**Supplemental Methods**

**Animal Procedures**

All animals were maintained at the Ecole Polytechnique Fédérale de Lausanne (EPFL) animal facility. For experimental analyses, littermates not expressing the Mx1Cre transgene were used as control mice (Controls). Genotyping of mice was performed on DNA isolated from toe biopsies using standard PCR buffer composition and reaction mixes. Conditioning of animals for bone marrow transplantation was performed as approved in study protocol VD1099. Donor animals used for subsequent enrichment of hematopoietic progenitors were injected intraperitoneally (i.p.) with 5-fluorouracil (150mg/kg bodyweight, Sigma-Aldrich, Cat# F6627). Mx1Cre induced gene inactivation was achieved performing five i.p. injections of 2μg/g body weight polyI:polyC (poly(I:C); Invivogen, Cat# tlrl-pic) at 2-day intervals. Genomic deletion of Tcf7 was assessed by PCR on total bone marrow cells (all primer sequences are listed in Supplemental Table 1). Induction of oncogenic Notch1 was assessed by flow cytometry detecting nuclear-localized enhanced GFP (eGFP).

**Compound Mouse Lines**

The N1IClox/lox, Tcf7lox/lox, β-cateninlox/lox and Mx1Cre lines were backcrossed 3 generations into 129S2/SvPasCrl (Charles River, France) to generate the following compound mouse strains: Notch1lox/lox Mx1Cre, Tcf7lox/lox Mx1Cre, N1IClox/lox Mx1Cre, N1IClox/+ Mx1Cre, N1IClox/+ Tcf7lox/lox, N1IClox/+ Tcf7lox/lox Mx1Cre, N1IClox/lox Tcf7lox/lox Mx1Cre, N1IClox/lox β-cateninlox/lox Mx1Cre. All primer sequences used for genotyping mouse lines are listed in Supplemental Table 1. B6.SJL-
Ptprcapo/BoyJ (B6-CD45.1)\textsuperscript{1} (Jackson Laboratories, ME) animals were bred and maintained at the EPFL’s animal facility.

**Generation of the Conditional Tcf7 Mouse Strain**

The conditional Tcf7 mouse line was generated as follows. The gene encoding Tcf1 (Tcf7) was conditionally targeted by the International Knockout Mouse Consortium EMMA (Project 37596). Exon 3 of Tcf7 was flanked by two loxP sites and deletion of this exon resulted in a nonsense frameshift mutation. The targeting strategy disrupts the β-catenin binding site (Ctnnb1) deleting the floxed exon 3 upon Cre-mediated recombination, corresponding to amino acid 33 through 69. Possible splicing events from exon 2 to exon 4, 5, 6, and 7 will result in out of frame proteins with premature stop codons, whereas splicing to exon 8 could possibly result in an in-frame protein in which amino acids 33 to 226 including a large portion of the high-mobility-group will be missing. Frozen embryos were shipped by EMMA and transferred into pseudo-pregnant females at the Center of PhenoGenomics EPFL, Lausanne. By crossing offspring with B6;SJL-Tg(ACTFLPe)9205Dym/J (ACTB:FLPe B6;SJL) Tg mice, the LacZ-Neo cassette flanked with Frt sites was excised, giving rise to the conditional Tcf7 allele.

**Generation of the TMe Transcription Factor Binding Site Mutant Mouse Strain**

The TMe mouse line was created by gene editing using the CRISPR-Cas9 technology\textsuperscript{2} in zygotes. Briefly, zygotes isolated from C57BL/6JRj mice were injected with a mixture of 2 sgRNAs and Cas9 mRNA. The sgRNAs targeted Cas9 to cut at loci on each side of the Tcf1 binding motif to induce a deletion mutation. The injected zygotes were cultured overnight and 2-cell stage embryos were transferred to pseudopregnant foster mice\textsuperscript{2,3}. For the TMe mouse line the CRISPR/Cas9 targets were (PAM underlined): 5’side-Target\textsubscript{87}:
GTAGGTCTAGGACTAGTGGG and 3’-side-Target_83: GGTGGGAGTGGGTTGGTG. The targets were evaluated for potential off-targets using E-CRISP (E-CRISP, RRID:SCR_019088). Among the resulting mice a founder carrying a 1068 bp deletion (GRCm38/mm10 chr15(+): 63270451-63271519) was identified by PCR techniques using primers for TMe:

336_FW1 Tcf7 BS 5’-ACTGCCTGACTTGACTCCCTAGC-3’
337_Rev1 Tcf7 BS Del 5’-GAAGTGGGAGTGGGTTGGTG-3’
338_Rev2 Tcf7 BS WT 5’-TGAGTCAGCGGGTTCTCTCTCT-3’

**Bone Marrow Chimeras and Transplantation Assays**

Bone marrow (BM) chimeric mice were generated as previously described. Reconstitution of transplanted animals was assessed by peripheral blood lymphocyte (PBL) analysis using flow cytometry 2-6 weeks post transplantation. Overall reconstitution and progression of T-ALL was monitored over time by PBL analysis until the endpoint of each experiment.

**Flow Cytometry and Cell Sorting**

Single cell suspensions were prepared from spleen, bone marrow, thymus or PBL of mice as previously described. For Western blot analysis for Tcf1 and Myc oncogenic Notch1-expressing T cells were sorted using a MoFlo Astrios EQ (Beckman Coulter). Purity of sorted subsets was >97%. Flow-cytometric data were acquired on a Gallios cytometer (Beckman Coulter) and analyzed using FlowJo v10.7.0. Cell viability was assessed using either 4’,6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) or Zombie UV™ dye (BioLegend) for sorting and LIVE/DEAD Fixable Aqua (Invitrogen) for analysis. All primary and secondary antibody conjugates are listed in the Supplemental Table 2.
**Isolation of human hematopoietic stem/progenitor cells**

Anonymized normal human cord blood (CB) samples were obtained with informed consent from women undergoing caesarian deliveries of full-term births according to protocols approved by the Research Ethics Board of the University of British Columbia and Children’s & Women’s Hospital of BC. CD34+ CB cells were obtained at >90% purity from pooled collections using a two-step RosetteSep/EasySep human CD34-positive selection kit (StemCell Technologies) according to the manufacturer’s protocols. CD34+ cells were seeded into 96-well round bottom plates and prestimulated in StemSpan SFEM II (StemCell Technologies, Cat# 09655) with 10 ng mL\(^{-1}\) human SCF (Cat# 300-07), 20 ng mL\(^{-1}\) human TPO (Cat# 300-18), 20 ng mL\(^{-1}\) human IGF2 (Cat# 100-12), and 10 ng mL\(^{-1}\) human FGF\(\alpha\) (Cat# 100-17A) (Peprotech) for 24 h.

**Cell culture and Cell Lines**

Cell culture of cell lines was performed under standard conditions in a humidified atmosphere at 37 °C under 5% CO\(_2\). The retroviral packaging cell line BOSC-23 (ATCC, Cat# CRL-11270, RRID:CVCL_4401) was purchased from ATCC and maintained in DMEM GlutaMAX Supplement, pyruvate (GIBCO, Cat# 31966047) supplemented with 10% FBS (GIBCO), 25mM HEPES buffer (\(N\)-2-hydroxyethylpiperazine-\(N'\)-2-ethanesulfonic acid, AMIMED, Cat# 5-31F00-H), 50µM 2-mercaptoethanol (Life Technologies, Cat# 31350010) and 10µg mL\(^{-1}\) Gentamicin (Life Technologies, Cat# 15710049), referred to as complete DMEM. The lentiviral packaging cell line HEK293T cells was obtained from D. Trono (EPFL, Lausanne, RRID:CVCL_0063) and maintained in complete DMEM. All primary tumor T-ALL cells and cell lines were grown in RPMI 1640 GlutaMAX Supplement (GIBCO, Cat# 61870010) supplemented with 10% FBS (GIBCO), 25mM HEPES buffer (\(N\)-2-hydroxyethylpiperazine-\(N'\)-2-ethanesulfonic acid,
AMIMED, Cat# 5-31F00-H), 50µM 2-mercaptoethanol (Life Technologies, Cat# 31350010) and Gentamicin (10µg mL\(^{-1}\); Life Technologies, Cat# 15710049) and referred to as complete RPMI 1640. Lineage-negative bone marrow cells were cultured in IMDM GlutaMAX\(^\text{TM}\) Supplement (GIBCO; Cat# 31980022) supplemented with 10% FBS (PAA), 25mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, AMIMED, Cat# 5-31F00-H), 50µM 2-mercaptoethanol (Life Technologies, Cat# 31350010), 10µg mL\(^{-1}\) Gentamicin (Life Technologies, Cat# 15710049) and growth factors: 10ng mL\(^{-1}\) murine IL-1β (Cat# 78035), 10ng mL\(^{-1}\) murine IL-3 (Cat# 78042), 100ng mL\(^{-1}\) murine Flt3L (Cat# 78011) and 100ng mL\(^{-1}\) murine SCF (Cat# 78064) (Stem Cell Technologies). Transduced CB cells were cultured on confluent monolayers of OP9-DL1 cells (RRID:CVCL_B218) in αMEM media supplemented with 20% FBS (GIBCO) plus 10 ng mL\(^{-1}\) human SCF (Cat# 300-07), 5 ng mL\(^{-1}\) human FLT3L (Cat# 300-19) and 3 ng mL\(^{-1}\) human IL-7 (Cat# 200-07) (Peprotech).

