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	ggplot2 (3.3.5) (https://github.com/tidyverse/ggplot2) pheatmap (v1.0.12) (https://github.com/raivokolde/pheatmap) CellQuest Pro software (BD Biosciences)
Data analysis	Cell Ranger (v3.0.2) (https://github.com/10XGenomics/cellranger) Seurat (v4.0.5) (https://satijalab.org/seurat) SlingShot(v2.17.0)(https://github.com/kstreet13/slingshot) fastp (v0.20.0) (https://github.com/OpenGene/fastp). Cutadapt (v1.18) (https://github.com/marcelm/cutadapt) TrimGalore (version 0.6.6)(https://github.com/FelixKrueger/TrimGalore) Hisat2 (v2.2.1) (https://github.com/DaehwanKimLab/hisat2) deeptools (v3.5.0) (https://deeptools.readthedocs.io/en/develop). bedtools (v2.29.2) (https://github.com/arq5x/bedtools). deepTools (v3.5.0) (https://github.com/deeptools/deepTools) DESeq2 (v1.30.0) (https://github.com/mikelove/DESeq2) Bowtie2 (v2.3.5.1) (https://github.com/BenLangmead/bowtie2) Picard (v1.118) (http://broadinstitute.github.io/picard). Homer (v4.10.4) (https://github.com/bastienwirtz/homer). SEACR(1.3-1)(https://github.com/FredHutch/SEACR) DiffBind(v3.4.11)(https://www.cruk.cam.ac.uk/core-facilities/bioinformatics-core/software/DiffBind) ChIPSeeker(v1.26.0) (https://github.com/YuLab-SMU/ChIPseeker).

the rank ordering of super-enhancers (ROSE) algorithm (https://github.com/stjude/ROSE).
HiC-Pro (v2.11.1) (https://github.com/nservant/HiC-Pro).
Juicerbox (v1.5.1) (https://github.com/aidenlab/Juicebox)
HiTC (v1.34.0) (https://github.com/bioinfo-pf-curie/HiTC).
matrix2compartment.pl (https://github.com/dekkerlab/cworld-dekker).
multilHiCcompare (v1.8.0) (https://github.com/dozmorovlab/multiHiCcompare)
Fit-Hi-C (v2.0.7) (https://github.com/ay-lab/fithic)
pgltools (v2.2.0) (https://github.com/billgreenwald/pgltools)
hic2cool (v0.8.3) (https://github.com/4dn-dcic/hic2cool).
coolpup.py (v0.9.5) (https://github.com/open2c/coolpuppy).

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

The raw sequence data of single-cell RNA-Seq, bulk Smart-seq2, CUT&Tag, Hi-C, ATAC-seq and ACC-seq reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database under the accession number: GSE313769 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE313769].
GEO accession codes (or SRA accession number) of the published data used in this study are as follows: SATB1 ChIP-seq, GSE90635 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE90635]; RNA-seq of T cell development, GSE109125 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE109125].

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

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 and number of replicates were based on standard practices in the field.
Data exclusions	No data were excluded
Replication	scRNA-seq analysis of Satb1+/+ and Satb1-/- CD69+DP thymocytes(2 replicates); Bulk Smart-seq2 analysis of Satb1+/+ and Satb1-/- for CD69-DP and CD69+DP thymocytes(4 replicates); SATB1, CTCF, RAD21, H3K4me3 and H3K27ac CUT&Tag analysis of Satb1+/+ and Satb1-/- for CD69-DP and CD69+DP thymocytes(3 replicates); ATAC-seq analysis of Satb1+/+ and Satb1-/- for CD69-DP and CD69+DP thymocytes(4 replicates);Hi-C analysis of Satb1+/+ and Satb1-/- CD69-DP and CD69+DP for thymocytes(4 replicates); ACC-seq analysis of Satb1+/+ and Satb1-/- for thymocytes(2 replicates). All replicates refer to independent biological replicates unless otherwise stated.
Randomization	Animal experiments were based on genotypes.
Blinding	Investigators were not blinded during experiments, because the analyses were based on predefined computational pipelines and objective quantitative measurements of the experimental data in this study. Analyses of temporal profiles of mouse tissues were performed blindly.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	Antibodies used for FACS: anti-CD3e-EFLUOR 450 (eBioscience 48-0032-80, Clone 17A2), anti-CD2-Percp-Cy5.5(Biolegend 100115, Clone RM2-5), anti-CD8a-FITC (Biolegend 100706, Clone 53-6.7), anti-CD4-APC (Biolegend 100516, Clone RM4-5), anti-CD5-EFLUOR 450 (Thermo Scientific 48-0051-82, Clone 57-7.3), anti-CD69-PE(BioLegend 104507, Clone H1.2F3), anti-TCRB-PE/CY5(BioLegend 109222, Clone H57-597). Antibodies for Immunofluorescence: anti-SATB1 (Rabbit monoclonal [EPR3951], abcam, ab109122), anti-SATB1-AF647(Mouse monoclonal[C-6], santa cruz, sc-376096) and anti-Rabbit-AF647(Mouse polyclonal, FD0144, Fdbio science, China). Antibodies for CUT&Tag: anti-SATB1 (Rabbit monoclonal [EPR3951], abcam, ab109122), anti-H3K4me3 (Rabbit monoclonal [MC315], Millipore, 04-745), anti-H3K27ac (Rabbit polyclonal, abcam, ab4729), anti-CTCF (Rabbit polyclonal, Millipore, 07-729), anti-RAD21 (Rabbit polyclonal [EPR22506-15] , abcam, ab217678).

