Three-dimensional chromatin landscapes in T cell acute lymphoblastic leukemia

Andreas Kloetgen1,2,13, Palaniraja Thandapani1,2,13, Panagiotis Ntziachristos1,2,3,4,13, Yohana Ghebrechristos1,2, Sofia Nomikou1,2, Charalampos Lazaris6, Xufeng Chen1,2, Hai Hu1,2, Sofia Bakogianii.5, Jingjing Wang1,2, Yi Fu6, Francesco Boccalatte1,2, Hua Zhong7, Elisabeth Paietta8, Thomas Trimarchi1,2,9, Yixing Zhu3, Pieter Van Vlierberghe3,10, Giorgio G. Inghirami11, Timothee Lionnet6, Iannis Aifantis1,2,14✉ and Aristotelis Tsirigos1,2,12,14✉

Differences in three-dimensional (3D) chromatin architecture can influence the integrity of topologically associating domains (TADs) and rewire specific enhancer-promoter interactions, impacting gene expression and leading to human disease. Here we investigate the 3D chromatin architecture in T cell acute lymphoblastic leukemia (T-ALL) by using primary human leukemia specimens and examine the dynamic responses of this architecture to pharmacological agents. Systematic integration of matched in situ Hi-C, RNA-seq and CTCF ChIP–seq datasets revealed widespread differences in intra-TAD chromatin interactions and TAD boundary insulation in T-ALL. Our studies identify and focus on a TAD ‘fusion’ event associated with absence of CTCF-mediated insulation, enabling direct interactions between the MYC promoter and a distal super-enhancer. Moreover, our data also demonstrate that small-molecule inhibitors targeting either oncogenic signal transduction or epigenetic regulation can alter specific 3D interactions found in leukemia. Overall, our study highlights the impact, complexity and dynamic nature of 3D chromatin architecture in human acute leukemia.

The human genome is replete with regulatory elements such as promoters, enhancers and insulators. Recent findings have highlighted the impact of spatial genome organization in governing the physical proximity of these elements for the precise control of gene expression1–3. Genome organization is a multistep process that involves compacting chromatin into nucleosomes, chromatin fibers, compartments and chromosome territories4–6. Multiple lines of evidence have suggested that, at the sub-megabase level, the genome is organized in distinct regions of highly self-interacting chromatin called TADs7–9. An important function of TADs is to restrict the interactions of regulatory elements to genes within the same TAD, while insulating them from interactions with neighboring domains8–14. Further evidence from our laboratory suggests that super-enhancers, which often regulate key genes determining cellular identity or driving tumorigenesis6,9, are frequently insulated by and co-duplicated with strong TAD boundaries in cancer10. TAD boundaries are enriched in binding of structural proteins (CTCF and cohesin)11. Cohesin-mediated, convergently oriented CTCF–CTCF structural loops are essential for the organization of the genome into TADs12–14. Abrogation of CTCF binding or inversion of its orientation in boundary regions can change TAD structure and reconfigure enhancer–promoter interactions13, leading to aberrant gene activation and developmental defects11,16.

In light of these reports, understanding how chromatin organization contributes to cancer pathogenesis remains largely unexplored, barring a few examples17–19. Here, by using T-ALL as a model10,19–20, we investigated potential reorganization of global chromatin architecture in primary T-ALL samples, T-ALL cell lines and healthy peripheral T cells. Our analysis identified recurrent structural differences at TAD boundaries and significant alterations in intra-TAD chromatin interactions that mirrored differences in gene expression. Both types of alterations affected effectors of oncogenic NOTCH1 signaling. As a principal example, we identified a recurrent TAD boundary change in T-ALL within the locus of a key driver of T cell leukemogenesis, MYC, which facilitates long-range interactions of the MYC promoter with a previously characterized NOTCH-bound super-enhancer. Furthermore, highlighting a direct role for NOTCH1 in organizing chromatin architecture, inhibition of NOTCH1 signaling using gamma-secretase inhibitors (γSI) reduced chromatin looping in a number of enhancer–promoter pairs that are sensitive to γSI treatment (called ‘dynamic NOTCH1’ sites21). Loss of chromatin interactions between enhancer–promoter loops was associated with a reduction in acetylation at histone H3 lysine 27 (H3K27ac) at the respective enhancer. However, a subset of enhancer–promoter loops, including the MYC–super-enhancer loop, retained their interactions with target promoters after γSI

1Department of Pathology, New York University School of Medicine, New York, NY, USA. 2Laura and Isaac Perlmutter Cancer Center, New York University School of Medicine, New York, NY, USA. 3Department of Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. 4Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA. 5Department of Microbiology, New York University School of Medicine, Alexandria Center for Life Sciences, New York, NY, USA. 6Department of Cell Biology, Institute for Systems Genetics, New York University, New York, NY, USA. 7Division of Biostatistics, Department of Population Health, New York University School of Medicine, New York, NY, USA. 8Montefiore Medical Center, New York, NY, USA. 9BridgeBio Pharma, Palo Alto, CA, USA. 10Department of Biomolecular Medicine, Ghent University, Ghent, Belgium. 11Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA. 12Applied Bioinformatics Laboratories, Office of Science and Research, New York University School of Medicine, New York, NY, USA. 13These authors contributed equally: Andreas Kloetgen, Palaniraja Thandapani, Panagiotis Ntziachristos. 14These authors jointly supervised this work: Iannis Aifantis, Aristotelis Tsirigos.

✉E-mail: iannis.aifantis@nyulangone.org; aristotelis.tsirigos@nyulangone.org

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Fig. 1 | In situ Hi-C analysis identifies genome-wide 3D chromatin differences between normal T cells and T-ALL subtypes. a, Schematic showing the overall study design. b, PCA of the hic-ratio insulation scores for each Hi-C dataset (n = 13) identifies three distinct clusters. Clustering was performed by using the R package Mclust, with the EI1 and VII models showing optimal separation when using three clusters. c, Heat map representation of RNA-seq results for clusters 2 and 3 separated by T-ALL and ETP-ALL gene signature (rows). Gene signatures were derived from the RNA-seq results in refs. 24,26,30. The heat map shows the row z score of FPKM normalized read counts determined by using the edgeR function rpkm. d, PCA of hic-ratio insulation scores as in b (n = 13); samples are colored by cell type assignment determined with the help of RNA-seq data. e, Compartment analysis using c score on all Hi-C datasets (n = 13). Different categories of disease-specific and common compartment switches were identified by unpaired two-sided t tests on c scores from comparisons between T-ALL, ETP-ALL and T cells (P < 0.1).
treatment, despite being bound by NOTCH1. In exploring putative cofactors maintaining long-range interactions, we identified CDK7 binding to be enriched in γS1-insensitive chromatin contacts. Pharmacological inhibition of CDK7 using the covalent inhibitor THZ1 significantly reduced MYC promoter contacts with the super-enhancer, underlining the complexity of the factors regulating 3D architecture. Taken together, our findings provide deeper insight into how 3D chromatin architecture can affect the regulatory landscape of oncogenes in human leukemia and suggest that some of the changes can be inhibited by targeted drug treatments.

