.12.50
rat CD2 - Biolegend cat#201303 lot#B293083 clone OX-34
RelA - Biolegend cat#653005 lot#B322869 clone 14G10A21
TCF7 - Biolegend cat#655207 lot#B293747 clone 7F11A10
TIM3 - Biolegend cat#345014 lot#B326803 clone F38-2E2
Vg9 TCR - Biolegend cat#331308 lot#B296104 clone B3
Vd2 TCR - Biolegend cat#331418 lot#B311264 clone B6

Western blot:
GAPDH – Cell Signaling Technologies cat#2118S lot#14 clone 14C10
IKKa – Cell Signaling Technologies cat#11930S lot#5 clone 3G12
IKKb – Cell Signaling Technologies cat#8943S lot#4 clone D30C6
NF-kB p65 – Cell Signaling Technologies cat#8242T lot#16 clone D14E12
phospho-NF-kB p65 Ser536 – Cell Signaling Technologies cat#3033T lot#17 clone 93H1
IkBa - Cell Signaling Technologies cat#4814T lot#17 clone L35A5
phospho-IkBa Ser32 - Cell Signaling Technologies cat#2859T lot#18 clone 14D4
NF-kB p100/p52 - Cell Signaling Technologies cat#4882T lot#6 clone 4882
RelB - Cell Signaling Technologies cat#4922T lot#3 clone C14E4

Validation

Flow cytometry:
BATF – Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.
CCR7 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD14 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD154 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD25 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD39 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD4 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD45RO - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD54 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD69 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD70 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD74 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
CD8 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
F(ab')2 – Species reactivity: mouse. From manufacturer's website: Based on immunoelectrophoresis and/or ELISA, the antibody reacts with the F(ab')2/Fab portion of mouse IgG. It also reacts with the light chains of other mouse immunoglobulins. No antibody was detected against the Fc portion of mouse IgG or against non-immunoglobulin serum proteins. The antibody has been tested by ELISA and/or solid-phase adsorbed to ensure minimal cross-reaction with human, bovine and horse serum proteins, but it may cross-react with immunoglobulins from other species.
FLAG - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
HLA-ABC - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
HLA-DR, DP, DQ - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
IL12B - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.
Ki-67 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by our Ki-67 staining protocol.
LAG3 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
LIGHT - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
LTA - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.
LTBR - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by

immunofluorescent staining with flow cytometric analysis.
MS4A3 – Species reactivity: human. From manufacturer's website: Intracellular flow cytometry - Human peripheral blood cells after erythrocyte lysis were fixed, permeabilized, and stained with CD20L (MS4A3) antibodies or with the corresponding REA Control (I) antibodies (left image) as well as with CD15 antibodies. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris were excluded from the analysis based on scatter signals.
NGFR - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
PD1 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
phospho-RelA – Species reactivity: human. From manufacturer's website: Intracellular staining (flow cytometry) (Routinely Tested).
rat CD2 - Species reactivity: rat. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
RelA - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis.
TCF7 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by intracellular flow cytometry using our True-Nuclear™ Transcription Factor Staining Protocol.
TIM3 - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Vg9 TCR - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Vd2 TCR - Species reactivity: human. From manufacturer's website: Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

Western blot:
GAPDH – Species reactivity: human. From manufacturer's website: GAPDH (14C10) Rabbit mAb detects endogenous levels of total GAPDH protein.
IKKa – Species reactivity: human. From manufacturer's website: IKKα (3G12) Mouse mAb recognizes endogenous levels of total IKKα protein.
IKKb – Species reactivity: human. From manufacturer's website: IKKβ (D30C6) Rabbit mAb recognizes endogenous levels of total IKKβ protein. This antibody does not cross-react with other IKK family members.
NF-kB p65 – Species reactivity: human. From manufacturer's website: NF-κB p65 (D14E12) XP® Rabbit mAb recognizes endogenous levels of total NF-κB p65/RelA protein. It does not cross react with other NF-κB/Rel family members.
phospho-NF-kB p65 Ser536 – Species reactivity: human. From manufacturer's website: Phospho-NF-kappaB p65 (Ser536) (93H1) Rabbit mAb detects NF-κB p65 only when phosphorylated at Ser536. It does not cross-react with the p50 subunit or other related proteins.
phospho-IkBa Ser32 - Species reactivity: human. From manufacturer's website: Phospho-IκBα (Ser32) (14D4) Rabbit mAb detects endogenous levels of IκBα only when phosphorylated at Ser32.
NF-kB p100/p52 - Species reactivity: human. From manufacturer's website: NF-kappaB2 p100 Antibody detects endogenous levels of p100, the precursor, and p52, the mature form of NF-kappaB2. The antibody does not cross-react with other family members.
RelB - Species reactivity: human. From manufacturer's website: RelB (C1E4) Rabbit mAb detects endogenous levels of total RelB protein