**Generation of Stable Primary Murine T-ALL Cell Lines**

Late-stage primary oncogenic Notch1-driven (retroviral: MigR1-N1ICD-IRES-eGFP for PR12 and PR13 or genetic: \(R26\ N1IC^{Δ/Δ}\) for T2T3) T-ALL tumor cells were isolated from bone marrow of T-ALL-bearing end-stage animals and expanded *in vitro* in complete RPMI 1640. The genotype of mouse PR12 T-ALL cell line is \(N1IC\ Tcf7^{Δ/Δ}\) and for PR13 \(N1IC\ Tcf7^{lox/lox}\), the T2T3 T-ALL cell line was derived from cells with the genetic composition of \(R26\ N1IC^{Δ/Δ}\ β\-catenin^{Δ/Δ}\) (\(N1IC\ β\-catenin^{Δ/Δ}\)).

**Plasmids, Retroviral and Lentiviral Vectors**

The retroviral packaging plasmid pCL\(^\text{ECO}\) for pCL retroviruses was obtained from Addgene\(^6\) (RRID:Addgene_12371). The coding sequence of the intracellular domain of the
mouse Notch1 receptor lacking the PEST domain (corresponding to amino acids 1751–2443) was created by PCR amplification from the mouse Notch1 cDNA template. The sequence was flanked by EcoRI restriction sites (underlined) by amplification with the following primer pairs:

**Forward 5’-CCGGAATTCCGCCACCATGGATCCCGCGCGCCAG-3’**

**Reverse 5’-CCGGAATTCTACAGTGATGTTGGTAGGGCTG-3’**

The modified N1ICD construct was cloned into the EcoRI site of the Migr1 vector, a pMSCV2.2-based retroviral vector containing an IRES-eGFP cassette and termed RV-N1ICD-IRES-eGFP. The retroviral vector Migr1 Tcf7-p45 eGFP (RV Tcf7 IRES eGFP) containing the cDNA of Tcf7 (p45 isoform) was obtained from H.H. Xue (University of Iowa). All sequences were verified by DNA sequencing.

The human NOTCH1 (ΔE allele) was obtained from J Aster (Boston), Harvard PlasmID. NOTCH1ΔE and GFP cDNAs were connected with equine rhinitis A virus 2A (E2A) peptide. The polycistronic cDNA was cloned into pRRL-cPPT/CTS-MNDU3-PGK-GFP-WPRE3 immediately downstream of the MNDU3 promoter. The construct was verified by DNA sequencing.

**Viral Particle Production and Transduction**

Retroviruses were packed and produced as previously described by transfection of BOSC-23 cells with the retroviral vectors and packaging plasmid pCL\(^{ECO}\)\(^{6}\). Retroviral titers were determined by serial dilution of concentrated retroviral stock aliquots using the NH10 cell line\(^{11}\). Retroviral particles (RVP) were transduced into lineage-negative bone marrow cells as previously described\(^{7}\). Briefly, RVP were transduced (1-5 RVP per cell) by spinoculation (45 min at 34 °C by centrifugation at 800g) into lineage-negative bone marrow cells in complete IMDM medium supplemented with growth factors (murine recombinant IL-1β 10ng mL\(^{-1}\), IL-3 10ng
mL\(^{-1}\), Fli3L 100ng mL\(^{-1}\) and SCF 100ng mL\(^{-1}\) and 10µg mL\(^{-1}\) polybrene (Sigma Aldrich, Cat# TR-1003-G).

High-titer lentiviral supernatants were produced by transient transfection of HEK293T cells using polyethyleneimine HCl MAX (Polysciences) with second-generation packaging/envelope vectors pCMV dR8.74 (RRID:Addgene_22036), pRSV-Rev (RRID:Addgene_12253), and pCMV VSV-G (RRID:Addgene_8454), followed by ultracentrifugal concentration (25,000 rpm for 90 min at 4 °C; Beckman SW32Ti rotor). Prestimulated CB cells were treated with 8 µM Cyclosporin H (Toronto Research Chemicals, Cat# C988920,) for 16 hours, subsequently transduced in 96-well plates coated with 5 µg/cm\(^2\) fibronectin (StemCell Technologies, Cat# 07159) by direct addition of concentrated viral supernatants and transferred to OP9-DL1 co-cultures 6 h later.

Western blot

Tcf1 (anti-Tcf1 antibody, Cell Signaling Technology, Cat# 2203, RRID:AB_2199302), β-catenin (anti-β-catenin antibody, BD Biosciences, Cat# 610153, RRID:AB_397554) and c-Myc (anti-c-Myc antibody, Abcam, Cat# ab32072, RRID:AB_731658) were analyzed by Western blotting of sorted cells and cell lines. Whole-cell lysates (RIPA buffer) separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Bio-Rad) were transferred to polyvinylidene difluoride membranes and incubated with indicated primary antibodies. Washed membranes were incubated with a secondary sheep anti-mouse IgG HRP conjugate (GE Healthcare, Cat# NXA931, RRID:AB_772209) and developed using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Cat# 32209). β-Actin (anti-β-Actin antibody, Abcam, Cat# ab8226, RRID:AB_306371) expression was analyzed as loading control.
ATAC-seq

Accessible chromatin mapping was performed using Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) as previously described\textsuperscript{12,13} using sorted $R26 \; N1IC^{\text{lox/}+} \; Tcf7^{\text{lox/lox}}$ (Controls), $R26 \; N1IC^{\Delta/+}$ (N1IC), $R26 \; N1IC^{\Delta/+} \; Tcf7^{\Delta/\Delta}$ (N1IC Tcf7$^{\Delta/\Delta}$), $R26 \; N1IC^{\Delta/+} \; TMe^{+/\Delta}$ (N1IC TMe$^{+/\Delta}$) and $R26 \; N1IC^{\Delta/+} \; TMe^{-/-}$ (N1IC TMe$^{-/-}$) LSK cells (50,000 cells each), with minor adaptations as detailed below. Cells were washed twice in 150μL and 50μL 1×PBS, resuspended in 50μL ATAC-seq lysis buffer, incubated for 10min on ice and centrifuged at 400g for 10 min at 4 °C. The pellet was incubated in the transposase reaction mix (25μL 2×TD buffer (Illumina), 2.5μL transposase (Illumina Cat# FC-121-1030) and 22.5μL nuclease-free water) for 30min at 37 °C with gentle agitation. After DNA purification with the MinElute PCR Purification kit (Qiagen, Cat# 28004) library was amplified with NEBNext High-Fidelity PCR Master Mix (NEB, Cat# M0541S) using custom Nextera primers. Library for sequencing was size selected with Agencourt AMPure XP beads (Beckman Coulter, Cat# A63880). DNA concentration was measured with an Invitrogen Qubit fluorometer (Life Technologies) and Agilent Fragment Analyzer. The libraries were sequenced by Gene Expression Core Facility of EPFL using the Illumina NextSeq 500 platform and the 75-bp paired-end configuration to obtain at least 35 million reads per sample, or for TMe at Novogene using the Illumina NovaSeq and the 150-bp paired-end configuration to obtain at least 100 million reads per sample.
RNA-seq

RNA was extracted from sorted cells using PicoPure RNA Isolation Kit (Thermo Fisher Scientific - Applied Biosystems, Cat# KIT0214). cDNA libraries were prepared and sequenced by the Gene Expression Core Facility of EPFL. Libraries were generated using TruSeq RNA Library Prep Kit v2 (Illumina, Cat# FC-122-1001). Strand-specific library construction and Illumina NextSeq 500 sequencing of paired-end 2x79nt or single-end 75nt reads were performed.

