

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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 / FACS data were collected using FACS Diva (v8.0.2). Sequencing Data were collected using Illumina NextSeq 500 or NovaSeq 6000
Data analysis	<p>FlowJo (v10.9.0) was used to analyze flow cytometry data.</p> <p>cellranger-arc (v2.0.2) was used to align single-cell multi-omic data to reference genomes/transcriptomes.</p> <p>R (v4.3.2) was used for analysis and data visualization.</p> <p>ArchR (v1.0.2) was used for scATAC-seq analysis.</p> <p>Seurat (v5.0.1) was used for scRNA-seq analysis.</p> <p>MACS2 (v2.2.9.1) was used to call ATAC-seq peaks.</p> <p>Tophat (v2.1.1) was used to align RNAseq data to mm10 reference transcriptome.</p> <p>Bowtie2 (v2.2.9) was used to align ChIP-seq and CUT&RUN data to mm10 reference genome.</p> <p>Samtools (v1.9) was used to process aligned reads and perform quality control.</p> <p>Subread (v2.0.1) was used to generate transcript counts for RNAseq data.</p> <p>EdgeR (v3.36.0) was used to perform differential gene expression analysis.</p> <p>Bulk RNA-seq data processing</p> <p>RNA-seq reads were mapped to the mm10 mouse genome assembly using TopHat (v2.1.1) with setting --microexon-search. Unmapped, unpaired and low quality reads (MAPQ ≤ 5) were removed using samtools (v1.9) view with settings -q 5 -f 2. Paired reads were counted for each gene using featureCounts from Subread (v2.0.1). 'Transcripts-per-million' (TPM) values were calculated for each gene to quantify the relative abundance of transcripts for clustering analysis. "Trimmed mean of M values" (TMM) was calculated for each gene for differential comparisons across samples using edgeR (v4.0.2) (calcNormFactors()). Common dispersions were estimated using estimateCommonDisp()</p>

and Benjamini-Hochberg false discovery rates (FDRs) were calculated for pairwise comparisons using `exactTest()`. Genes exhibiting $FDR \leq 0.05$ were regarded as significant.

Multiome data quality-control

Following sequencing, bcl files were converted to fastq using `cellranger-arc` (v2.0.2) `mkfastq`. FASTQ files were aligned to the mm10 or hg38 genome assembly using `cellranger-arc` `count`. ATAC-seq fragment files were used as inputs to ArchR76 (v1.0.2) analysis pipeline in R (v4.3.2). Transcript count matrices were used as inputs to the Seurat gene expression analysis pipeline. For gene expression quality control, cells with $nFeature_RNA \geq 250$ & $\leq 6,000$, $nCount_RNA \leq 25,000$ and $percent_mitochondrial \leq 25$ were included for downstream analyses. Transcript counts were log-normalized. For ATAC-seq quality control, cells with $n_ATAC_Fragments \geq 3,000$ & $TSS_Score \geq 10$ were included for downstream analyses. Doublet inference was conducted using ArchR's `addDoubletScores()`, and presumed doublets were excluded. Cells that passed each filter were admitted for downstream analyses. Finally, based on gene expression markers, contaminating cells (thymocytes) and putative mTEC mimetic cells were excluded from analysis. In the WT multiome (Fig. 1), an additional cluster of cells exhibiting uncharacteristically low TSS enrichment score was excluded.

Multiome data processing

Dimensionality reduction, ATAC-seq clustering, projections, pseudotime, transcription factor motif enrichment, and transcription factor footprinting were performed using the ArchR pipeline with default parameters. For UMAP plots overlaid with continuous color scales, MAGIC (v2.0.3) imputation was used for data smoothing to facilitate better visualization. MAGIC-imputed values were only used for UMAP display purposes; imputed values were used nowhere else in the analysis of scATAC-seq or scRNA-seq datasets (e.g. violin plots, heat maps, etc.). For scATAC-seq peak calling, 500 bp ATAC-seq peaks were called using MACS2 (v2.2.9.1) and corresponding ArchR wrapper function `addReproduciblePeakSet()` with the following arguments: `groupBy = Clusters`, `reproducibility = 1`, `peaksPerCell = 1000`, `minCells = 100`, `excludeChr = c(chrM, chrY)`, `shift = -75`, `extsize = 150`, `cutOff = 0.1`, `additionalParams = --nomodel --nolambda --extendSummits = 250`, `promoterRegion = c(2000, 100)`. Fraction of fragments within peaks (WIP) was computed automatically as a product of this function. Subnucleosomal and mononucleosomal fractions for each cell or sample were computed as the fraction of the cell's ATAC-seq fragments whose length $L \leq 100$ bp (subnucleosomal) or $100 < L \leq 200$ bp (mononucleosomal). Differential chromatin accessibility analysis across peaks was performed using ArchR's `getMarkerFeatures()` with the following arguments: `useMatrix = PeakMatrix`, `bias = c(TSSEnrichment, log10(nFragments))`, `testMethod = wilcoxon`.