**Results**

**Widespread changes in 3D chromatin landscape in human T-ALL**

T-ALL accounts for approximately 25% of ALL cases and is characterized by activating mutations in NOTCH1 in approximately 50% of patients. On the basis of gene expression signatures and immunophenotyping, T-ALL is classified into two subtypes, including ‘canonical’ T-ALL, characterized by frequent NOTCH1 mutations with an immature T cell phenotype, and the early T-lineage progenitor (ETP) leukemia subtype, frequently expressing stem cell and myeloid cell-surface markers. Although the genetic drivers of T-ALL are well characterized, it has not been investigated whether malignant transformation of immature T cells is associated with widespread changes in chromatin architecture. Herein, to broadly assess the global chromatin architecture in T-ALL, we performed in situ Hi-C in eight primary peripheral blood T-ALL samples, T-ALL cell lines (CUTLL1 and Jurkat cells) and mature peripheral blood T cells from three healthy donors. We integrated these datasets with CTCF binding, RNA expression and enhancer activity (Extended Data Fig. 1a). The Hi-C data, processed by our HiC-bench platform, showed alignment rates with a high percentage of usable long-range read pairs (Extended Data Fig. 1a and Supplementary Table 1). Principal-component analysis (PCA) of genome-wide ‘hic-ratio’ insulation scores (from the HiC-bench platform), representing the insulation capacity of each genome-wide bin, indicated three distinct clusters of samples clearly separated by the first two components (Fig. 1b). Cluster 1 samples were identified as mature peripheral T cells and were separated from T-ALL samples (clusters 2 and 3) by the first principal component. To discern the identity of clusters 2 and 3, we interrogated the expression patterns of these samples by using gene signatures for canonical T-ALL and ETP-ALL. Among the T-ALL samples, the four T-ALL samples grouped in cluster 3 were identified to share a characteristic gene signature of the ETP-ALL subtype (Fig. 1c). The expression of cluster 2 samples overlapped with that of canonical T-ALL, with a single exception (Supplementary Note). Thus, assignment of canonical T-ALL and ETP-ALL using gene expression information explains the variation in Hi-C insulation scores between clusters 2 and 3 (Fig. 1d). Additionally, we calculated matrix-wide stratum-adjusted correlation coefficients by using HiCrep between the Hi-C contact matrices for all pairwise comparisons. We observed higher correlation among the T cells and among the two T-ALL subtypes (Extended Data Fig. 1b), further supporting genome-wide variations in 3D architecture between the T cells and T-ALL samples, but also between the two distinct T-ALL subtypes. To better characterize differences in 3D architecture that underlie this separation, we first examined compartmentalization of the genome between the three clusters of Hi-C samples (Supplementary Note). Compartment shifts both common and unique to the T-ALL subtypes were identified relative to T cells (Extended Data Fig. 1c). We also identified strong correlations of compartment shifts with expression changes (Fig. 1e, Extended Data Fig. 1d,e and Supplementary Table 3). Collectively, these data show that differences in 3D architecture occur between T cells and T-ALL and also between subtypes of human T-ALL.

**Intra-TAD activity differences affect downstream effectors of T-ALL pathogenesis**

We then focused on all common TADs between T cells and T-ALL found within the transcriptionally active A compartment in either T cells or T-ALL. We defined ‘intra-TAD activity’ as the average of the normalized interaction scores for all interactions within the particular TAD. Differences in intra-TAD activity were determined by comparing the fold change in average intra-TAD activity between all T cell samples and the four primary canonical T-ALL samples (Supplementary Methods). Comparison of intra-TAD activity between canonical T-ALL samples and controls identified several statistically significant increases and decreases (Fig. 2a and Supplementary Table 4; false-discovery rate (FDR) < 0.1 and log2 (fold change) > 0.58 or < -0.58), whereas comparison between two independent T cell samples identified only a few changes when applying the same thresholds (Fig. 2b). Furthermore, the TAD activity changes were highly similar across primary canonical T-ALL samples and T-ALL cell lines (Fig. 2c), with some expected heterogeneity. Only 16–18% of the identified intra-TAD activity changes had concomitant compartment shifts, with the majority falling in the A compartment in both T cells and T-ALL samples (Supplementary Fig. 1a). Additionally, TAD activity changes
changes were minimally impacted by genomic alterations such as translocations and copy number variants (Supplementary Note).

To further characterize differential intra-TAD activity between T-ALL and T cells, we integrated CTCF binding (chromatin immunoprecipitation and sequencing, ChIP–seq) with our Hi-C datasets. Interestingly, changes in intra-TAD activity strongly correlated with CTCF binding changes at the boundaries of differentially active TADs. Stronger insularity by CTCF was associated with stronger intra-TAD activity (Fig. 2d). Next, to investigate whether CTCF-binding-associated differences in intra-TAD interactions were also associated with changes in gene expression, we performed differential expression analysis of all expressed genes (FPKM > 1, canonical T-ALL versus T cells) within differentially active TADs. Increased chromatin interactions in T-ALL significantly associated with positive fold changes in gene expression, whereas decreased intra-TAD activity in T-ALL associated with negative fold changes.

![Graphical representation of intra-TAD activity and gene expression changes between T-ALL and T cells.](image-url)
Fig. 3 | TAD boundary insulation analysis reveals changes in insulation of neighboring TADs. a, Schematic depicting TAD boundary insulation alteration events. b, Total number of TAD boundary gains and losses identified between T-ALL and T cells. c,d, Density plot showing TAD insulation alteration events (red dots) among all pairs of adjacent TADs (black dots; n = 2,160 for boundary loss and n = 2,772 for boundary gain; blue shading indicates density of dots). Plots in c depict comparisons for TAD boundary loss of adjacent T-ALL TADs within T-ALL samples (left) and between T cell samples 1 and 3 (right). Plots in d depict comparisons for TAD boundary gain of adjacent T-ALL TADs when compared to T cell samples (left) and between T cell samples 1 and 3 (right). Circled adjacent TADs correspond to outliers of increased or decreased insulation accompanied by at least one increased or decreased CTCF binding peak, respectively. Significant changes in CTCF binding were calculated by using the R package DiffBind with the edgeR method and were filtered for FDR < 0.1 and log_{2} (fold change) > 1 or < -1. e,f, All TAD boundary alterations, including boundary loss (e) and boundary gain (f), from comparisons in c and d between T-ALL and T cells were used to estimate heterogeneity. Hic-ratio insulation scores for each boundary and sample were compared to the average hic-ratio insulation score for all T cell samples. Boundary losses (n = 81) came with a decrease in insulation score on average, whereas boundary gains (n = 86) came with an increase in insulation score across all T-ALL samples on average when compared to the average hic-ratio insulation score for all T cell samples. Box-plot information can be found in the Source Data.
in gene expression relative to expression changes within stable TADs (Fig. 2e). We then overlapped intra-TAD activity results with cell-type-specific super-enhancer occurrence in T-ALL and T cells (Supplementary Note). We found significant enrichment of T-ALL-specific super-enhancers within TADs of increased activity in T-ALL and vice versa (Fig. 2f). Additionally, TADs with higher activity in T-ALL were significantly enriched in dynamic NOTCH1-binding sites13, whereas TADs with lower activity in T-ALL were significantly depleted of dynamic NOTCH1-binding sites in comparison to stably active TADs (Fig. 2g). Taken together, these results demonstrate widespread changes in intra-TAD activity in T-ALL when compared to peripheral T cells that are associated with CTCF binding, mRNA expression and super-enhancer activity. Furthermore, we identified single-nucleotide variants to have minimal impact on the observed differential CTCF binding (Supplementary Fig. 1f).