ChIP-seq

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) was performed on chromatin from 20,000 LSK cells. Sorted cells were cross-linked with 1% methanol-free formaldehyde (Pierce Life Technologies, Cat# 28906), quenched with 0.125 M glycine and frozen at -80 °C and stored until further processing. ChIP reaction was performed with Diagenode True MicroChIP Kit (Diagenode, Cat# C01010130) with modifications of the manual detailed below. Lysed samples were sonicated using Diagenode Bioruptor Pico (Diagenode, Cat# B01060010). Sheared chromatin was immunoprecipitated with 1μg H3K27ac antibody (Diagenode, Cat# C15410196, RRID:AB_2637079) and 1μg CTCF antibody (Diagenode, Cat# C15410210, RRID:AB_2753160). Eluted and decross-linked DNA was purified with Diagenode MicroChIP DiaPure columns (Diagenode, Cat# C03040001) and eluted in 30μL of Nuclease-Free water.

ChIP and input libraries for sequencing were prepared with MicroPlex Library Preparation Kit (Diagenode, Cat# C0101134). Size selection steps were performed with Agencourt AMPure XP magnetic beads (Beckman Coulter, Cat# A63880). Subsequently, ChIP-seq library was quantified with Agilent Fragment Analyzer. The libraries were sequenced by Gene Expression
Core Facility of EPFL using the Illumina NextSeq 500 platform and the 75-bp paired-end configuration to obtain at least 30 million reads per sample.

**ChIP-qPCR**

Briefly, chromatin samples were prepared from 10 million 1% formaldehyde fixed cells, sonicated in 200μL of 1% SDS lysis buffer with Diagenode Bioruptor Plus (Diagenode, Cat# B01020001) and immunoprecipitated with 2μg antibody recognizing Tcf1 (Cell Signaling Technology, Cat# 2203, RRID:AB_2199302) and 2μg of mouse IgG (Diagenode, Cat# C15400001-100, RRID:AB_2722553). Antibody-chromatin complexes were captured with Pierce protein A magnetic beads (Pierce Life Technologies, Cat# 88845), washed with low salt buffer, high salt buffer, LiCl wash buffer, and eluted. After reversal of cross-linking, RNase and proteinase K treatment were performed and DNA was purified with Diagenode MicroChIP DiaPure columns (Diagenode, Cat# C03040001). Enrichment analysis was performed by qPCR with 7900HT Fast qPCR.

**In situ Hi-C**

Low input in situ Hi-C was performed on 150,000 LSKs with Arima-HiC Kit (Arima) according to the manufacturers’ protocols. DNA fragmentation was performed on Covaris E220 Evolution sonicator (Covaris, Cat# 500429) with default settings for 400bp DNA size distribution. Libraries for sequencing were prepared with Swift Biosciences Accel-NGS 2S Plus DNA Dual Indexing Library Kit (Swift Biosciences, Cat# 21024 and #28096). Size selection steps were performed with Agencourt AMPure XP magnetic beads (Beckman Coulter, Cat# A63880). Library amplification was performed following DNA quantification with Invitrogen Qubit Fluorometer and KAPA Library Quantification Kit (Roche, Cat# 07958927001). Prepared
libraries were sequenced using the sequencing platforms at Novogene with Illumina NovaSeq 6000 S4 and the 150 bp paired-end reads generating around 700 million paired reads per library.

**Reverse ChIP**

The reverse ChIP assays were performed as previously described\(^\text{14}\). Briefly, the TMe DNA bait was generated using sequences by PCR from human genomic DNA using a TMe biotinylated forward primer (5’-GGTAAAGTCTAGACAG-3’) and an unmodified TMe reverse primer (5’-CAGTGTATCTTATCC-3’). The DNA baits were conjugated to streptavidin beads and incubated with nuclear protein extracts from DND-41. Non-conjugated beads were used as negative control. TMe pulled down proteins were analyzed by mass spectrometry at the Proteomics Unit of the Josep Carreras Leukemia Research Institute. The MS/MS spectra were searched against the Swissprot human database using Andromeda within MaxQuant (v.1.7.6.0). A 1% False Discovery Rate (FDR) cut off was applied at both peptide and protein level using a standard target-decoy database strategy. All the protein groups labeled as "Reverse", "Only identified by Site" and "Potential contaminant" were excluded from the analysis. Four hundred and fifty-nine proteins were recovered from DND-41 extracts. Then, proteins were filtered out that were present in more than 2% of the experiments of the Contaminant Repository for Affinity Purification (CRAPome) database\(^\text{15}\). Finally, 52 TMe associated proteins in DND-41 were identified.
3C-qPCR

Analysis of locus-locus interactions between the promoter of Myc and the NMe in sorted N1IC CD8⁺, N1IC CD4⁺CD8⁺ and N1IC TMe⁻/⁻ CD4⁺CD8⁺ was performed as previously described¹⁶ with minor modifications. Briefly, DNA from 10 million cells was fixed with 2% methanol-free formaldehyde and lysed with buffer containing NP-40. 1000 U of HindIII (NEB, Cat# R3104M) restriction enzyme digestion was performed first overnight, then additional 1000 U of enzyme were added for 4h. Ligation with 10000U of T4 DNA ligase (NEB, Cat# M0202M) was performed for 20h at 16°C. Quantification of genomic interactions was performed with BAC controls, using qPCR with TaqMan probe (IDT, /56-FAM/AAG CCC TGC CCT TCA GGA +G+G+C /3IABkFQ/), Myc promoter primer (GTCCGACTCGCCTCACTCAG) and NMe primer (GTGTGTACGGTGATTGTTCACCC). The experiment was performed with three biological replicates.

Bioinformatic analyses:

TMe Evolutionary Conservation Analysis

The evolutionary conservation of TMe sequences was analysed using the DIALIGN TF tool (DIALIGN, RRID:SCR_003041)¹⁷. TMe sequences from 26 vertebrate species were aligned and putative transcription factor binding sites were identified in highly conserved regions. Evolutionary conservation scores were calculated at individual alignment sites using PhastCons¹⁸. The TMe phylogenetic tree was generated using the iTOL tool (iTOL, RRID:SCR_018174)¹⁹.
RNA-seq

Adapter sequences and low-quality ends were removed with cutadapt (1.9.1) and reads were aligned to the mouse genome build mm10 using HISAT2 (2.0.3beta) aligner\textsuperscript{20}. Raw counts per gene were calculated with featureCounts (1.4.4) and genes with average TPM (Transcripts Per kilobase Million) < 1 were filtered out. Between-sample normalization was performed using the EdgeR (3.28.0) TMM method. Normalized log2 transformed counts (voom) were subsequently analyzed for differential expression (eBayes, limma 3.42.2), p-values were FDR corrected\textsuperscript{21}. Pathway over-representation analysis (ORA) was performed in the genes significantly (FDR < 0.05) induced (LFC > 1) or repressed (LFC < -1) using GO and KEGG biological pathway database from mSigDB (version 7.0)\textsuperscript{22}. P-values were computed by hypergeometric test and FDR corrected.

ChIP-seq

Adapter sequences and low-quality ends were removed with cutadapt as needed and reads were aligned to the mouse genome build mm10 using BWA-MEM (0.7.17)\textsuperscript{23}. Properly paired good quality reads were filtered with samtools (1.9) using samtools view -b -h -f 0x2 -q 2. Peaks were called using MACS2 (2.1.1.20160309)\textsuperscript{24} using macs2 callpeak -t -f BAMPE -g mm -B --nomodel --nolambda -q 0.05 --keep-dup all --call-summits (for CTCF ChIP) or --broad --broad-cutoff 0.1 (for H3K27ac ChIP).

ATAC-seq

Mouse ATAC-seq reads were processed as follows: adapter sequences and low-quality ends were removed with cutadapt, and reads were aligned to mouse genome build mm10 using BWA-MEM. Properly paired good quality reads were filtered with samtools view -b -h -f 0x2 -
q 2, and subsequently used for peak calling using MACS2 using macs2 callpeak -t -f BAMPE -g mm -B --nomodel --nolambda -q 0.05 --keep-dup all --broad --broad-cutoff 0.1.

Human cord blood ATAC-seq sequencing reads were trimmed using atropos (1.1.21) to remove the Illumina TruSeq adapter sequences. Trimmed reads were aligned to the human genome build hg38 using BWA-MEM. The aligned reads were filtered to include reads from chromosomes 1 - 22, X, and Y et al. using samtools and duplicates were marked using “MarkDuplicates” from Picard (2.18.27). ATAC-seq peaks were called with MACS2 with the same parameters. For the previously published human cord blood CD34+ samples used as control, ATAC-seq sequencing reads from patient 1 were trimmed to keep the first 38 bp to match the length of the ATAC-seq reads from patient 3. The subsequent steps until peak calling were performed as for the other human cord blood ATAC-seq data.

Transcription factor binding motif and footprints

Transcription factor footprints were predicted based on the aligned ATAC-seq reads using the TOBIAS (0.12.4) snakemake pipeline with default parameter values and options. Transcription factor motifs were downloaded from JASPAR (JASPAR 2020 non-redundant vertebrate CORE PFMs) and human and mouse evidence-based gene annotations were downloaded from GENCODE.