Validation	All the antibodies used were validated by the manufacturers and checked by us by FACS or Immunofluorescence or CUT&Tag.
	Validation statements on the manufacturer's website for all primary antibodies used in this study were as follows:
	anti-CD3e-EFLUOR 450 (eBioscience 48-0032-80, Clone 17A2): mouse(+);
	anti-CD2-Percp-Cy5.5(Biolegend 100115, Clone RM2-5): mouse(+);
	anti-CD8a-FITC/PE (Biolegend 100706, Clone: 53-6.7): mouse(+);
	anti-CD4-APC (Biolegend 100516, Clone:RM4-5): mouse(+);
	anti-CD69-PE(BioLegend 104507, Clone H1.2F3): mouse(+);
	anti-CD5-EFLUOR 450 (Thermo Scientific 48-0051-82, Clone 57-7.3): mouse(+);
	anti-TCRB-PE/CY5(BioLegend 109222, Clone H57-597): mouse(+).
	anti-SATB1 (Rabbit monoclonal [EPR3951], abcam, ab109122): Mouse(+), Rat(+), Human(+)
	anti-SATB1-AF647(Mouse monoclonal[C-6], santa cruz, sc-376096):Mouse(+), Rat(+), Human(+)
	anti-Rabbit-AF647(Mouse polyclonal, FD0144, Fdbio science, China): Rat(+)
	anti-H3K4me3 (Rabbit monoclonal [MC315], Millipore, 04-745): Mouse(+), Human(+)
	anti-H3K27ac (Rabbit polyclonal, abcam, ab4729): Mouse(+), Rat(+), Human(+)
	anti-CTCF (Rabbit polyclonal, Millipore, 07-729): mouse(+), human(+),, canine(+),, rat(+),, primate(+),
	anti-RAD21 (Rabbit polyclonal [EPR22506-15] , abcam, ab217678): Mouse(+), Rat(+), Human(+)

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Satb1fl/fl Vav-Cre and Satb1fl/fl CD4-Cre mice were generated as previously described, and used in this study as Satb1-cKO mice. OT-I male mice were originally purchased from and raised in a local core facility. Satb1-Ires-eGFP mice were made by Beijing Weitongda Biotechnology Co., Ltd. All mouse strains used in this study were maintained on a C57BL/6 background. Age- and sex-matched littermate controls were used for all experiments unless otherwise indicated. All experiments involving mice were performed using protocols approved by Southern Medical University Animal Studies Committee. Animals were housed and bred in a specific pathogen-free animal facility. The housing condition is a light-tight chamber at a constant temperature (23±1°C), humidity (55±10%), and 12-h light/12-h dark (LD) cycles
Wild animals	No wild animals were used in the study.
Reporting on sex	Both male and female mice were used unless otherwise indicated. Age- and sex-matched littermate controls were used for all experiments. Sex was not analyzed as a biological variable because the study was designed to examine genotype-dependent effects in thymocyte development; no apparent sex-specific trend was observed.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	The animal study was reviewed and approved by the Animal Ethics Committee of Southern Medical University (L2022158)

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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=GSE313769
Files in database submission	GSM9374870-GSM9374928
Genome browser session (e.g. UCSC)	no longer applicable

Methodology

Replicates	Both of samples are 3 replications.
Sequencing depth	Every samples about 1E7 reads.
Antibodies	anti-SATB1 (Rabbit monoclonal [EPR3951], abcam, ab109122), anti-H3K4me3 (Rabbit monoclonal [MC315], Millipore, 04-745), anti-H3K27ac (Rabbit polyclonal, abcam, ab4729), anti-CTCF (Rabbit polyclonal, Millipore, 07-729), anti-RAD21 (Rabbit polyclonal [EPR22506-15] , abcam, ab217678).
Peak calling parameters	Peak calling for CUT&Tag datasets was conducted using SEACR (https://github.com/FredHutch/SEACR) under the stringent mode. A uniform SEACR threshold of 0.02 was applied to H3K27ac, H3K4me3, CTCF, Rad21 and SATB1.
Data quality	Differential peaks were identified using DiffBind (v3.4.11) with two key criteria: false discovery rate (FDR) cutoff ≤ 0.05 and absolute log2 fold change (|log2FC|) ≥ 1.

Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	Thymus, spleen, mesenteric lymph nodes, inguinal lymph nodes, and axillary lymph nodes from 6-8 weeks mice were ground in MACS buffer (1×PBS, 0.5% BSA, 2mM EDTA) and filtered with 40-um nylon mesh. Red blood cells were lysed in RBC buffer (Biolegend, USA) for 10 minutes at room temperature.
Instrument	Flow cytometry was carried out on a BD FACS LSR Fortessa, or a FACS Aria3.
Software	Data were acquired using BD FACSDiva software and analyzed using FlowJo.
Cell population abundance	Purity of sorted CD4+CD8+CD69- or CD4+CD8+CD69+ DP cell populations was over 95% as checked by flow cytometry
Gating strategy	For all experiments, cells were first gated by FSC/SSC to exclude debris,followed by gating SSC-A and SSC-H to eliminate nonsinglets. Then, target cell populations for further analysis were gated by cell surface marker.

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