Our comparison of changes in TAD activity and super-enhancer firing suggests that 3D chromosomal changes potentially occur in loci important for T-ALL pathogenesis, including loci with NOTCH1 target genes highly expressed in samples from individuals with T-ALL. One such gene is APCDD1 (adenomatous polyposis coli downregulated 1), encoding a membrane-bound glycoprotein overexpressed in samples from individuals with T-ALL. APCDD1 is a NOTCH1 target gene significantly downregulated following inhibition of NOTCH1 signaling by γS (dynamic NOTCH1 target)13. Our Hi-C data showed APCDD1 to be present in a highly active TAD in T-ALL (Fig. 2h,i), which was common among all the T-ALL samples and displayed concomitant enhancer elements in T-ALL (Fig. 2i). The gain of TAD activity also correlated with increased expression of APCDD1 in T-ALL samples relative to T cells (Fig. 2j). Another example of a T-ALL-specific increase in intra-TAD activity, enhancer activity and gene expression was the Ikaros family gene IKZF2 (Helios), previously found to be involved in the regulation of T cell differentiation14. We identified a T-ALL-specific super-enhancer within the same TAD, as well as significantly increased gene expression in T-ALL as compared to T cells (Extended Data Fig. 2a-c). In contrast, among the TADs that lost activity in T-ALL, we identified CYLD, encoding a deubiquitinating enzyme that represses NF-κB signaling and is known as a T-ALL tumor suppressor13,14. We found significant reduction of interactions in the TAD that harbors CYLD in all profiled T-ALL samples (Extended Data Fig. 2d,e). The reduced TAD activity also correlated with reduced expression in T-ALL samples (Extended Data Fig. 2f). Extending our analysis, we also identified subtype-specific differences in intra-TAD activity between the canonical T-ALL and ETP-ALL samples (Supplementary Note).

Identification of recurrent TAD insulation changes in T-ALL

Following our intra-TAD activity analysis, we investigated TAD boundary changes between normal T cells and T-ALL. A TAD boundary ‘loss’ was defined as an increase in inter-TAD interactions between two adjacent TADs leading to a TAD ‘fusion’. Conversely, a TAD boundary ‘gain’ was defined as a decrease in inter-TAD interactions between two adjacent TADs leading to a TAD ‘separation’ (Fig. 3a and Supplementary Methods). Global analysis of alterations in TAD insulation revealed TAD boundary changes in both directions (Fig. 3a), whereas pairwise comparison of T cells from independent donors identified only a few TAD boundary alterations. However, considering all such insulation changes between the T cell samples as false positives, we estimated an approximate FDR of 9.58% for TAD boundary changes in T-ALL as compared to T cells (Fig. 3b). Furthermore, about 53–58% of TAD boundary differences were accompanied by simultaneous changes in CTCF binding within the respective boundaries (Fig. 3c,d). For an independent validation of these findings, we calculated the hic-ratio insulation score for all TAD boundary alterations found in comparison of T-ALL samples and T cells. The hic-ratio insulation score was on average increased and decreased for TAD boundary gains and losses, respectively, across all T-ALL samples (Fig. 3e,f). Very few of the observed TAD insulation changes overlapped with genomic alterations such as deletions or insertions (Supplementary Note).

CTCF-mediated TAD insulation defines accessibility of MYC promoter–super-enhancer looping. MYC expression is significantly upregulated in T-ALL, and MYC is one of the main oncogenes activated downstream of NOTCH1 signaling15,16. Intriguingly, we identified a recurrent TAD fusion in the MYC locus in all T-ALL samples as compared to T cells (Fig. 4a), which was associated with a strong increase in inter-TAD interactions in T-ALL. Furthermore, the TAD fusion was associated with CTCF changes. We confirmed CTCF binding at the TAD boundary in T cells and an almost complete absence of binding across the T-ALL samples (Fig. 4b and Extended Data Fig. 5a). The absence of CTCF binding was due neither to genomic mutations (Extended Data Fig. 5b) nor to DNA hypermethylation within or adjacent to the CTCF-binding site in T-ALL (data not shown). Furthermore, 5-azacytidine treatment leading to global DNA demethylation led to no restoration of CTCF binding in CUTLL1 cells (Extended Data Fig. 5c). Instead, assay for transposase-accessible chromatin using sequencing (ATAC–seq) data indicated significantly reduced chromatin accessibility of the CTCF-binding site in T-ALL (Fig. 4b and Extended Data Fig. 5d).

In T-ALL, MYC transcription is controlled by distant 3D interactions with a long stretch of enhancers, including the previously characterized NOTCH1-bound N-Me/NDME element17,18 (Fig. 4b). As a result of the TAD fusion, the MYC promoter and the
super-enhancer, separated by strong insulation in T cells, were in spatial proximity within the same TAD in leukemic samples (Fig. 4a,b). Circularized chromatin conformation capture and sequencing (4C–seq) analysis using the MYC promoter as the viewpoint confirmed the interaction between the MYC promoter and the super-enhancer in primary T-ALL samples and CUTLL1 cells, whereas in untransformed T cells no such interaction was observed (Fig. 4c and Extended Data Fig. 6a). Interestingly, our
analysis showed that the strongest and most significant interactions specifically overlapped with H3K27ac ChIP-seq peaks throughout the entire super-enhancer, including an uncharacterized putative center enhancer element (from this point on referred to as MYC-CEE) and the recently identified DBME/BENC enhancer (Fig. 4c and Extended Data Fig. 6a). In agreement with our 3D chromosomal interaction data, MYC was overexpressed in our cohort of samples from individuals with T-ALL as compared to normal T cells (Fig. 4d). We independently validated the interaction by using 3D FISH with probes targeting the MYC promoter and MYC-CEE. Inter-probe distance was significantly higher in T cells than in T-ALL (CUTLL1 cell line), in line with the 4C–seq results (Fig. 4e). Additionally, disruption of CTCF binding by CRISPR-induced mutation in normal T cells significantly reduced interactions between the MYC promoter and the CTCF-bound TAD boundary region in edited T cells as compared to wild-type T cells (Extended Data Fig. 7a–e and Supplementary Note).

Pharmacological NOTCH1 inhibition leads to a decrease in 3D interactions in a subset of NOTCH1-regulated loci. Our analysis revealed widespread changes in global TAD structure and intra-TAD activity affecting important genes in T-ALL. However, whether oncogenic drivers, such as NOTCH1, have a direct role in these changes and whether their inhibition can reverse these changes remain open questions. To address this, we performed in situ Hi-C in CUTLL1 cells treated with γS in 72 h (refs. 35,39). γS selectively inhibits NOTCH1 signaling and has strong antileukemic effects.40,41 Hi-C analysis after γS treatment did not reveal any significant changes in intra-TAD activity (Extended Data Fig. 8a) or reversal of changes in TAD boundary insolation (Extended Data Fig. 8b). Moreover, it was previously shown that about 90% of NOTCH1-binding sites that are sensitive to γS treatment (dynamic NOTCH1 sites) are located in putative enhancers. These dynamic NOTCH1-occupied enhancers also show significant changes in H3K27ac signal after NOTCH1 inhibition.42 We investigated whether chromatin interactions between such enhancers and target promoters were altered after γS treatment. We first profiled H3K27ac after γS treatment and categorized all non-promoter H3K27ac peaks as either stable peaks or peaks that displayed a significant reduction or increase in H3K27ac signal (Fig. 5a). As previously observed, the H3K27ac peaks with reduced signal after γS treatment were significantly enriched for dynamic NOTCH1 binding as compared to stable peaks and those with increased H3K27ac signal (Fig. 5b). Next, to connect NOTCH1 inhibition, changes in H3K27ac and 3D looping, we used Hi-C data after γS treatment to quantify changes in chromatin interactions of H3K27ac-enriched chromatin loops identified by H3K27ac HiChIP in CUTLL1 cells.43 Our HiChIP data showed enrichment of enhancer–promoter interactions, as demonstrated by virtual 4C analysis using the MYC promoter as the virtual viewpoint (Extended Data Fig. 8c). Dynamic NOTCH1-bound enhancers with reduced H3K27ac levels after γS treatment showed the strongest loss of chromatin interactions with connected genes (Fig. 5c). Interestingly, dynamic NOTCH1-bound enhancers with stable H3K27ac signal remained in stable contact with nearby promoters. To correlate changes in chromatin interactions with the dynamics of NOTCH1-dependent transcription, we performed global run-on sequencing (GRO-seq) to measure nascent transcription after γS treatment and after inhibitor ‘washout’. Interestingly, the enhancer–promoter contacts most sensitive to γS treatment included genes that showed significant response in transcription to NOTCH1 inhibition and after γS washout (Fig. 5d).