Differential analysis and pathway enrichment

Differential analysis of chromatin accessibility or binding was performed using EdgeR within the DiffBind R package (2.16.2). Chromatin peaks were called significantly changed if FDR was below the cutoff of 0.01. Heatmaps of significantly changed peaks were produced with the genomation R package or EnrichedHeatmap R package version (1.18.2). Pathway
enrichment analysis was performed using rGREAT (1.20.0)\textsuperscript{32} and the following geneset collections: Gene Ontology Biological Process, Phenotype Data and Human Disease, KEGG. Ontologies with a fold enrichment $> 2$ and FDR 0.05 were reported.

**In-situ Hi-C data analysis**

**Creation of Hi-C contact maps.** Hi-C paired-end sequencing reads for each sample were filtered and aligned to the mouse genome build mm10 using the Juicer pipeline 1.6\textsuperscript{33} using default parameters and a file containing the restriction site coordinates of the restriction enzymes used in the Arima-HiC kit (cutting at GATC and GANTC) which was generated with the generate_site_positions.py script provided with Juicer. Aligned Hi-C contacts with an alignment score $> 30$ (MAPQ $> 30$) from individual replicates were combined using the “mega.sh” script provided with Juicer. Chromatin contact maps from pooled data were created (in .hic format) using the “pre” command. In this step, contact matrices were obtained at multiple resolutions: custom resolutions ($2.5$ kb and $5 – 25$ kb with a $1$ kb increase) and at default resolutions ($50$, $100$, $250$, $500$ kb, $1$, and $2.5$ Mb). Contact maps were visualized with Juicebox\textsuperscript{33}.

**Correlation between Hi-C contact maps.** Contact matrices at $1$ Mb resolution with Knight-Ruiz normalization were obtained for each chromosome from each sample to obtain the correlation between Hi-C contact maps. The contact matrices of each chromosome from the same sample were combined to create genome-wide contact matrices for each sample. The Pearson correlation between the genome-wide, normalized counts of each sample was calculated using R. Hierarchical clustering of the Pearson correlation matrix was performed in R with the hclust function, default parameters, and $(1 - \text{Pearson correlation})$ as the dissimilarity measure.
Chromatin compartment analysis. Compartments were analyzed using the Calder algorithm\textsuperscript{34} at 10 kb resolution. Briefly, for each chromosome, Calder computes a similarity measure between each pair of bins (10kb) of the Hi-C map defined as the Fisher’s z-transformed correlations of whole-chromosome interactions. A chromosome is then partitioned into compartment domains showing high intra-domain similarity while low inter-domain similarity. Compartments are then hierarchically clustered based on inter-domain interactions, ignoring their physical order on the chromosome. A normalized rank value between 0 and 1 is assigned to each domain (the direction is determined by the sign of correlation with gene density), to identify its position within the dendrogram. Domain ranks were shown highly correlated with gene density and active histone mark intensities\textsuperscript{34}. The classification in eight compartments (B.2.2, B.2.1, B.1.2, B.1.1, A.2.2, A.2.1, A.1.2, A.1.1) was generated by cutting the domain hierarchy at the third level of the dendrogram, starting from the top (the hierarchy is built as a binary tree). To find regions that switch compartments between two conditions, we started by computing the differences of Calder rank (denoted as $\Delta$Calder\_rank) for each bin. A random $\Delta$Calder\_rank, capturing the noise possibly arising from technique fluctuation, was derived by first merge the two Hi-C maps then sampling 10 random Hi-C maps with similar total contacts from the merged map and finally computing the $\Delta$Calder\_rank among these maps. Consecutive bins with observed $\Delta$Calder\_rank subtracted by random $\Delta$Calder\_rank greater than 0.1 were designated as regions having compartment switch.

Identification and analysis of chromatin domains. TADs were called using TopDom\textsuperscript{35} on the 10kb resolution Hi-C maps with default parameters. TAD boundary changes between conditions were quantified by using TADcompare (1.0.0) and the condition specific contact matrices at 10Kb resolution. Contact matrices were extracted from the *.hic files using Juicer
Tools dump command without normalization. Changes were considered significant if the absolute differential boundary scores ("gap scores") > 2.

**Chromatin domain boundaries CTCF enrichment analysis.** CTCF coverage tracks (bigWig) were generated from ChIP-seq read alignments using the bamCoverage tool from deepTools 2.0\textsuperscript{36} with a bin size set to 10bp and a RPGC normalization of the number of reads per bin.

**Identification of chromatin loops.** Chromatin loops were identified similarly to\textsuperscript{37}. Chromatin contacts were called at 2.5 and 5 to 25 kb resolutions (with a 1 kb increase) with HiCCUPS from Juicer tools\textsuperscript{38} using parameter values as described in Greenwald et al. 2019 for resolutions 5 to 25Kb, and -p 4, -i 7 and -d 10000 for 2.5Kb resolution. For resolutions between 2.5 and 10 kb, chromatin loops within 20 kb were merged together using pgltools (2.2.0)\textsuperscript{39}. For resolutions higher than 10 kb, loops which were called within twice the size of the loop anchors (i.e. resolution) were merged together using pgltools. The merged loops called at each resolution were then combined and subsequently merged if they occurred within 20 kb. At each merging event, chromatin loops which were called in at least three different resolutions were retained. Finally, the chromatin loop called at the highest resolution and with the lowest "FDR donut" value was kept for downstream analyses. Condition-specific chromatin loops were identified by using pgltools intersect with the \texttt{--v} option to obtain loops that do not overlap with loops from a different condition. For functional annotation of chromatin loops, each anchor of chromatin loops was associated to a gene if the anchor boundary occurred within 2.5 kb either upstream or downstream of a gene TSS. For visualization of chromatin loops, Hi-C contact maps, TADs, RNA-seq, ChIP-seq, ATAC-seq and CTCF binding sites, we used the Sushi R package (1.27.0).

**H3K27ac at chromatin loop anchors.** We analyzed the H3K27ac levels at chromatin loop which had anchors associated to a gene TSS. For each chromatin loop anchor that was
associated to a TSS, we plotted the normalized H3K27ac signal present at the other end of the chromosome loop. H3K27ac heatmaps were produced with the EnrichedHeatmap using the following parameters: extend = 2500, w = 50, mean_mode = "w0", and background = 0.

**Enhancer-promoter analysis.** We studied differential enhancer-promoter interactions (EPI) between three comparison groups: N1IC vs Controls, N1IC Tcf7Δ/Δ vs Controls, and N1IC Tcf7Δ/Δ vs N1IC. We focused on differentially expressed genes in each comparison group. We first computed EPI strength for each gene under each condition. The EPI strength was defined as the maximum contact value of the gene promoter with its four nearest enhancers within a +/- 1Mb flanking window. The choice of four nearby enhancers is based on an empirical observation that they are the most probable direct interaction partner of the promoter. We used the distance-normalized contact value that is corrected for the bias of genomic distances (by specifying the observed/expected option using Juicer tools33, such that a contact value greater than 1 indicates contact enrichment. An enhancer-promoter was considered as differentially interacting if the difference of EPI strength is greater than 1. The analysis was done at resolution of 5kb.

**Software**

Genomic data was visualized with Integrative Genomics Viewer 2.7.2 (IGV, RRID:SCR_011793) and Juicebox 1.11.0840. Quantitative data and statistical analysis was performed with GraphPad Prism 9 (RRID:SCR_002798). Flow cytometric data was analyzed with FlowJo v10.7.1 (RRID:SCR_008520). Representation of data was generated in Adobe Illustrator 2021 (RRID:SCR_010279).
**Quantification and Statistical analysis**

p-values were calculated using unpaired two-tailed Student’s *t*-test or one-way ANOVA with GraphPad Prism 9. Statistical difference analyses of Kaplan-Meier survival curves were performed using Log-rank (Mantel-Cox) test. Unless otherwise stated, bioinformatic statistical analyses were performed using R version 4.0.3 (RRID:SCR_001905) and Bioconductor 3.12. To highlight statistical significances in figures, the following annotations for p values: *, p-value<0.05; **, p-value<0.01; ***, p-value<0.001; ****, p-value<0.0001 were used.
Supplemental Tables