Analyses comparing aTSGpos and aTSGneg mTECs

Cells from Early Mature, Mid Mature and Late Mature clusters expressing any aTSG_i>0 were selected as the aTSGpos cohort and a size-matched cohort of aTSGneg cells was sampled randomly from the remaining cells from the same 3 clusters. These cohorts were then used as inputs to `getMarkerFeatures()` in ArchR for differential accessibility of peaks between aTSGpos and aTSGneg mTECs. For local OOP and WIP analysis, ATAC-seq fragments within peaks and outside of peaks from aTSGpos and aTSGneg cohorts were intersected with a ± 5 kb sliding window with 1 kb increments, normalized to the total number of ATAC-seq fragments per cell, and tallied in each window within a region flanking aTSG_i. For TSG co-expression analysis, the probability of detecting each aTSG_i neighboring TSG_0 within the specified length scale (or a randomly selected alternative aTSG as a control) was computed for each of the aTSGpos and aTSGneg cohorts.

CUT&RUN data processing

CUT&RUN reads were mapped to mm10 mouse genome assembly using Bowtie2 (v2.2.9) with settings `--local --very-sensitive-local --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 700`. PCR duplicates were removed using Picard (v2.21.8) `MarkDuplicates REMOVE_DUPLICATES=true VALIDATION_STRINGENCY=LENIENT`. Reads with MAPQ scores below 30 were purged and excluded from downstream analysis using `samtools` (v1.9) `view -b -q 30 -f 2 -F 1804`. Peaks were called for each sample using MACS2 (v2.2.7.1) with settings `--shift 0 --extsize 200 --nomodel --call-summits --keep-dup all -p 0.01`. For each sample, a 301 bp fixed-width peak set was generated by extending the MACS2 summits by 150 bp in both directions. Peaks were ranked by significance (MACS2 peak score) and overlapping peaks with lower peak scores were removed iteratively to create non-overlapping sample peak sets. Peaks mapping to chrY as well as any that spanned genomic regions containing "N" nucleotides were removed. Robust peaks were defined by a 'score-per-million' (SPM) (each peak score divided by the sum of all peak scores in the sample, divided by 1 million), and we only retained peaks with an SPM value ≥ 5 . 'p53 CUT&RUN peaks' were defined by further filtering for peaks that overlapped with known p53-binding motifs (HOMER2 (v5.1)) from samples with characterized p53 activity (mTEC samples). CUT&RUN fragment counts across regions of interest were normalized by the number of unique fragments in the sample library.

ChIP-seq data processing

ChIP-seq reads were mapped to mm10 mouse genome assembly using Bowtie2 (v2.2.9) with settings `--very-sensitive -X 2000`. PCR duplicates were removed using Picard (v2.21.8) `MarkDuplicates REMOVE_DUPLICATES=true VALIDATION_STRINGENCY=LENIENT`. Reads with MAPQ scores below 30 were purged and excluded from downstream analysis using `samtools` (v1.9) `view -b -q 30 -F 1796`. ChIP-seq read counts were normalized by the number of unique reads in the sample library.

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](#)

Original raw scATAC-seq, scRNA-seq and bulk RNA-seq data have been deposited to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus: accession numbers GSE274320, GSE274324, GSE290716 and GSE301724. Additional Gene Expression Omnibus accession numbers for published datasets used in this study include GSE53111, GSE102526, GSE234331, GSE194253, GSE231681, and GSE92597. mm10 and hg38 reference genomes were used for mouse and human genomic data respectively.