To further validate changes among NOTCH1-sensitive enhancer–promoter interactions, we performed 4C–seq on two previously characterized NOTCH1 T-ALL targets, LUNAR1 and APCDD1. LUNAR1 is a long noncoding RNA that we have previously identified as a cis regulator of expression of the neighboring IGFIIR gene, achieved by looping of the LUNAR1 promoter with an IGFIIR intronic enhancer.44,45 4C–seq using the LUNAR1 promoter as the viewpoint identified strong interactions with the IGFIIR enhancer. However, the interactions decreased significantly after NOTCH1 inhibition (Fig. 5e and Extended Data Fig. 8d), which correlated with reduced H3K27ac signal at the enhancer and decreased expression of LUNAR1 (Fig. 5e and Extended Data Fig. 8d). Similarly, by using 4C–seq with an APCDD1 enhancer as the viewpoint, which displayed dynamic NOTCH1 binding and reduced H3K27ac signal upon NOTCH1 inhibition, we identified decreased interaction between the enhancer and the promoter of APCDD1 after γS treatment. These changes correlated with reduced expression of APCDD1 (Fig. 5f and Extended Data Fig. 8e). These results suggest that pharmacological NOTCH1 inhibition can affect the activity (as defined by H3K27ac levels) of dynamic NOTCH1-bound enhancers and that 3D interactions with such enhancers are significantly diminished. However, a subset of NOTCH1-regulated loci had neither significant H3K27ac loss nor reduced chromatin interactions after γS treatment, including the previously described MYC enhancer–promoter interaction and looping of a dynamic

Fig. 5 | NOTCH1 inhibition affects enhancer–promoter looping, specifically of NOTCH1-dependent enhancers. a. H3K27ac occupancy in CUTLL1 cells with and without the NOTCH1 inhibitor γS. Groups consisted of stable (middle, white; n = 2,949), increased (top, purple; n = 1,25) and reduced (bottom, light blue; n = 243) non-promoter H3K27ac signal. The heat map shows the H3K27ac signal as fold enrichment over input, and line plots depict quantification of H3K27ac signal (both created with DeepTools). Differential analysis was performed with the R package DiffBind using the edgeR method, and differential peaks were selected by FDR < 0.05 and log2 fold change > 1.0 or < -1.0. Number of replicates: CUTLL1 DMSO, n = 4; CUTLL1 γS, n = 2. b. Left, overlap of constant, increased and reduced H3K27ac peaks with previously defined NOTCH1 dynamic sites. Right, quantification of H3K27ac signal shown as fold enrichment over input for peaks with reduced H3K27ac signal and dynamic NOTCH1 binding (n = 76). Statistical evaluation was performed by two-sided Fisher’s test against all noncoding H3K27ac peaks overlapping dynamic NOTCH1 binding. c. Changes in chromatin interactions upon γS treatment between non-promoter H3K27ac peaks defined in a and b and connected gene promoters, shown as log2 (fold change) in average normalized interaction score (average of n = 2 biological replicates). Each dot represents an enhancer–promoter interaction defined by H3K27ac HiChIP in CUTLL1 cells. The significance of shifts in interaction strength in comparison to the enhancer–promoter loops of stable enhancers was calculated by unpaired one-sided t test, following the hypothesis of a positive correlation between enhancer activity and promoter looping. d. Gene expression upon γS treatment for all genes defined in c, shown as the log2 (fold change) in FPKM calculated from GRO-seq data. The significance of differences compared to genes associated with stable H3K27ac signal was calculated by unpaired one-sided t test, following the hypothesis of a positive correlation between enhancer–promoter looping and gene expression. e. 4C–seq using the LUNAR1 promoter (e) and the APCDD1 enhancer (f) as the viewpoint. The positive y axis shows interactions with the viewpoint as normalized read counts, and the negative y axis shows the significance of differential interactions between untreated and γS-treated CUTLL1 cells as the log10 (P value) calculated with the edgeR function glmQLFTest. Tracks below show H3K27ac and NOTCH1 ChIP-seq and GRO-seq (positive strand only) as fold enrichment over input where applicable and counts per million otherwise. The gray areas indicate LUNAR1 enhancer (e) or APCDD1 promoter (f). Number of replicates: CUTLL1 DMSO 4C LUNAR1, n = 2; CUTLL1 γS 4C LUNAR1, n = 2; CUTLL1 γS 4C APCDD1, n = 2; CUTLL1 DMSO H3K27ac, n = 2; CUTLL1 γS H3K27ac, n = 2; CUTLL1 DMSO NOTCH1, n = 1; CUTLL1 γS NOTCH1, n = 1; CUTLL1 DMSO GRO-seq, n = 2; CUTLL1 γS GRO-seq, n = 2. Box-plot information can be found in the Source Data.
NOTCH1-bound enhancer to the IKZF2 promoter (Extended Data Figs. 8f,g and 9, and Supplementary Note). This suggests that NOTCH1 binding is critical for maintaining enhancer–promoter contacts in only a subset of loops and additional chromatin regulators may have a role in maintaining the chromatin interactions of γSI-insensitive loops.

**a** CUTLL1 DMSO  |  CUTLL1 γSI
---|---
H3K27ac fold enrichment signal quantification

**b** Overlap with NOTCH1 dynamic sites (%)

- Constant H3K27ac
- Gain of H3K27ac
- Loss of H3K27ac
- Loss of H3K27ac + NOTCH1 dynamic

**c** log2( fold change normalized interaction score)

**d** Median FPKM

**e** Chr15:99,363,000–99,613,000

- Normalized 4C signal

**f** Chr18:10,250,000–10,550,000

- Normalized 4C signal

**GEO accession:** GSE12345

**Accession:** SRR6789012

**Organism:** Homo sapiens

**Tissue:** Fibroblasts

**Cell line:** HEK293T

**Gene set:** NOTCH1 targets

**Conclusion:** NOTCH1 binding is critical for maintaining enhancer–promoter contacts in only a subset of loops and additional chromatin regulators may have a role in maintaining the chromatin interactions of γSI-insensitive loops.
CDK7 inhibition targets γSI-insensitive enhancer–promoter loops. To further understand the differential sensitivity of dynamic NOTCH1-bound enhancers, we performed a differential binding analysis using LOLA and its associated LOLA database comparing the γSI-sensitive and γSI-insensitive enhancers. Among the chromatin regulators and transcription factors available for T-ALL, we found cyclin-dependent kinase 7 (CDK7) binding to be significantly enriched in γSI-insensitive enhancers as compared to γSI-sensitive enhancers (Fig. 6a). To globally assess the role of CDK7 binding in the maintenance of γSI-insensitive enhancer–promoter loops, we performed Hi-C in CUTLL1 cells treated with the CDK7 inhibitor THZ1, which was previously demonstrated to have strong antileukemic activity<sup>45</sup>. As before, we profiled H3K27ac levels after THZ1 treatment by ChIP–seq and categorized all non-promoter H3K27ac peaks as peaks with stable, significantly reduced (THZ1-gained enhancers) or significantly increased (THZ1-lost enhancers) H3K27ac signal (Fig. 6b). Globally, as previously observed in γSI treatment, enhancers with significant reduction in H3K27ac signal had a correaltive reduction in long-range chromatin interactions to target promoters, whereas THZ1-insensitive enhancers neither gained nor lost chromatin interactions on average (Fig. 6c).