Supplemental Table 1 - Genotyping Primers

| Primer                  | Sequence                  |
|------------------------|---------------------------|
| 204_N1 5' lox FW       | CTGAGGCCTAGAGCCTTGAA      |
| 205_N1 3' lox RV       | TGTGGGACCAGAATGTTAGG      |
| 219_Mx1-Cre            | GGCAGGGCTCTCAGTGATTC      |
| 220_Cre2L21            | CTTGGCAGCCTCTGAACATGTC    |
| 336_FW1 TMe_BS         | ACTGCCCTGAATGACTCCCTAGC   |
| 337_Rev1 TMe_BS_Del    | GAAATGGGAGTGGTTGGTTTG     |
| 338_Rev2 TMe_BS_WT     | TGGATCAAGCGGTTCCTCTTT     |
| 3251_Ef(3251) Tcf7 lox sense | GGAAGCTGACCCCTGTAGTG     |
| 3253_Err(3253) Tcf7 lox as | CTGGTTTCCTAGCACTGCAAG    |
| 3249_L3r2 Tcf7 Del as  | TAGCCTAGAAGACCTGACCTG     |
| 155_Rosa26 WT sense    | AAAGTCGCTCTGAGTTGGAT      |
| 156_Rosa26 Neo as      | GCCGAAGTTTGTCCCTCAACC     |
| 157_Rosa26 WT as       | GGAGCGGGAGAAATGGATATG     |
| 005_b-cat lox sense RM41 | AAGGATAGGTGAAAGTTGGTT    |
| 006_b-cat lox as RM42  | CACCATGCTCTGCTATCC        |

Supplemental Table 2 – Antibodies

| Antibodies                          | SOURCE                        | CAT.No. - IDENTIFIER                      |
|-------------------------------------|-------------------------------|------------------------------------------|
| anti-Biotin, Streptavidin PE Texas Red - Flow Cytometry | Invitrogen Molecular Probes | Cat# SA1017                               |
| anti-mouse B220, PE Texas Red - Flow Cytometry | Thermo Fisher Scientific | Cat# RM2617; RRID:AB_10372805             |
| anti-mouse B220, AlexaFluor 700 - Flow Cytometry | Thermo Fisher Scientific | Cat# 56-0452-82; RRID:AB_891458           |
| anti-mouse B220, Pacific Blue, clone RA3-6B2 - Flow Cytometry | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                       |
| anti-mouse B220, FITC, clone RA3-6B2 - Flow Cytometry | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                       |
| anti-mouse CD117, APC - Flow Cytometry | Thermo Fisher Scientific | Cat# 17-1171-81; RRID:AB_469429           |
| anti-mouse CD117, APCeF780 - Flow Cytometry | Thermo Fisher Scientific | Cat# 47-1171-80; RRID:AB_1272213          |
| anti-mouse CD11b, PE-Cy7 - Flow Cytometry | Thermo Fisher Scientific | Cat# 25-0112-81; RRID:AB_469587           |
| anti-mouse CD11b, AlexaFluor 700 - Flow Cytometry | Thermo Fisher Scientific | Cat# 56-0112-82; RRID:AB_657585           |
| Antigen                          | Supplier                      | Cat#/RRID                          |
|---------------------------------|-------------------------------|-----------------------------------|
| anti-mouse CD11b, FITC, clone M1/70 - Flow Cytometry | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A |
| anti-mouse CD135, PE - Flow Cytometry | Thermo Fisher Scientific | Cat# 12-1351-83; RRID:AB_465860 |
| anti-mouse CD150, PE-Cy5 - Flow Cytometry | BioLegend | Cat# 115912; RRID:AB_493598 |
| anti-mouse CD19, AlexaFluor 700 - Flow Cytometry | Thermo Fisher Scientific | Cat# 56-0193-80; RRID:AB_837082 |
| anti-mouse CD25, APCeF780 - Flow Cytometry | Thermo Fisher Scientific | Cat# 47-0251-82; RRID:AB_1272179 |
| anti-mouse CD34, eF660 - Flow Cytometry | Thermo Fisher Scientific | Cat# 50-0341-80; RRID:AB_10609352 |
| anti-mouse CD4, PE-Cy7 - Flow Cytometry | Thermo Fisher Scientific | Cat# 25-0041-82; RRID:AB_469576 |
| anti-mouse CD4, AlexaFluor 700 - Flow Cytometry | Thermo Fisher Scientific | Cat# 56-0041-80; RRID:AB_494001 |
| anti-mouse CD4, FITC, clone YTS191.1 - Flow Cytometry | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A |
| anti-mouse CD44, PE - Flow Cytometry | Thermo Fisher Scientific | Cat# 12-0441-82; RRID:AB_465664 |
| anti-mouse CD44, PE-Cy7 - Flow Cytometry | Thermo Fisher Scientific | Cat# 12-0441-82; RRID:AB_465664 |
| anti-mouse CD45.1, Pacific Blue - Flow Cytometry | BioLegend | Cat# 110722; RRID:AB_492866 |
| anti-mouse CD45.1, AlexaFluor 700 - Flow Cytometry | BioLegend | Cat# 110724; RRID:AB_493733 |
| anti-mouse CD45.2, PerCp Cy5.5 - Flow Cytometry | BioLegend | Cat# 109828; RRID:AB_893350 |
| anti-mouse CD45.2, Pacific Blue - Flow Cytometry | BioLegend | Cat# 109820; RRID:AB_492872 |
| anti-mouse CD48, Biotin - Flow Cytometry | Thermo Fisher Scientific | Cat# 13-0481-82; RRID:AB_466470 |
| anti-mouse CD71, PE - Flow Cytometry | Thermo Fisher Scientific | Cat# 12-0711-81; RRID:AB_465739 |
| anti-mouse CD8, AlexaFluor 700 - Flow Cytometry | Thermo Fisher Scientific | Cat# 56-0081-80; RRID:AB_494006 |
| anti-mouse CD8, Alexa647, clone | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A |
| Antibody/Reagent                                                                 | Supplier                              | Cat#/RRID                          |
|---------------------------------------------------------------------------------|---------------------------------------|------------------------------------|
| YTS169.4 - Flow Cytometry                                                       | conjugated at EPFL following standard protocols - ute.koch@epfl.ch |                                    |
| anti-mouse CD8, FITC, clone YTS169.4 - Flow Cytometry                          | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                |
| anti-mouse Gr1, AlexaFluor 700 - Flow Cytometry                                | Thermo Fisher Scientific              |                                    |
| anti-mouse Gr1, PerCp Cy5.5 - Flow Cytometry                                    | Thermo Fisher Scientific              | Cat# 45-5931-80; RRID:AB_906247     |
| anti-mouse Gr1, FITC, clone RB6-8C5 - Flow Cytometry                           | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                |
| anti-mouse IgM, eF660 - Flow Cytometry                                          | Thermo Fisher Scientific              |                                    |
| anti-mouse IgM, FITC - Flow Cytometry                                           | Thermo Fisher Scientific              | Cat# 56-5931-82; RRID:AB_494007     |
| anti-mouse Sca1, PE-Cy7 - Flow Cytometry                                        | Thermo Fisher Scientific              |                                    |
| anti-mouse Sca1, Pacific Blue - Flow Cytometry                                  | BioLegend                             | Cat# 108120; RRID:AB_493273        |
| anti-mouse Ter119, APCeF780 - Flow Cytometry                                   | Thermo Fisher Scientific              | Cat# 47-5921-80; RRID:AB_1548794    |
| anti-mouse Ter119, FITC, clone Ter119 - Flow Cytometry                         | EPFL_UPRAD - the antibody was purified from hybridoma supernatants and conjugated at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                |
| anti-mouse NK1.1, clone PK136 - in vivo NK cell depletion                       | EPFL_UPRAD - the antibody was purified from hybridoma supernatants at EPFL following standard protocols - ute.koch@epfl.ch | N/A                                |
| anti-Tcf1 (human, mouse), clone C63D9 – Western and ChiP                      | Cell Signaling Technology             | Cat# 2203; RRID:AB_2199302         |
| anti-beta-catenin (dog, chicken, human, mouse, rat) - Western                  | BD Biosciences                        | Cat# 610153; RRID:AB_397554        |
| anti-actin (mouse, rat, human) - Western                                        | Abcam                                 | Cat# ab8226; RRID:AB_306371        |
| anti c-Myc (mouse, rat, human), clone Y69 - Western                            | Abcam                                 | Cat# ab32072; RRID:AB_731658       |
| anti-mouse IgG HRP conjugate - Western                                         | GE Healthcare                         | Cat# NXA931; RRID:AB_772209        |
| anti-human CD5, BV421 - Flow Cytometry                                         | BD Biosciences                        | Cat# 562646; RRID:AB_2737700       |
| anti-human CD7, PE-CF594 - Flow Cytometry                                      | BD Biosciences                        | Cat# 562541; RRID:AB_2737642       |
Supplemental Table 3 - DiffBind FDR/FC statistics for ATAC-seq analysis performed on sorted murine LSKs for Myc promoter (Figure 6A, left panel), NMe (red) and TMe (green) (Figure 6A, right panel).

| Myc locus ATAC N1IC vs Controls (Figure 6A) | log₂FC | p-value | FDR |
|--------------------------------------------|--------|---------|-----|
| Myc                                        |        |         |     |
| NMe                                        | 4.13   | 1.31E-11| 6.31E-10 |
| TMe                                        | 2.58   | 1.56E-11| 7.38E-10 |

Supplemental Table 4 - DiffBind FDR/FC statistics for TOBIAS footprinting and ATAC-seq analysis performed human CD34+ cord blood cells (Figure 6E).

| TOBIAS footprinting (Figure 6E) | N1IC_score | Controls_score | N1IC vs Controls log₂FC |
|---------------------------------|------------|----------------|--------------------------|
| IL2RA                           | 0.57087    | 0.00549        | 4.15537                  |

| IL2RA locus ATAC NOTCH1 vs Controls (Figure 6E) | log₂FC | p-value | FDR |
|-------------------------------------------------|--------|---------|-----|
| IL2RA                                           | 1.71   | 8.33E-18| 3.37E-17 |
References

1. Charbonneau H, Tonks NK, Walsh KA, Fischer EH. The leukocyte common antigen (CD45): a putative receptor-linked protein tyrosine phosphatase. *Proceedings of the National Academy of Sciences*. 1988;85(19):7182–7186.