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	Thymus fragments were obtained from one 12 week-old male patient with no known genetic abnormalities undergoing standard of care cardiac surgery. Patient sex was reported by the attending physician.
Reporting on race, ethnicity, or other socially relevant groupings	Information on race, ethnicity, etc. was not provided.
Population characteristics	One 12 week-old with no known genetic abnormalities undergoing cardiac surgery.
Recruitment	Thymic tissue was excised during the course of standard of care cardiac surgery.
Ethics oversight	All human studies were in compliance with the Declaration of Helsinki and conducted in accordance with Institutional Review Board (IRB) protocol 20-1392 approved by the Biological Sciences Division and the University of Chicago Medical Center Institutional Review Boards at the University of Chicago and protocol 2020-203 approved by the Advocate Aurora Health Research Subject Protection Program and Advocate Aurora Health Care Institutional Review Board.

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 statistical method was used to determine sample size. The number of replicates performed was determined based on those of previous related studies: e.g. Fang et al., Nature 2024 PMID: 38480882; Givony et al., Nature 2023 PMID: 37674082; Gamble et al., Nat Immunol 2024 PMID: 38632339; Zhou W et al., Sci Immunol 2022 PMID: 35594339; Hosokawa et al., J Exp Med 2021 PMID: 34180951; Shin et al., PNAS 2021 PMID: 33479171; Shin et al., Nat Immunol 2023 PMID: 37563311.
Data exclusions	No animals or samples were excluded from analysis. Low-quality reads were excluded from processing. Low-quality and duplicate barcodes were excluded from analysis. Thymic mimetic cells were not considered.
Replication	All results from single-cell multi-omic assays were reproduced across one C57BL6 mouse, one pair of WT and sex-matched p53-cHyper mice, and one human sample. All other experimental findings were reliably reproduced with at least 2 biologically independent replicates.
Randomization	Pairs of sex-matched littermates housed in the same cages were randomly selected for each experimental perturbation. Relevant control samples were processed together and in parallel with all perturbation samples. No additional method of randomization was used.
Blinding	Investigators were not blinded to experimental group allocations because the same investigators performed genotyping, tissue harvest, experimental procedures and/or analyses. However, all controls and perturbations were performed on sex-matched littermates. Additionally, all experimental and bioinformatic processing for control and perturbation groups was performed identically, together and in parallel for each replicate.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

For flow cytometry, the following antibodies were used:

Pacific Blue anti-mouse CD45 (clone 30-F11, 1:100) BioLegend Cat# 103125, RRID:AB_493536
 APC/Cyanine 7 anti-mouse CD45 (clone 30-F11, 1:100) BioLegend Cat# 103115, RRID:AB_312980
 APC/Cyanine 7 anti-mouse EPCAM (clone G8.8, 1:50-1:100) BioLegend Cat# 118217, RRID:AB_1501158
 PE anti-mouse Ly-51 (clone 6C3, 1:100) BioLegend Cat# 108307, RRID:AB_313364
 APC anti-mouse I-A/I-E (clone M5/114.15.2, 1:100) BioLegend Cat# 107613, RRID:AB_313328
 PE anti-mouse I-A/I-E (clone M5/114.15.2, 1:100) BioLegend Cat# 107607, RRID:AB_313322
 eFlour 660 anti-mouse AIRE (clone 5H12, 1:500) Invitrogen Cat #50-5934-80, RRID:AB_2574257
 Pacific Blue anti-mouse CD4 (clone GK1.5, 1:100) BioLegend Cat# 100428, RRID:AB_493647
 APC anti-mouse CD8a (clone 53-6.7, 1:100) BioLegend Cat# 100711, RRID:AB_312750
 APC/Cyanine 7 anti-mouse CD8a (clone 53-6.7, 1:100) BioLegend Cat# 100713, RRID:AB_312752
 PE anti-mouse CD69 (clone H1.2F3, 1:100) BioLegend Cat# 104508, RRID:AB_313111
 APC anti-mouse CD62L (clone MEL-14, 1:100) BioLegend Cat # 104412, RRID:AB_313099
 APC anti-mouse CD25 (clone PC61, 1:100) BioLegend Cat# 102012, RRID:AB_312861
 PE anti-mouse/human CD44 (clone IM7, 1:100) BioLegend Cat# 103007, RRID:AB_312958
 FITC anti-mouse TCR-β (clone H57-597, 1:100) BioLegend Cat# 109206, RRID:AB_313429
 PE anti-mouse FOXP3 (clone FJK-16s, 1:100) Invitrogen Cat# 12-5773-80, RRID:AB_465936