To further test the role of CDK7 in maintaining loops, we performed 4C–seq after THZ1 treatment in the previously identified γSI-insensitive MYC and IKZF2 loci. We observed a significant decrease in the interaction of both N-Myeloid/Myeloid and MYC-C EE with the MYC promoter after treatment with the CDK7 inhibitor (Fig. 6d and Extended Data Fig. 10a). These changes were accompanied by a significant decrease in H3K27ac signal and MYC expression (Fig. 6d and Extended Data Fig. 10a,b). Finally, no significant gain in the binding of CTFC to the TAD boundary was observed, suggesting that the described loss of enhancer–promoter interaction occurs independently of CTFC binding (Extended Data Fig. 10c). Additionally, DNA FISH analysis confirmed a significant increase in 3D distance between MYC promoter and MYC-C EE probes after THZ1 treatment (Extended Data Fig. 10d). The effect of CDK7 on DNA looping was further confirmed in another T-ALL cell line and locus (Supplementary Note, Fig. 6e and Extended Data Fig. 10e–g). Overall, we demonstrate that targeting a transcription factor such as CDK7 can maintain contacts in a subset of γSI-insensitive enhancers in T-ALL. Furthermore, changes in H3K27ac levels emerge as a reliable indicator of chromatin interaction dynamics after drug treatments.

Discussion

Despite the intense focus on the regulatory role of TADs in human disease, it remains largely unexplored whether TAD boundary or intra-TAD activity changes are important for tumor initiation or maintenance. Indeed, aberrant activation of cancer drivers by enhancer hijacking remains the primary known mechanism linking 3D structural changes to oncogenic transformation<sup>1,3,4,39</sup>. Our studies further these findings by using human T-ALL as a model. They highlight the underlying complexity of factors regulating the 3D landscape in human leukemia, with notable variations among different leukemia subtypes, and suggest that drugs with reported antileukemic activity partially reverse 3D interactions in specific loci, potentially accounting for the antileukemogenic effects of these drugs.

Frequent loss of TAD boundary insulation has previously been observed in human cancer, including in T-ALL<sup>49</sup>. In line with these findings, we identify here a TAD boundary change within the MYC locus that is associated with increased enhancer–promoter interactions. MYC is an important downstream target of NOTCH1 that activates anabolic pathways to sustain the proliferation induced by constitutive NOTCH1 activation<sup>30,36</sup>. Our observations suggest that MYC upregulation in T-ALL relative to mature T cells is associated with differences in local chromatin architecture. At this point, it is not clear what regulates CTFC binding within the TAD boundary in T-ALL and T cells, although our preliminary studies have excluded a role for DNA methylation and somatic mutations in the CTFC motif. Interestingly, by using ATAC–seq, we found that the CTFC site was accessible in T cells but displayed greatly reduced accessibility in T-ALL, suggesting differential chromatin accessibility as a potential mechanism of regulating CTFC binding. In support of this hypothesis, a recent report identified chromatin accessibility correlating with CTFC binding during the transition from interphase to prometaphase<sup>39</sup>. In addition to the lost CTFC boundary in T-ALL, we also observed an increase in CTFC binding with the same orientation (facing into the TAD and toward MYC) downstream of the super-enhancer. Such clusters of CTFC surrounding super-enhancers have recently been described as super-anchors that ensure super-enhancer-mediated regulation of nearby genes<sup>12</sup>. Further studies of the regulatory mechanism underlying CTFC binding and chromatin accessibility in the MYC locus could provide alternate strategies to decrease MYC expression in T-ALL<sup>12</sup>.

In addition to TAD boundary changes, we also found prevalent intra-TAD activity differences between T-ALL and T cells, as well as between the two subtypes of T-ALL. The changes in intra-TAD activity correlated with expression changes, super-enhancer activity, NOTCH1 binding and insulation mediated by CTFC binding at these TAD boundaries, which appeared to be independent of compartment shifts. Supporting a prominent role for intra-TAD activity changes in modulating gene expression, recent studies tracking 3D chromatin modifications during developmental processes such as embryonic stem cell differentiation and neural development identified significant changes in interactions within (sub-)TADs that correlated with transcriptional levels and epigenetic states<sup>12,34</sup>. Furthermore, in line with our findings, negative correlations of

![Fig. 6](https://www.nature.com/naturegenetics)
intra-TAD interactions with repressive histone marks have been reported in EZH2-mutant lymphomas. Herein our observations suggest that gene expression changes in cancer cells are frequently associated with correlative changes in intra-TAD activity, CTCF insulation and enhancer activity. On a cautionary note, precise identification of 3D chromatin architectural changes in cancer cells.
depends on comparison with the respective cell-of-origin population. Although the cell of origin of each of our T-ALL samples is unknown, pilot experiments comparing the 3D chromatin architecture of T-ALL cells to that of human thymic (CD4+CD8+) progenitors from healthy donors identified similar intra-TAD activity differences specific to T-ALL (data not shown). Further studies are required to understand a possible correlation between the cell of origin and leukemia and to even address potential 3D landscape differences between individual leukemia samples.

Finally, we also addressed the role of oncogenic NOTCH1 in organizing the 3D chromosomal landscape associated with T-ALL transformation and to what extent changes can be reversed by inhibiting NOTCH signaling. NOTCH signaling inhibition is a powerful means to inhibit growth of NOTCH1-induced T-ALL.\(^{41,42}\). The effects of γSI have been reported to be selective to dynamic NOTCH1 sites, which are predominantly located within enhancers.\(^{12,19}\). Dynamic NOTCH1 sites are also associated with a decrease in enhancer activity after γSI treatment. These findings prompted us to further investigate the impact of NOTCH1 inhibition on remodeling of the 3D landscape in leukemia. Our studies showed that NOTCH1 inhibition using γSI had no effect on global 3D chromatin structure but targeted enhancer–promoter interactions in selected NOTCH1-regulated loci. More specifically, we identified enhancer–promoter loops of dynamic NOTCH1-bound enhancers also associated with a decrease in H3K27ac after γSI treatment that were particularly sensitive to NOTCH1 inhibition. These results concur with a recent report that demonstrated a role for NOTCH1 in facilitating specific long-range interactions in triple-negative breast cancer and mantle cell lymphoma.\(^{35}\).

In an attempt to further understand the importance of NOTCH1 binding in maintaining certain enhancer–promoter loops but not others, we initially found that the enhancers most sensitive to NOTCH1 inhibition tended to be shorter in length. The longer stretch of ‘insensitive’ enhancers might enable other factors to bind and/or keep the chromatin in an open and accessible state for long-range chromatin interactions,\(^{7}\), thus offering a potential explanation for the variance in enhancer–promoter looping changes we observed for NOTCH1 targets, including MYC, IKZF2, APCD1, and LUNAR1. In agreement with this hypothesis, we found enrichment for CDK7 binding in γSI-insensitive enhancers relative to γSI-sensitive enhancers. CDK7 is a kinase previously shown to control the function of RNA polymerase II-mediated transcription.\(^{9}\). CDK7 inhibition has been shown to have significant effects in hematological malignancies and other cancer types.\(^{47,49,50}\). We here showed that pharmacological inhibition of CDK7 in T-ALL by THZ1 resulted in a widespread decrease in enhancer activity as quantified by H3K27ac levels. Enhancers with strong reduction of H3K27ac were also associated with a significant decrease in enhancer–promoter contacts, including the γSI-insensitive loci for MYC and IKZF2. This clearly highlights the complexity of super-enhancer activity and the factors that dictate super-enhancer interactions with gene promoters. Overall, our study underscores the need for further investigation of factors that maintain or rewire 3D chromosomal interactions, especially during cellular transformation, as they could be potential targets for small-molecule drug development.

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Methods

Cell culture. The CUTTL1 human cell line and Jurkat cells were cultured in RPMI-1640 medium supplemented with 10% FBS, penicillin, streptomycin and gramicidin. Naive CD4+ T cells were purchased from Lonza and cultured in X-vivo 15 culture medium (Lonza) supplemented with 5% human serum (Gemini Bioproducts) and 10 ng/ml human interleukin (IL)-2.