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293FT were from Thermo Fisher. Jurkat, Nalm6, Capan2 and BxPC3 were obtained from ATCC.
Authentication	No authentication was performed. Cell lines were used as targets for T cell recognition and therefore only the expression of target antigen(s) was of interest.
Mycoplasma contamination	Cell lines were routinely tested for mycoplasma contamination (Lonza MycoAlert Plus) and found to be negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Buffy coats from anonymous healthy donors were purchased from the New York Blood Center.
Recruitment	Healthy donors were recruited by the New York Blood Center under a broad consent covering in vitro laboratory research. Blood products for research are byproduct of normal donations. Diffuse large B cell lymphoma patients were recruited the Perlmutter Cancer Center. The samples were de-identified and no additional information (including age/race/gender) was provided or sought by the authors.
Ethics oversight	The use of cancer patients' PBMC has been approved by the Perlmutter Cancer Center Institutional Review Board. Healthy donor buffy coats were collected by and purchased from the New York Blood Center under an IRB-exempt protocol. All donors provided informed consent.

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	Flow cytometry for cell surface and intracellular markers All antibodies and dyes used for flow cytometry are listed in Supplementary Table 3. For CD25 (IL2RA) and CD154 (CD40L) quantification, T-cells were re-stimulated with CD3/CD28 Activator (6.25 ul per 10^6 cells) for 6 hours (CD154 staining in CD8+) or for 24 hours prior to staining (CD25 staining in both CD4+ and CD8+, and CD154 staining in CD4+). For Ki-67 and 7-AAD staining, T-cells were rested overnight in media without IL-2 and then activated with CD3/CD28 antibodies for 24h. In other cases, T-cells were stained without stimulation. For detection of secreted proteins, T-cells were stimulated for 24 h with CD3/CD28 Activator (LTA, LIGHT), and protein transport inhibitors brefeldin A and monensis were included for the last 6 h of stimulation (IL12B, LTA, LIGHT). First, the cells were harvested, washed with PBS and stained with LIVE/DEAD Violet cell viability dye for 5 minutes at room temperature in the dark, followed by surface antibody staining for 20 minutes on ice. After surface antibody staining (where applicable) the cells were washed with PBS and acquired on Sony SH800S cell sorter or taken for intracellular staining. For intracellular staining, the cells were resuspended in a fixation buffer (Fixation Buffer, Biolegend for IL12B and MS4A3 staining; True-Nuclear Transcription Factor Fix, Biolegend for BATF and FLAG staining; FoxP3/Transcription Factor Fixation Reagent, eBioscience for Ki-67) and incubated at room temperature in the dark for 1h. Following the incubation, the cells were washed twice in the appropriate permeabilization buffer and stained with the specific antibody or isotype control for 30 minutes in the dark at room temperature. Finally, the cells were washed twice in the appropriate permeabilization buffer and acquired on Sony SH800S cell sorter. For cell cycle analysis, the cells were further stained with 0.5 ug/mL 7-amino-actinomycin D (7-AAD) for 5 minutes immediately before acquisition. Gating was performed using appropriate isotype, fluorescence minus one and biological controls. Typically, 5,000-10,000 live events were recorded per sample. Flow cytometry detection of phosphorylated proteins T-cells were rested for 24 h in media without IL-2 prior to detection of phosphorylated proteins. The rested cells were stimulated with CD3/CD28 antibodies for indicated times. Immediately after the stimulation period the cells were fixed with a pre-warmed fixation buffer (Biolegend) for 15 minutes at 37C and washed twice with the cell staining buffer (PBS + 2% FBS). Then the cells were resuspended in the residual volume and permeabilised in 1 mL of pre-chilled True-Phos Perm Buffer (Biolegend) while vortexing. The cells were incubated in the True-Phos Perm Buffer for 60 minutes at -20C. After permeabilization the cells were washed twice with the cell staining buffer and stained with anti-CD4, anti-CD8, anti-RelA and anti-phospho-RelA antibodies (or isotype controls) for 30 minutes at room temperature. Following staining, the cells were washed twice in the cell staining buffer and acquired on Sony SH800S cell sorter. Gating was performed on CD4+ or CD8+ cells, and the levels of RelA and phospho-RelA were determined using appropriate isotype and biological controls.
Instrument	Sony SH800S
Software	Data acquisition: Cell Sorter Software v 2.1.5; data analysis: FlowJo v 10.7.1 (BD)
Cell population abundance	100% - all flow cytometry experiments were performed on transduced and fully selected populations.
Gating strategy	Lymphocytes were first selected based on FSC/SSC parameters, followed by dead cell exclusion (LIVE/DEAD Violet positive). Where applicable, subsequent gating was performed to include CD8+ or CD4+ populations. Gating was performed using appropriate fluorescence minus one, isotype or biological controls.

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