BUV563 anti-mouse CD45 (clone 30-F11, 1:100) Invitrogen Cat# 365-0451-82, RRID:AB_2925377
 BV605 anti-mouse CD104 (clone 346-11A, 1:200) BD Biosciences Cat# 743080, RRID:AB_2741272
 AF532 anti-mouse IA/IE (clone M5/114.15.2, 1:50) Invitrogen Cat# 58-5321-82, RRID:AB_2811913
 PE anti-mouse GP2 (clone 2F11-C3, 1:10) MBL Cat# D278-5
 AF700 anti-mouse CD177 (clone 1171A, 1:25) R&D Cat# FAB8186N
 eFlour450 anti-mouse Ly-6D (clone 49-H4, 1:200) Invitrogen Cat# 48-5974-80, RRID:AB_2574089
 BV785 anti-mouse Sca-1 (clone D7, 1:200) BioLegend Cat# 108139, RRID:AB_2565957
 BUV805 anti-mouse Ki-67 (clone SolA15, 1:100) Invitrogen Cat# 368-5698-82, RRID:AB_2896151
 Biotin anti-mouse SynCAM (clone 3E1, 1:100) MBL Cat# CM004-6
 PE-Vio770 anti-mouse CD171 (L1CAM) (clone 555, 1:25) Miltenyi Cat# 130-102-135

BV421 Donkey anti-Rabbit (polyclonal, 1:50/1:100) Jackson Immuno Cat# 711-675-152, RRID:AB_2651108
 Rabbit anti-mouse/human MDM2 (clone EPR22256-98, 1:25) Abcam Cat# ab259265

APC/Cyanine 7 anti-human EPCAM (clone 9C4, 1:50-1:100) BioLegend Cat# 324245, RRID:AB_2783193
 FITC anti-human CD45 (clone 2D1, 1:100) BioLegend Cat#368507, RRID:AB_2566367
 PE anti-human LY51/CD249 (clone 2D3/APA, 1:100) BD Biosciences Cat# 553735, RRID:AB_395018
 APC anti-HLA-DRA (clone L243, 1:100) BioLegend Cat# 307609, RRID:AB_314687

Validation

All antibodies were previously validated by their manufacturers. Links to relevant citations listed on vendor sites and additional validation information provided below:

Pacific Blue anti-mouse CD45 (clone 30-F11, BioLegend Cat# 103125, RRID:AB_493536): <https://doi.org/10.4049/jimmunol.140.11.3851>
 APC/Cyanine 7 anti-mouse CD45 (clone 30-F11, BioLegend Cat# 103115, RRID:AB_312980): <https://doi.org/10.4049/jimmunol.140.11.3851>
 APC/Cyanine 7 anti-mouse EPCAM (clone G8.8, BioLegend Cat# 118217, RRID:AB_1501158): <https://doi.org/10.4049/jimmunol.148.2.590>
 PE anti-mouse Ly-51 (clone 6C3, BioLegend Cat# 108307, RRID:AB_313364): <https://doi.org/10.1084/jem.165.3.920>
 APC anti-mouse I-A/I-E (clone M5/114.15.2, BioLegend Cat# 107613, RRID:AB_313328): <https://doi.org/10.1146/annurev.immunol.15.1.821>
 PE anti-mouse I-A/I-E (clone M5/114.15.2, BioLegend Cat# 107607, RRID:AB_313322): <https://doi.org/10.1146/annurev.immunol.15.1.821>
 eFlour 660 anti-mouse AIRE (clone 5H12, Invitrogen Cat #50-5934-80, RRID:AB_2574257): <https://doi.org/10.1084/jem.165.3.920>