2. Pritchard CEJ, Kroese LJ, Huijbers IJ. Direct Generation of Conditional Alleles Using CRISPR/Cas9 in Mouse Zygotes. *Site-Specific Recombinases: Methods and Protocols*. 2017;21:35–35.

3. Yang H, Wang H, Shivalila CS, et al. One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. *Cell*. 2013;154(6):1370–1379.

4. Heigwer F, Kerr G, Boutros M. E-CRISP: fast CRISPR target site identification. *Nat Methods*. 2014;11(2):122–123.

5. Wilson A, MacDonald HR, Radtke F. Notch 1–Deficient Common Lymphoid Precursors Adopt a B Cell Fate in the Thymus. *Journal of Experimental Medicine*. 2001;194(7):1003–1012.

6. Naviaux RK, Costanzi E, Haas M, Verma IM. The pCL vector system: rapid production of helper-free, high-titer, recombinant retroviruses. *Journal of Virology*. 1996;70(8):5701–5705.

7. Wendorff AA, Koch U, Wunderlich FT, et al. Hes1 Is a Critical but Context-Dependent Mediator of Canonical Notch Signaling in Lymphocyte Development and Transformation. *Immunity*. 2010;33(5):671–684.

8. Liu Z, Chen O, Wall JBJ, et al. Systematic comparison of 2A peptides for cloning multi-genes in a polycistronic vector. *Sci Rep.* 2017;7(1):2193.

9. Giambra V, Jenkins CR, Wang H, et al. NOTCH1 promotes T cell leukemia-initiating activity by RUNX-mediated regulation of PKC-θ and reactive oxygen species. *Nature Medicine*. 2012;18(11):1693–1698.

10. Barde I, Salmon P, Trono D. Production and Titration of Lentiviral Vectors. *Current Protocols in Neuroscience*. 2010;53(1):4.21.1–4.21.23.

11. Ruedl C, Khameneh HJ, Karjalainen K. Manipulation of immune system via immortal bone marrow stem cells. *International Immunology*. 2008;20(9):1211–1218.

12. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods*. 2013;10(12):1213–1218.

13. Corces MR, Trevino AE, Hamilton EG, et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. *Nature Methods*. 2017;14(10):959–962.

14. Belver L, Yang AY, Albero R, et al. GATA3-Controlled Nucleosome Eviction Drives MYC Enhancer Activity in T-cell Development and Leukemia. *Cancer Discov*. 2019;9(12):1774–1791.

15. Mellacheruvu D, Wright Z, Couzens AL, et al. The CRAPome: a contaminant repository for affinity purification–mass spectrometry data. *Nat Methods*. 2013;10(8):730–736.

16. Hagège H, Klous P, Braem C, et al. Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat Protoc.* 2007;2(7):1722–1733.

17. Al Ait L, Yamak Z, Morgenstern B. DIALIGN at GOBICS—multiple sequence alignment using various sources of external information. *Nucleic Acids Research*. 2013;41(W1):W3–W7.
18. Siepel A, Bejerano G, Pedersen JS, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.* 2005;15(8):1034–1050.
19. Letunic I, Bork P. Interactive tree of life (iTOl) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research.* 2016;44(W1):W242–W245.
20. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol.* 2019;37(8):907–915.
21. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological).* 1995;57(1):289–300.
22. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences.* 2005;102(43):15545–15550.
23. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv:1303.3997 [q-bio].* 2013;
24. Zhang Y, Liu T, Meyer CA, et al. Model-based Analysis of ChIP-Seq (MACS). *Genome Biology.* 2008;9(9):R137.
25. Didion JP, Martin M, Collins FS. Atropos: specific, sensitive, and speedy trimming of sequencing reads. *PeerJ.* 2017;5:e3720.
26. Parmentier R, Moussy A, Chantalat S, et al. Selective silencing rather than targeted activation of gene expression underlies fate choice in human hematopoietic stem cells. *bioRxiv.* 2020;2020.09.09.289751.
27. Bentsen M, Goymann P, Schultheis H, et al. ATAC-seq footprinting unravels kinetics of transcription factor binding during zygotic genome activation. *Nat Commun.* 2020;11(1):4267.
28. Fornes O, Castro-Mondragon JA, Khan A, et al. JASPAR 2020: update of the open-access database of transcription factor binding profiles. *Nucleic Acids Research.* 2020;48(D1):D87–D92.
29. Frankish A, Diekhans M, Ferreira A-M, et al. GENCODE reference annotation for the human and mouse genomes. *Nucleic Acids Research.* 2019;47(D1):D766–D773.
30. Stark R, Brown G. DiffBind: Differential binding analysis of ChIP-Seq peak data. 71.
31. Gu Z, Eils R, Schlesner M, Ishaque N. EnrichedHeatmap: an R/Bioconductor package for comprehensive visualization of genomic signal associations. *BMC Genomics.* 2018;19(1):234.
32. Gu Z. rGREAT: Client for GREAT Analysis. Bioconductor version: Release (3.12); 2020.
33. Durand NC, Shamim MS, Machol I, et al. Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments. *Cell Systems.* 2016;3(1):95–98.
34. Liu Y, Nanni L, Sungalee S, et al. Systematic inference and comparison of multi-scale chromatin sub-compartments connects spatial organization to cell phenotypes. *Nat Commun.* 2021;12(1):2439.
35. Shin H, Shi Y, Dai C, et al. TopDom: an efficient and deterministic method for identifying topological domains in genomes. *Nucleic Acids Res.* 2016;44(7):e70.
36. Ramirez F, Ryan DP, Grüning B, et al. deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Research.* 2016;44(W1):W160–W165.
37. Greenwald WW, Li H, Benaglio P, et al. Subtle changes in chromatin loop contact propensity are associated with differential gene regulation and expression. *Nature Communications.* 2019;10(1):1054.
38. Rao SSP, Huntley MH, Durand NC, et al. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. *Cell*. 2014;159(7):1665–1680.

39. Greenwald WW, Li H, Smith EN, et al. Pgltools: a genomic arithmetic tool suite for manipulation of Hi-C peak and other chromatin interaction data. *BMC Bioinformatics*. 2017;18(1):207.

40. Robinson JT, Turner D, Durand NC, et al. Juicebox.js Provides a Cloud-Based Visualization System for Hi-C Data. *Cell Systems*. 2018;6(2):256-258.e1.
Supplemental Fig. 1. Physiologic T cell development requires Notch1 and Tcf1 whereas Tcf7 is dispensable in Notch1-driven T-ALL.