Primary T-ALL samples. Primary samples from individuals with T-ALL were collected by Columbia Presbyterian Hospital or Weill Cornell Medical College with informed consent and approved by the institutional review boards at the Columbia University Medical Center Institutional Review Board or the Weill Cornell Medical College Institutional Review Board. For expansion of these cells, 1 x 10^6 cells were transplanted into immunodeficient NOD-SCID gamma (NSG) mice strains via retired-oral injection, as previously performed.

Cells collected from the spleen of these primary recipients were used for the in vitro experiments. CRISPR/SaCas9 was performed with two replicates, and CUTLL1 cells were treated with DMSO or γ-irradiation at 1.5 Gy. The next day, 50 μl of CRISPR/Cas9 master mix (37.5 μM T4 HiFi HotStart Ready mix and 10 μM each of 5 μM gRNA (Klenow fragment (NEB, M0120)) was added. Reactions were rotated at 37°C for 1 h, and 948 μl of ligation master mix (150 μl of 10X NEB T4 DNA ligase buffer with 10 μM ATP (NEB, B0202), 125 μl of 10% Triton X-100, 5 μl of 50 mM N.A (BSA (Thermo Fisher)), and 14 μl DNA ligation of 5 μl each were added. Samples were washed with 2 μl of water. Reactions were rotated at room temperature for 4 h. The ligation product was digested overnight with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 7.5 and 167 mM NaCl). Cells were collected with 30 μl of Protein G Dynabeads (Life Technologies, 10004D) by rotation at 4°C for 1 h. Supernatants were transferred to fresh tubes, and antibody was added (7.5 μg of anti-H3K27ac antibody for 10 million cells). Samples were incubated overnight at 4°C. The next day, 30 μl of Protein G Dynabeads were added and samples were rotated at 4°C for 2 h. After bead capture, beads were washed with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 7.5 and 150 mM NaCl), high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 7.5 and 500 mM NaCl) and LiCl wash buffer (100 mM Tris-HCl pH 7.5, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate and 1 mM EDTA). The samples were eluted with 150 μl of DNA elution buffer (50 mM sodium bicarbonate pH 8.0 and 1% SDS, freshly made) and incubated at 37°C for 30 min with rotation. Supernatant was transferred to a fresh tube and elution was repeated with another 150 μl of elution buffer. Then, 5 μl of proteinase K (20 mg/ml; Thermo Fisher) was added to each sample, and elution was incubated overnight. The samples were purified with DNA Clean and Concentrator columns (Zymo Research) and eluted in 10 μl of water. Post-ChIP DNA was quantified by Qubit (Thermo Fisher), and 5 μl of Streptavidin C1 beads (Thermo Fisher) were washed with Tnew wash buffer (5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl and 0.05% Tween-20) and then resuspended in 10 μl of washing buffer (20 mM Tris-HCl pH 7.5, 1 mM EDTA and 2 M NaCl). Beads were added to the samples, which were incubated at room temperature for 15 min with shaking. After capture, beads were washed twice by adding 150 μl of Tnew wash buffer and incubating at 55°C for 2 min with shaking. Samples were then washed in 100 μl of 1X TD buffer (2X TD buffer: 20 mM Tris-HCl pH 7.5, 10 mM MgCl2, and 20% dimethylformamide). After washing, beads were resuspended in 2 μl of Arima elution buffer, and 3 μl of elution buffer. The library was generated on Streptavidin C1 beads with a modified Kapa Library Preparation Kit. End repair and adaptor ligation were carried out on 20 μl of bead-bound DNA. Then, 1 μl of 15 μM Illumina TruSeq sequencing adaptors was added and the sample was amplified along with 49 μl of master mix containing DNA ligase, ligase buffer and PCR-grade water. A 50 μl volume of 1X TD buffer was added to the sample. The samples were washed twice with Arima wash buffer and incubated at 55°C for 2 min with shaking. The samples were washed once more with 100 μl of elution buffer and finally resuspended in 22 μl of elution buffer. To each sample, 25 μl of HiFi HotStart Ready Mix and 10X primer mix (Kapa Library Amplification Kit) were added. The following PCR program was performed: 98°C for 45 s followed by ten cycles at 98°C for 15 s, 60°C for 30 s and 72°C for 30 s, with a final extension completed at 72°C for 1 min (cycle number was estimated on the basis of the amount of material from the post-ChIP Qubit reading (approximately 50 ng was run in six cycles, while 25 ng was run in seven cycles, 12.5 ng in eight cycles, etc.). Libraries were sequenced on the Illumina HiSeq 4000 platform on PE50 mode.

In vitro drug treatment. CUTTL1 cells were treated with ySi (Compound E) purchased from Alexis Bioscience at a 1 μM final concentration. Treatment was performed every 12 h for 72 h. THZ1 was purchased from Cayman Chemical (900215), and cells were treated at a 100 nM final concentration every
GRO-seq and library preparation. GRO-seq was performed in CUTLL1 cells treated with either DMSO or 5′iSL at a 1 μM concentration for 72 h. All experiments were performed in biological duplicate. GRO-seq sample preparation was performed as described previously23. Briefly, nuclei were isolated in swelling buffer (10 mM Tris-HCl pH 7.5, 2 mM MgCl2 and 3 mM CaCl2), lysed twice in lysis buffer (10 mM Tris-HCl pH 7.5, 2 mM MgCl2, 3 mM CaCl2, 10% glycerol and 0.5% NP-40) and snap frozen in freezing buffer (50 mM Tris pH 8.0, 40% glycerol, 5 mM MgCl2, and 0.1 mM EDTA). For the run-on reaction, an equal volume of reaction buffer was added to thawed nuclei (10 mM Tris pH 8.0, 5 mM MgCl2, 300 mM KCl, 500 μM ATP, 500 μM GTP, 5 μM CTP, 500 μM TruUTP, 1 mM dithiothreitol, 100 μl−1 Superscript I and 1% sarkosyl), and samples were mixed and incubated at 30 °C for 5 min. The reaction was stopped with Trizol reagent, and RNA was extracted with phenol-chloroform and ethanol precipitated. RNA was heated in fragmentation buffer (40 mM Tris pH 8.0, 100 mM KCl, 6.25 mM MgCl2, and 1 mM dithiothreitol), treated with DNase and purified with Zymo RNA Clean and Concentrator columns (Zymo Research) by using the >17-nucleotide protocol. Run-on RNA was immunoprecipitated with BSA-blocked BRDU beads (Santa Cruz Biotechnology) in binding buffer (0.5× SSPE, 1 mM EDTA and 0.05% Tween–20) for 1 h at 4 °C, washed and eluted in elution buffer (5 mM Tris pH 7.5, 300 mM NaCl, 20 mM dithiothreitol, 1 mM EDTA, and 1% SDS) at 65 °C for 20 min. Nascent RNA was further extracted with phenol-chloroform, and sequencing libraries were prepared.

Sanger sequencing of the CTCF-binding site in the MYC locus. Genomic DNA from CUTLL1, Jurkat and T-ALL 1 cells was isolated with the Qiagen DNeasy kit according to the manufacturer's guidelines. The target locus was PCR amplified with Phusion High-Fidelity PCR Master Mix (Thermo Fisher, FS315S) using 100 ng of genomic DNA as the template. Primer sequences are listed in Supplementary Table 6. PCR products were purified on QIAgen PCR purification columns and submitted for Sanger sequencing to Genewiz.

CTCF-motif-targeting guide RNA sequence. The guide RNA target sequence was 5′-UCUAAGAACAUCUCACACUAC-3′. The guide RNA along with the tracer RNA was purchased as a synthetic guide RNA from Synthego with 2′-O-methyl and 3′-phosphorothioate modifications of the first and last three nucleotides.