pubmed.ncbi.nlm.nih.gov/2815252/
 Pacific Blue anti-mouse CD4 (clone GK1.5, BioLegend Cat# 100428, RRID:AB_493647): <https://doi.org/10.1146/annurev.iy.07.040189.003051>
 APC anti-mouse CD8a (clone 53-6.7, BioLegend Cat# 100711, RRID:AB_312750): [https://doi.org/10.1016/1074-7613\(94\)90075-2](https://doi.org/10.1016/1074-7613(94)90075-2)
 APC/Cyanine 7 anti-mouse CD8a (clone 53-6.7, BioLegend Cat# 100713, RRID:AB_312752): [https://doi.org/10.1016/1074-7613\(94\)90075-2](https://doi.org/10.1016/1074-7613(94)90075-2)
 PE anti-mouse CD69 (clone H1.2F3, BioLegend Cat# 104508, RRID:AB_313111): <https://doi.org/10.4049/jimmunol.141.2.369>
 APC anti-mouse CD62L (clone MEL-14, BioLegend Cat # 104412, RRID:AB_313099): <https://doi.org/10.1073/pnas.87.6.2244>
 APC anti-mouse CD25 (clone PC61, BioLegend Cat# 102012, RRID:AB_31286): <https://pubmed.ncbi.nlm.nih.gov/3934270/>
 PE anti-mouse/human CD44 (clone IM7, BioLegend Cat# 103007, RRID:AB_312958): [https://doi.org/10.1016/0092-8674\(89\)90639-9](https://doi.org/10.1016/0092-8674(89)90639-9)
 FITC anti-mouse TCR- β (clone H57-597, BioLegend Cat# 109206, RRID:AB_313429): <https://pubmed.ncbi.nlm.nih.gov/2467936/>
 PE anti-mouse FOXP3 (clone FJK-16s, Invitrogen Cat# 12-5773-80, RRID:AB_465936): this antibody was validated via relative expression: <https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/12-5773-82>
 BUV563 anti-mouse CD45 (clone 30-F11, Invitrogen Cat# 365-0451-82, RRID:AB_2925377): <https://doi.org/10.4049/jimmunol.140.11.3851>
 BV605 anti-mouse CD104 (clone 346-11A, BD Biosciences Cat# 743080, RRID:AB_2741272): <https://pubmed.ncbi.nlm.nih.gov/3940637/>
 AF532 anti-mouse IA/IE (clone M5/114.15.2, Invitrogen Cat# 58-5321-82, RRID:AB_2811913): <https://doi.org/10.1146/annurev.immunol.15.1.821>
 PE anti-mouse GP2 (clone 2F11-C3, MBL Cat# D278-5): <https://doi.org/10.1038/nature08529>
 AF700 anti-mouse CD177 (clone 1171A, 1:25) R&D Cat# FAB8186N <https://doi.org/10.1038/s41467-023-44348-y>
 eFlour450 anti-mouse Ly-6D (clone 49-H4, Invitrogen Cat# 48-5974-80, RRID:AB_2574089): <https://doi.org/10.1016/j.celrep.2018.11.069>
 BV785 anti-mouse Sca-1 (clone D7, BioLegend Cat# 108139, RRID:AB_2565957): <https://doi.org/10.1084/jem.164.3.709>
 BUV805 anti-mouse Ki-67 (clone SolA15, Invitrogen Cat# 368-5698-82, RRID:AB_2896151): This antibody was validated via cell treatment and knockout validation <https://www.thermofisher.com/antibody/product/Ki-67-Antibody-clone-SP6-Recombinant-Monoclonal/MA5-14520>
 Biotin anti-mouse SynCAM (clone 3E1, MBL Cat# CM004-6): <https://doi.org/10.4049/jimmunol.176.2.1238>
 PE-Vio770 anti-mouse CD171 (L1CAM) (clone 555, Miltenyi Cat# 130-102-135): <https://doi.org/10.1002/j.1460-2075.1984.tb01753.x>
 BV421 Donkey anti-Rabbit (polyclonal, Jackson Immuno Cat# 711-675-152, RRID:AB_2651108): <https://doi.org/10.1038/s41388-025-03318-y>
 Rabbit anti-mouse/human MDM2 (clone EPR22256-98, Abcam Cat# ab259265): <https://doi.org/10.3390/biom11111695>
 APC/Cyanine 7 anti-human EPCAM (clone 9C4, BioLegend Cat# 324245, RRID:AB_2783193): <https://pubmed.ncbi.nlm.nih.gov/2463074/>
 FITC anti-human CD45 (clone 2D1, BioLegend Cat# 368507, RRID:AB_2566367): <https://doi.org/10.1093/jnci/65.1.33>
 PE anti-human LY51/CD249 (clone 2D3/APA, BD Biosciences Cat# 553735, RRID:AB_395018): <https://doi.org/10.21769/BioProtoc.4865>
 APC anti-HLA-DRA (clone L243, BioLegend Cat# 307609, RRID:AB_314687): <https://doi.org/10.4049/jimmunol.137.2.490>