A) Controls Notch1^{lox/lox} Mx1Cre Tcf7^{lox/lox} Mx1Cre Tcf7^{D/Δ} Mx1Cre Tcf7 deletion

B) Thymocytes number

CD4 and CD8 thymic T cells

Immature thymic T cells

B220 IgM Thymic B cells

10 weeks post-induction

CD45.2 [%]
Supplemental Fig. 1 – Physiologic T cell development requires Notch1 and Tcf1 whereas Tcf7 is dispensable in Notch1-driven T-ALL. A, Schematic representation of conditional deletion: Tcf7
\(^{lox/lox}\) or Notch1
\(^{lox/lox}\) (Controls, black, n=15), Notch1
\(^{lox/lox}\) Mx1Cre (orange, n=9) or Tcf7
\(^{lox/lox}\) Mx1Cre (blue, n=11) mice. Lower panel confirms genomic deletion in total bone marrow (BM) cells of the targeted Tcf7 locus by PCR. B, Quantification of absolute number of thymocytes in all experimental groups. Data are represented as mean ± SEM. Unpaired t-test, ****, p-value<0.0001. C-H, Phenotypic flow cytometric analysis of thymic cells 4-6 weeks post induction. Left panels (C,E,G) depict representative plots of thymocytes isolated from Controls, Notch1
\(^{Δ/Δ}\) Mx1Cre and Tcf7
\(^{Δ/Δ}\) Mx1Cre animals, with quantification of absolute numbers on the right for D, CD4\(^+\), CD8\(^+\) and CD4\(^+\) CD8\(^+\) T cells, F, immature double negative (DN, CD4\(^-\) CD8\(^-\)) thymic T cells and H, immature (B220\(^+\) IgM\(^-\)) and mature (B220\(^+\) IgM\(^+\)) thymic B cells. Data are represented as mean ±SEM. Unpaired t-test, ***, p-value<0.01; ****, p-value<0.0001. I, Total protein analyses for Tcf1, β-catenin and β-actin on isolated BM GFP\(^+\) cells and T-ALL cells of experimental samples: N1IC, N1IC Tcf7
\(^{Δ/Δ}\) and N1IC
\(^{Δ/Δ}\) β-catenin
\(^{Δ/Δ}\). J, Phenotypic flow cytometric analysis of CD45.2\(^+\) transplanted cells in peripheral blood of R26 N1IC
\(^{lox/+}\) Tcf7
\(^{lox/lox}\) (Controls, black, n=6), R26 N1IC
\(^{Δ/+}\) Mx1Cre (N1IC
\(^{Δ/+}\), red, n=8), R26 N1IC
\(^{Δ/+}\) Tcf7
\(^{Δ/Δ}\) Mx1Cre (N1IC Tcf7
\(^{Δ/Δ}\), blue, n=5) chimeras, 10 weeks after poly(I:C) induction. Data are represented as mean ± SEM. Unpaired t-test, *, p-value<0.05; **, p-value<0.01.
Supplemental Fig. 2. Differential gene expression analysis of bone marrow progenitors (LSKs) for Notch1- and Tcf1-dependency in T-ALL initiation.

A

Significantly regulated genes

B

GOBP repressed by \textit{N1IC} but not in \textit{N1IC Tcf7}^{\Delta/\Delta}

C

\textit{RV eGFP IRES eGFP (Controls)}

\textit{RV Tcf7 IRES eGFP (Tcf7)}

\textit{RV N1ICD IRES eGFP (N1ICD)}

D

\textbf{Controls}  \hspace{1cm}  \textbf{Tcf7}  \hspace{1cm}  \textbf{N1ICD}

E

CD4/CD8 cells in bone marrow

F

\textbf{Controls}  \hspace{1cm}  \textbf{Tcf7}  \hspace{1cm}  \textbf{N1ICD}

\textbf{48, 50 kDa}  \hspace{1cm}  \textbf{Tcf-1}

\textbf{42 kDa}  \hspace{1cm}  \textbf{\beta-Actin}
Supplemental Fig. 2 – Differential gene expression analysis of bone marrow progenitors (LSKs) for Notch1- and Tcf1-dependency in T-ALL initiation. A, Quantitative analysis of differentially expressed genes in comparisons between experimental groups: upregulated - green and downregulated - grey (FDR<0.05, -2>FC>2). B, Enrichment of biological pathways from Gene Ontology biological process (GOBP) collection in genes repressed by N1IC and Tcf7 from RNA-seq on LSK cells. Top 20 pathways are shown. p-values were calculated with Fisher’s exact test. C, Schematic representation of retroviral-based overexpression experiment: Bone marrow progenitors infected with MigR1 viruses overexpressing eGFP IRES eGFP (Controls, black, n=4), Tcf7 IRES eGFP (Tcf7, turquoise, n=5) or N1ICD IRES eGFP (N1ICD, red, n=3). Depicted timepoints show analysis performed at experimental endpoints (W - weeks). D, Phenotypic flow cytometric analysis of eGFP+ CD4 and CD8 T cells derived from bone marrow at endpoint. E, Quantification of absolute T cell numbers in bone marrow. Data are represented as mean ± SEM. One-way ANOVA, ***, p-value<0.001. F, Total protein analyses for Tcf1 and β-actin on isolated bone marrow cells of experimental groups: Controls (n=2), Tcf7 (n=4) and N1ICD (n=2).
Supplemental Fig. 3. Differential genetic accessibility analysis by ATAC-seq of bone marrow progenitors (LSKs).

A

Significantly regulated accessibility

B

GOBP of accessibility repressed by N1IC in Tcf7 dependent manner

C

TF binding motifs enriched in accessibility dependent on N1IC vs Controls

D

TF binding motifs enriched in accessibility dependent on N1IC Tcf7/ΔΔ vs N1IC
Supplemental Fig. 3 - Differential genetic accessibility analysis by ATAC-seq of bone marrow progenitors (LSKs). A, Quantitative analysis of differentially accessible genetic loci in comparisons between experimental groups: upregulated - green and downregulated - grey (FDR<0.01). B, Enrichment of biological pathways from Gene Ontology biological process (GOBP) collection regulated as decreased accessibility proximal to genes by N1IC and Tcf7 from ATAC-seq on LSK cells. Top 20 pathways are shown. p-values were calculated with Fisher’s exact test. C,D, Analysis of transcription factor (TF) binding motifs in CIS-BP at loci with increased (red) or decreased (grey) accessibility in comparison between ATAC-seq on: C, N1IC vs Controls and D, N1IC Tcf7 Δ/Δ vs N1IC LSKs.
Supplemental Fig. 4. Identification of 3D chromatin interactions by in situ Hi-C.

A

Sequenced read pairs

| Compartment | Controls | N1IC | N1IC Tcf7 Δ/Δ |
|-------------|----------|------|---------------|
| Normal paired | 31.20% | 29.77% | 30.40% |
| Chimeric paired | 56.20% | 58.34% | 57.90% |
| Chimeric ambiguous | 11.50% | 10.99% | 10.70% |
| Unmapped | 1.10% | 0.90% | 1.00% |

\[ \sum = 1,513,976,070 \]

Alignable (Normal + Chimeric Paired)

| Compartment | Controls | N1IC | N1IC Tcf7 Δ/Δ |
|-------------|----------|------|---------------|
| Unique reads | 80.40% | 78.00% | 81.10% |
| PCR duplicates | 18.90% | 21.30% | 18.20% |
| Optical duplicates | 0.70% | 0.70% | 0.70% |

\[ \sum = 1,433,194,564 \]

B

Hi-C contacts

| Compartment | Controls | N1IC | N1IC Tcf7 Δ/Δ |
|-------------|----------|------|---------------|
| 72.42% |
| 9.38% |
| 18.20% |
| 71.30% |
| 10.20% |
| 18.50% |

\[ \sum = 1,494,131,556 \]

C

Pearson correlation of contact maps

D

Rep 1 vs N1IC vs Controls

E

Compartment switching N1IC Tcf7 Δ/Δ vs Controls

F

Tspan9

Controls vs N1IC vs N1IC Tcf7 Δ/Δ
Supplemental Fig. 4 – Identification of 3D chromatin interactions by in situ Hi-C. A,B, Quality control analysis by Juicer depicting distribution of sequenced reads, alignable reads and generated Hi-C contacts for Controls, N1IC and N1IC Tcf7 Δ/Δ lineage Sca1+ cKit+ (CD117+) LSKs. C, Juicebox-generated contact matrices from chromosome 11: the whole chromosome, at 250 kb resolution (left panel) and chromatin loops at the resolution of 5 kb (right panel) shown for Controls LSKs vs bone marrow HSCs (GSE119347). The 1D regions corresponding to a contact matrix are indicated in the diagrams below and at right. The intensity of each pixel represents the normalized number of contacts between a pair of loci. Maximum intensity: 1349 (left), 14 (right). D, Hierarchical clustering of Pearson correlation analysis on the KR-normalized contact matrices at 1 Mb resolution. E, Chromatin sub-compartment rank switching between N1IC Tcf7 Δ/Δ versus Controls quantified on the left for inactive (0), active (7) and inactive (-7) sub-compartment (left panels). Association with gene expression differences (FDR<0.1) for genes within dynamic compartments is shown in right panels. Unpaired Wilcoxon test, ****, p-value<0.0001. F, Expression of Tspan9 measured as transcripts per million (TPM) from RNA-seq on LSKs. Data are represented as mean ±SEM. One-way ANOVA, *, p-value<0.05; **, p-value<0.01.
Supplemental Fig. 5. Dynamics of TADs regulated by Notch1 and Tcf1.