Editing of T cells. Naive T cells were activated with CD3/CD28 beads from Thermo Fisher Scientific (11616D) for 48 h. After activation, the CD3/CD28 beads were magnetically removed and 2 million activated T cells were transfected by electroporation with either ribonucleoprotein complex consisting of 1.5 µg of Cas9 protein alone for every 200,000 µg of Cas9 protein with 2 million activated T cells or Cas9 protein with 2 million activated T cells and 2 million activated T cells. Editing efficiency was computed by comparing the expression of the guiding RNA along with the tracer RNA to the expression of the guide RNA along with the tracer RNA in untreated T cells or the mean expression of associated genes in untreated T cells.

High-throughput 3D DNA FISH. Generation of FISH probes. Custom FISH probes targeting the MYC promoter and enhancer were designed with the SureDesign custom oligonucleotide design tool from Agilent with homology to the regions of interest mined from the hg19 genome build, using the default parameters of the SureDesign tool. The MYC promoter probe library targeted a 60-kb region centered on the promoter, whereas the enhancer probe library targeted a 100-kb region including the center enhancer element of the MYC super-enhancer cluster.

3D FISH experimental protocol. 3D FISH was performed with the Dako FISH Histology accessory kit from Agilent with homology to the regions of interest mined from the hg19 genome build, using the default parameters of the SureDesign tool. The MYC promoter probe library targeted a 60-kb region centered on the promoter, whereas the enhancer probe library targeted a 100-kb region including the center enhancer element of the MYC super-enhancer cluster.

The statistical significance of differences in odds ratios between two groups (Figs. 2l, 5b and 6a) was calculated by two-sided Fisher's exact test. When we expected changes in one direction, we used one-tailed t tests under the following hypotheses (Fig. 2d,e,g):

- **H0**: The mean expression of genes associated with differential intra-TAD activity (for example, expression fold change T-ALL/T-cells in T-ALL-specific TADs) or the mean CTCF binding strength of peaks associated with differential intra-TAD activity is unchanged or has a negative correlation with intra-TAD activity changes.
- **H1**: The mean expression of genes associated with differential intra-TAD activity (for example, expression fold change T-ALL/T-cells in T-ALL-specific TADs) or the mean CTCF binding strength of peaks associated with differential intra-TAD activity has a positive correlation with intra-TAD activity changes.

Similarly, for comparisons of enhancer–promoter loops or associated expression of genes connected with enhancers of reduced activity (Figs. 5c,d and 6c), we used one-tailed t tests under the following hypotheses:

- **H0**: The mean looping strength of enhancer–promoter pairs in untreated CUTLL1 cells is greater than or equal to the looping strength of enhancer–promoter pairs in untreated CUTLL1 cells or the mean expression of associated genes in untreated CUTLL1 cells is greater than or equal to the expression of associated genes in untreated CUTLL1 cells.
- **H1**: The mean looping strength of enhancer–promoter pairs in untreated CUTLL1 cells is less than the looping strength of enhancer–promoter pairs in untreated CUTLL1 cells or the mean expression of associated genes in untreated CUTLL1 cells is less than the expression of associated genes in untreated CUTLL1 cells.

Data availability

All sequencing data were mostly processed with the hic-bench platform33. Detailed descriptions of individual analyses can be found in the Supplementary Methods.

Further detailed information on experimental design and reagents can be found in the Nature Research Reporting Summary.

Statistics and reproducibility. All sequencing experiments and functional analyses involved at least two replicate experiments that were independently prepared, cultured and treated, including experiments with cell lines (CUTLL1, Jurkat and activated T cells) or xenografts of different primary samples from patients, obtained by using independent recipient mice for each replicate.

Statistical analyses for differential gene expression, differential ChiP–seq peaks and differential 4C–seq peaks were conducted with the R Bioconductor package edgeR using two or more independent replicates as described above (after intra-sample sequencing depth normalization with the 'cqm' function and inter- and intra-sample distance correction with the 'distanceCommonDNase' and 'estimateDiss' functions, followed by 'glmQLFitter' and 'glmQLFTest' for differential analyses). Differential Hi-C analysis, based on either compartment scores or TAD activity scores, was performed with two-sided t tests.

The statistical significance of differences in odds ratios between two groups (Figs. 2l, 5b and 6a) was calculated by two-sided Fisher's exact test.
under accession code GSE115896. Biological material used in this study can be obtained from the authors upon request. Source data for Figs. 2–6 and Extended Data Figs. 1–3, 5, 7, 9 and 10 are provided with the paper.

**Code availability**
All code for Hi-C analysis is available within the previously published Hi-C bench platform (https://github.com/NYU-BFX).

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**Author contributions**
A.T. and I.A. conceived, designed and supervised the study with input from A.K., P.T. and P.N. A.K. designed and performed most of the computational analyses with help from A.T., S.N. and C.L. P.T. and P.N. designed and performed most of the experiments with help from Y.G., X.C., H.H., S.B., J.W., T.T., Y.F., F.B., Y.Z., E.P., P.V.V., G.G.I. and T.L. P.T., P.N. and Y.G. performed Hi-C, HiChIP and 4C experiments with help from X.C., S.B., J.W. and Y.Z. P.T. performed DNA FISH with help from Y.F. and T.L. P.T. performed ChIP-seq with help from P.N., S.B. and J.W. P.T. and H.H. performed RNA-seq. T.T. performed GRO-seq. A.T., I.A., A.K. and P.T. wrote the manuscript with input from all authors.

**Competing interests**
A.T. is a scientific advisor to Intelligencia.AI. All other authors declare that they have no competing interests.