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

Mice used in this study were housed in pathogen-free facilities at the University of Chicago or Stanford University. All mice were housed in positively pressurized individually ventilated cage racks and changed in biological safety cabinets. Cage supplies are sanitized using hot water (180°F). Bedding and shredded paper enrichment were autoclaved and cages were provided with irradiated food. Reverse Osmosis water was provided by an automated watering system directly to each cage. Rodent housing rooms were maintained at a 12-hour light / 12-hour dark cycle. Temperature and humidity were within the Guide for the Care and Use of Laboratory Animals recommended ranges: 68-79°F and 30-70% humidity.

All experiments and animal use procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.

B6.129-Trp53L^{SL}-L25Q,W26S,F53Q,F54S heterozygous mice^{27,61} were provided by Laura Attardi (Stanford University) and were bred with B6-Foxn1Cre homozygous mice⁶² purchased from Jackson Laboratories to generate Trp53L^{SL}-L25Q,W26S,F53Q,F54S/wt; Foxn1Cre/wt and Trp53wt/wt; Foxn1Cre/wt littermates. Trp53f/f mice were purchased from Jackson Laboratories and bred with B6-Foxn1Cre mice to generate Trp53f/f; Foxn1Cre/wt mice. C57BL/6J mice were purchased from Jackson Laboratories. mTECs and thymocytes were harvested at 4-5 weeks of age. Sex-matched littermates were used for all comparisons of genetic perturbations.

Wild animals

No wild animals were used in this study.

Reporting on sex

Samples were derived from both male and female animals. Sex-matched littermates were used for all perturbations.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All experiments and animal use procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.

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

Plants

Seed stocks	No plants were used in this study.
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

<input checked="" type="checkbox"/> Confirm that both raw and final processed data have been deposited in a public database such as GEO .	
<input checked="" type="checkbox"/> Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.	
Data access links <i>May remain private before publication.</i>	All genomics data produced in this study are publicly available on GEO under the accession number GSE290716.
Files in database submission	Raw FASTQ files, MACS2 peak bed files, and normalized BigWig files are available on GEO: GSE290716.
Genome browser session (e.g. UCSC)	https://genome.ucsc.edu/s/ngamble/antiP53_CUTRUN_mTECs

Methodology

Replicates	Two independent biological replicates were generated for each experimental condition. Representative individual replicates were used for heat maps and histograms. Original FASTQ files, MACS2 peak bed files, and normalized BigWig files for all conditions and replicates are publicly available on GEO: GSE290716
Sequencing depth	50bp paired-end reads. Unique reads for each sample: anti-P53 CUT&RUN mTEClo Rep1: 57,657,219 anti-P53 CUT&RUN mTEChi Rep1: 63,136,557 anti-P53 CUT&RUN mTEClo Rep2: 59,417,428 anti-P53 CUT&RUN mTEChi Rep2: 50,050,248
Antibodies	Leica NCL-L-p53-CM5p
Peak calling parameters	Peaks were called for each sample using MACS2 (v2.2.9.1) with settings --shift 0 --extsize 200 --nomodel --call-summits --keep-dup all -p 0.01. For each sample, a 301 bp fixed-width peak set was generated by extending the MACS2 summits by 150 bp in both directions
Data quality	See ATAC-seq and CUT&RUN data analysis section in Methods.
Software	Bowtie2 (v2.2.9) was used to align sequencing data to mm10 reference genome. Picard (v2.21.8) was used to mark and purge duplicates. Samtools (v1.9) was used to process aligned reads and perform quality control. MACS2 (v2.2.9.1) was used to call ATAC-seq, ChIP-seq and CUT&RUN peaks. bedGraph2BigWig (v4) was used to convert bedGraph files to BigWig file format. Bedtools (v2.27.1) was used for intersection, subtraction, and additional analysis of genomic regions.