A) TADs overlapping with CTCF

B) Gene expression in TADs

C) Grap2

D) Controls

E) N1IC Tcf7 Δ/Δ
Supplemental Fig. 5 – Dynamics of TADs regulated by Notch1 and Tcf1. **A,** Quantification of the proportion of identified TADs with confirmed CTCF ChIP-seq signal at the boundaries shown for: Controls, N1IC and N1IC Tcf7 Δ/Δ analyzed at ±25 kb windows around TAD. **B,** TAD analysis with TopDom and TADCompare at 10 kb resolution focusing on TAD boundary changes between N1IC versus Controls (left panel) and N1IC vs N1IC Tcf7 Δ/Δ for association with significant gene expression differences (adjusted p-values<0.05). **C,** Expression of Grap2 measured as transcripts per million (TPM) from RNA-seq on LSKs. Data are represented as mean ±SEM. One-way ANOVA, *p*-value<0.05. **D,E,** Schematic depiction of Notch1- and Tcf1-dependent TADs regulating the expression of Grap2 gene. Hi-C matrix at 10 kb resolution is shown on top, below TopDom and TADCompare analyzed TADs are shown together with boundary score visualization. IGV profiles for CTCF and ATAC-seq are shown for **D,** Controls and **E,** N1IC Tcf7 Δ/Δ LSKs. Tracks were group-scaled. Representation of genetic loci is depicted below the tracks.
Supplemental Fig. 6. Analysis of chromatin loops regulated by Notch1 and Tcf1.

A. **N1IC-specific gene-anchored loops**

| Gene       | Controls vs N1IC | N1IC vs N1IC Tcf7+/− | Fold change (adj. p-value) | Fold change (adj. p-value) |
|------------|------------------|-----------------------|-----------------------------|-----------------------------|
| Pdgfrb     | 5.04E10          | 3.48E-08              | -2.06E23                    | 0.00343                     |
| Kras       | 1.47E10          | 0.00097               | 1.04E31                     | 0.0336                      |
| Abi1       | 1.22E34          | 0.02563               | -1.01E47                    | 0.8774                      |
| Nras       | 1.21E38          | 0.0178                | -1.00E41                    | 0.9766                      |
| Jaks       | 1.20E76          | 0.03535               | -1.23E26                    | 0.02983                     |

B. **Human phenotype N1IC Tcf7+/−-specific loop**

C. **N1IC Tcf7+/− specific gene-anchored loops**

D. Differential EPI strength correlation with gene expression analysis

E. **N1IC vs N1IC Tcf7+/− EPI**

F. **N1IC Tcf7+/− vs Controls EPI**

G. Differential EPI and compartment shift N1IC vs Controls

H. Differential EPI and compartment shift N1IC Tcf7+/− vs Controls

I. Differential EPI and compartment shift N1IC Tcf7+/− vs N1IC

J. **Gpr174**

K. Controls

L. **N1IC**
Supplemental Fig. 6 – Analysis of chromatin loops regulated by Notch1 and Tcf1. A, List of differentially expressed genes in N1IC-specific chromatin loops anchored in proximity to transcription start site (TSS). Analysis of expression by RNA-seq is shown with fold change and adjusted p-value for experimental comparisons. Values of adjusted p-value were calculated with Fisher’s exact test. B, Gene enrichment analysis for N1IC Tcf7 Δ/Δ-specific gene-associated loops from human phenotype catalogue. Top 10 pathways are shown from ontologies with differential gene expression FDR<0.05. p-values were calculated with Fisher’s exact test. C, List of differentially expressed genes in N1IC Tcf7 Δ/Δ-specific chromatin loops anchored in proximity to TSS. Analysis of expression by RNA-seq is shown with fold change and adjusted p-value for experimental comparisons. Values of adjusted p-value were calculated with Fisher’s exact test. D, Quantification of identified enhancer-promoter interactions (EPI) for Controls, N1IC and N1IC Tcf7 Δ/Δ LSKs. Consistency in direction of change between EPI strength and changes in gene expression is calculated. E,F, Gene-annotated scatterplot for differential enhancer-promoter interactions (EPI, x-axis) for differentially expressed genes (log2FC, y-axis) in comparison E, N1IC vs N1IC Tcf7 Δ/Δ and F, N1IC Tcf7 Δ/Δ vs Controls. Dots for genes with differential EPI >1 are colored. G-I, EPI analysis at 5 kb resolution with interaction strength >1 for genes with consistent change in the expression and association with sub-compartment changes genome-wide identified by Calder. J, Expression of Gpr174 measured as transcripts per million (TPM) from RNA-seq on LSK cells. Data are represented as mean ±SEM. One-way ANOVA, *, p-value<0.05. K, Representation of 5kb interacting regions between the indicated genomic coordinates color coded based on their EPI strength value (top). Identified enhancer (grey line) and promoter (yellow line) interaction is enlarged in top left corner. IGV CTCF, chromatin accessibility and H3K27ac profiles are shown for all experimental conditions (bottom). Tracks were group-scaled. Schematic representation of genetic loci is depicted below the profiles.
Supplemental Fig. 7. Regulation of Myc expression in bone marrow-derived LSKs and human T-ALL.

A

**Myc murine LSKs**

B

Controls

C

ATAC-seq DND-41 ENCLB946ZTU

D

α-TCF1 ChIP

E

Peptide Spectrum Match

% sequence coverage
Supplemental Fig. 7 – Regulation of Myc expression in bone marrow-derived LSKs. A, Expression of Myc measured as TPM (Transcripts per kilobase Million) with induced expression by N1IC and Tcf7 from RNA-seq on murine lineage- Sca1+ c-Kit+ (CD117+) LSK cells. Barplots from the left to right of each graph: Controls, N1IC, N1IC Tcf7 Δ/Δ. Unpaired t-test, *, p-value<0.05; **, p-value<0.01. B, In situ Hi-C contact matrices at 10 kb resolution depicting identified chromatin interactions of Myc promoter for Controls and N1IC. Tracks below, ChIP-seq for H3K27ac with identified chromatin loops IGV profiles shown for all experimental groups. Tracks were group-scaled. C, IGV chromatin accessibility profiles for NMe and TMe in human DND-41 T-ALL cells (ENCLB946ZTU). Tracks were group-scaled. D, ChIP-qPCR analysis for TCF1 binding in human DND-41 T-ALL cells (n=3) and IgG Controls (n=2) at NMe (red) and TMe (green). Data are represented as mean ±SEM. E, Reverse ChIP identification of potential TMe binding factors. TMe DNA bait was incubated in the presence of nuclear extracts from DND-41 cells and recovered peptides were analyzed by mass spectrometry.
Supplemental Fig. 8. Physiological analysis of hematopoietic lineages in mice with genomic deletion of Tcf1-regulated Myc enhancer (TMe).

A

Hematopoietic progenitors

B

Bone marrow cellularity

Thymic cellularity

C

Lineage

cKit Sca1

D

Hematopoietic lineages

E

Hematopoietic progenitors

F

Live CD71 Ter119

CD11b Gr1

B220 IgM

G

Controls TMe+/- TMe-/

H

Controls TMe+/- TMe-/

CD44 CD4 CD71

CD25
Supplemental Fig. 8 – Physiological analysis of hematopoietic lineages in mice with genomic deletion of Tcf1-regulated Myc enhancer (TMe). A, Schematic representation of the phenotypic analysis of bone marrow (BM) and thymus, using CRISPR-edited transgenic mouse models: Controls (black, n=3), Tcf1-regulated Myc enhancer +/- (TMe +/-, orange, n=4) or Tcf1-regulated Myc enhancer -/- (TMe -/-, green, n=7) mice. B, Quantification of the absolute cellularity in BM (left panel) and thymi (right panel) of all experimental groups. C, Phenotypic flow cytometric analysis of BM progenitors. Panels depict representative plots of gating strategy applied for all experimental groups, shown for Controls. Parental populations are shown above the plots. D, Absolute numbers of the BM progenitors from flow cytometric-based analysis in panel C. Data are represented as mean ± SEM. Unpaired t-test, *, p-value<0.05. E, Absolute numbers of hematopoietic lineages in BM from flow cytometric-based analysis in panel F. Data are represented as mean ± SEM. F, Phenotypic flow cytometric analysis of hematopoietic lineages in BM. Panels depict representative plots of gating strategy applied to all experimental groups, shown for Controls. Parental populations are shown above the plots. G,H, Phenotypic flow cytometric analysis of thymocytes. Left panels depict representative plots of gating strategy for all experimental groups, shown for Controls, with absolute numbers in the right panels for G, CD4+, CD8+ and CD4+ CD8+ T cells and H, immature double negative (DN, CD4- CD8-) thymic T cells gated from hematopoietic lineage population. Data are represented as mean ± SEM.