Flow Cytometry

Plots

Confirm that:	
<input checked="" type="checkbox"/> The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).	
<input checked="" type="checkbox"/> 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).	
<input checked="" type="checkbox"/> All plots are contour plots with outliers or pseudocolor plots.	
<input checked="" type="checkbox"/> A numerical value for number of cells or percentage (with statistics) is provided.	

Methodology

Sample preparation

For all flow cytometry, cells were incubated for 15 minutes at 4°C with relevant antibody mix diluted in T cell FACS buffer (2% FCS, 0.5% BSA, 10 mM EDTA in PBS).

Thymocytes:

Thymi from 4-5 week-old mice were removed and small cortical incisions made prior to mechanical agitation with wide-bore glass pipettes in DMEM/F-12 (Gibco) to liberate thymocytes.

Splenocytes:

Spleen and lymph nodes from 4-6 week-old mice were isolated in RPMI 1640 (Gibco) supplemented with 10% FCS and 1% Penicillin-Streptomycin (10% RPMI). Cells were liberated by mincing with syringe plunger and filtered through 40 um strainer.

Murine thymic epithelial cells:

Thymi from 4-5 week-old mice were harvested and connective tissue was removed. Stromal tissue was perforated using scissors and incubated with rotation in DMEM-F12 (Gibco) at room temperature for 10 min to liberate thymocytes. Remaining stromal tissue was enzymatically digested (0.5 mg/mL Collagenase D (MilliporeSigma), 0.2 mg/mL DNaseI (MilliporeSigma), 0.5 mg/mL Papain (Worthington Biochemical)). Cells were stained with anti-EpCAM antibodies conjugated to APC-Cy7 (BioLegend, clone 9C4) and EpCAM+ cells were enriched via positive selection using magnetic anti-Cy7 beads (Miltenyi). Intracellular staining for AIRE was performed using the eBioscience FoxP3 transcription factor staining kit (Invitrogen) according to manufacturer's instructions.

Human thymic epithelial cells:

Thymus fragments were obtained from a 12 week-old patient with no known genetic abnormalities undergoing standard of care cardiac surgery. Patient was de-identified upon receipt with written informed consent for release of genomic sequence data in accordance with IRB protocols 20-1392 from the University of Chicago and 2020-203 from Advocate Aurora Health. Connective tissue was removed and remaining tissue was minced, then incubated with rotation in DMEM-F12 (Gibco) at 4°C for 20 min to liberate thymocytes. Stromal tissue was enzymatically digested using 0.5 mg/mL Collagenase D (MilliporeSigma) and 0.2 mg/mL DNase I (MilliporeSigma) at 37°C for 20 min. Remaining fragments were incubated with rotation in 0.5 mg/mL Papain (Worthington), 0.25 mg/mL Collagenase D and 0.1mg/mL DNase I at 37°C for 20 min. Cells were stained with anti-EpCAM antibodies conjugated to APC-Cy7 (BioLegend, clone 9C4) and EpCAM+ cells were enriched via positive selection with magnetic anti-Cy7 beads (Miltenyi).

Instrument

Flow cytometry and FACS were performed using BD FACS Symphony S6, BD FACSAria Fusion, or BD FACSAria II equipped with a 100 um nozzle.

Software

BD FACS Diva (v8.0.2) and FlowJo (v10.9.0) were used for data collection and analysis respectively.

Cell population abundance

Post-sort fractions were analyzed to have > 95% of the relevant cell population. See representation of gating strategies and population abundance in Extended Data.

Gating strategy

Cells were gated based on FSC-A and SSC-A to exclude debris and doublets were excluded by gating on FSC-A / FSC-H. Dead cells were excluded using DAPI (Invitrogen). Supplemental gating strategies are provided in Extended Data and Supplementary Information.

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