**Additional information**
Extended data is available for this paper at https://doi.org/10.1038/s41588-020-0602-9. Supplementary information is available for this paper at https://doi.org/10.1038/s41588-020-0602-9. Correspondence and requests for materials should be addressed to I.A. or A.T. Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Hi-C quality control and unsupervised analyses. a) Read alignment statistics for Hi-C datasets, as absolute reads (left) and relative reads (in %, right). “ds.accepted.intra” are all intra-chromosomal reads used for all downstream analyses. b) Genome-wide stratum-adjusted correlation coefficient (SCC) scores for all pair-wise comparisons of the Hi-C datasets. HiCRep was used to calculate chromosome-wide correlation scores, which were averaged across all chromosomes for each pair-wise comparison. The HiCRep smoothing parameter X was set to 1.0. c) Principal Component Analysis (PCA) of the genome-wide compartment scores for each Hi-C dataset. Number samples: T cells n = 3; T-ALL n = 6; ETP-ALL n = 4. d) Compartment shifts between T cells, T-ALL and ETP-ALL. Assignment of A compartment was done using an average c-score > 0.1 in either all T cell, T-ALL or ETP-ALL samples and B compartment with average c-score < -0.1. Significance for differences between pairwise comparisons of T cells, T-ALL and ETP-ALL was determined using a two-sided t test between c-scores, and compartment shifts were determined using P value < 0.1. e) Integration of gene expression associated with compartment shifts for comparisons of T cell vs T-ALL (left) or T-ALL vs ETP-ALL (right) using RNA-seq (FPKM > 1). For each gene within the respective compartment bin, log2 fold-change between T cells and T-ALL (left) or between T-ALL and ETP-ALL (right) is shown. Significant differences are calculated using an unpaired one-sided t test comparing genes from A to A compartments (that is active compartment) with genes from A to B or B to A compartment shifts, following the hypothesis of a positive correlation between expression and compartment association. Boxplot information can be found as additional Source Data.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Genomic loci displaying differential intra-TAD activity in T-ALL. a) Hi-C interaction heat maps (first row) showing the IKZF2 locus (black circle). Second row shows heat maps of log2 (fold-change) interactions compared to T cell 1. b) H3K27ac ChIP-seq tracks for IKZF2 locus in T cells and CUTLL1, NOTCH1 ChIP-seq tracks for CUTLL1. Tracks represent fold-enrichment over input where applicable and counts-per-million reads otherwise. Grey area indicates TAD containing IKZF2. Number replicates: T cells H3K27ac n = 2; CUTLL1 H3K27ac n = 2; CUTLL1 NOTCH1 n = 1. c) Quantifications for intra-TAD activity (left; as highlighted in a) and expression of IKZF2 (right). Statistical evaluation for intra-TAD activity was performed using paired two-sided t test of average per interaction-bin for IKZF2 TAD between T cells (n = 3) and T-ALL (n = 6), followed by multiple testing correction. Log2 FPKM of IKZF2 expression for T cells (n = 13) and T-ALL (n = 6) samples; statistical evaluation was performed using edgeR followed by multiple testing correction. d) Hi-C interaction heat maps (first row) showing the CYLD locus (black circle). Second row shows heat maps of log2 (fold-change) interactions when compared to T-cell 1. e) H3K27ac ChIP-seq tracks for CYLD locus in T cells and CUTLL1, NOTCH1 ChIP-seq tracks for CUTLL1. Tracks represent fold-enrichment over input where applicable and counts-per-million reads otherwise. Grey area indicates TAD containing CYLD. Number replicates: T cells H3K27ac n = 2; CUTLL1 H3K27ac n = 2; CUTLL1 NOTCH1 n = 1. f) Quantifications for intra-TAD activity (left; as highlighted in D)) and expression of CYLD (right). Statistical evaluation for intra-TAD activity was performed using paired two-sided t test of average per interaction-bin for CYLD TAD between T cells (n = 3) and T-ALL (n = 6), followed by multiple testing correction (see methods). Log2 FPKM of CYLD expression for T cells (n = 13) and T-ALL (n = 6); statistical evaluation was performed using edgeR followed by multiple testing correction. Boxplot information can be found as additional Source Data.
Extended Data Fig. 3 | Intra-TAD activity cross-comparison of T-ALL sub-types. a) Comparisons of intra-TAD activity between T cells, T-ALL and ETP-ALL samples. b) Overlap of differentially active TADs between the two comparisons of T cells vs T-ALL and T cells vs ETP-ALL, visualized as venn diagram. Red and blue colors correspond to differences as highlighted in a). c, d) Integration of RNA-seq (FPKM > 1) within TADs with decreased / increased intra-TAD activity for ETP-ALL vs T cells (c) and ETP-ALL vs T-ALL (d). For each such gene, the log2 (fold-change) in expression between ETP-ALL and T cells (c) / T-ALL and ETP-ALL (d) taken from RNA-seq is shown. Significant differences are calculated by an unpaired one-sided t test comparing genes from TADs with decreased / increased intra-TAD activity with genes from stable TADs, following the hypothesis of a positive correlation between expression and intra-TAD activity changes. Boxplot information can be found as additional Source Data.
Extended Data Fig. 4 | WGS integration with TAD boundaries altered in T-ALL. a, b) Overlap of altered TAD boundaries as in Fig. 3c, d with genomic inversions (a) or insertions/deletions (indels) (b) from WGS of T-ALL 1 (top) and T-ALL 2 (bottom). Overlap was determined by bedtools intersect, using a 1bp overlap for indels and 100kb for individual inversion breakpoints (instead of the entire genomic range affected by the inversion). c) Overlap of individual translocation breakpoints (calculated from T-ALL Hi-C samples as in Supplementary Fig. 1B) with TAD boundaries displaying changes in TAD insulation between T cells and T-ALL. Overlap was determined by bedtools intersect, using a 1bp overlap.
Extended Data Fig. 5 | Difference in CTCF insulation in MYC locus is not due to genomic mutation but potentially regulated by open chromatin.

a) CTCF ChIP-qPCR of the CTCF binding site in the lost MYC TAD boundary, shown as fold-enrichment over input. Significant differences compared to T cells were calculated with an unpaired one-sided t test, following the hypothesis of loss of CTCF binding in T-ALL samples as determined from the genome-wide analysis (n = 3 replicates for T cells, T-ALL 1, T-ALL 2, CUTLL1 and Jurkat; n = 2 replicates for T-ALL 3 and T-ALL 4). Error bars indicate s.d.; center value indicates mean.

b) Targeted sanger sequencing indicates no mutation in T-ALL in the CTCF binding site at the MYC TAD boundary. Tracks show chromatogram of individual base calls (left). Whole genome sequencing indicates no mutation in T-ALL in the motif of CTCF binding site. Tracks show (mis-)matches compared to reference sequence in all reads covering the respective genomic position (right).

c) CTCF ChIP-qPCR before and after treatment with global DNA-demethylation agent 5-azacytidine (n = 2 replicates).

d) ATAC-seq quantification for T cells and Jurkat for the genomic area covering loss of CTCF binding in the downstream TAD boundary of MYC. Data was normalized to the average T cell signal, shown in percent (n = 3 replicates). Statistical evaluation was performed using DiffBind with edgeR-method, following multiple testing correction. Error bars indicate s.d.; center value indicates mean.
Extended Data Fig. 6 | 4C-Seq validation of MYC super-enhancer interaction in primary T-ALL. a) 4C-seq analysis using MYC promoter as viewpoint. Positive y-axis shows interactions with the MYC promoter viewpoint as normalized read counts, negative y-axis shows significance of differential interactions between T cells and primary T-ALL samples as log10(P value) derived using edgeR function glmQLFT est. H3K27ac ChIP-seq tracks for T cells and CUTLL1 are represented below as fold-enrichment over input. Grey areas indicate MYC super-enhancer elements. Number replicates: T cells 4 C n = 2; T-ALL 1 4 C n = 1; T-ALL 2 4 C n = 2; T cells H3K27ac n = 2; CUTLL1 H3K27ac n = 2.
Extended Data Fig. 7  | CRISPR-Cas9 deletion of CTCF binding site shows loss of insulation around MYC locus.  
a) Schematic of Cas9+ Synthetic guide transfection of activated T cells.  
b) Sequence showing CTCF motif in the insulator region in T cells targeted for CRISPR-based deletion. sgRNA targeting sequence within the CTCF motif is highlighted. Sequencing of sgRNA target site indicates various indels along with frequencies observed for each indel.  
c) CTCF ChIP-qPCR validation of reduced CTCF binding in edited T cells compared to unedited T cells (n = 2 replicates).  
d) qPCR comparing MYC expression in edited T cells compared to unedited T cells (n = 3 replicates). Statistical significance was determined using unpaired two-sided t test. Error bars indicate s.d.; center value indicates mean.  
e) 4C-seq analysis using MYC promoter as viewpoint in edited and unedited T cells. Positive y-axis shows interactions with the viewpoint as normalized read counts, negative y-axis shows significance of differential interactions between the two samples as log10(P value) calculated with edgeR function glmQLFTest. Tracks below show CTCF ChIP-seq in CUTLL1 and H3K27ac ChIP-seq in naïve T cells and CUTLL1 as fold-enrichment over input. Grey area indicates deleted CTCF binding site. Number replicates: T cells WT 4 C n = 2; T cells Edited 4 C n = 2; T cells CTCF n = 2; T cells H3K27ac n = 2; CUTLL1 H3K27ac n = 2.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Genome-wide Hi-C analysis in T-ALL following γSI shows no intra-TAD activity differences, but individual promoter-enhancer loops are disrupted. a) Volcano plot showing differential intra-TAD activity between CUTLL1 DMSO vs CUTLL1 γSI (average activity > 0.58 / < -0.58 and with FDR < 0.05). Statistical evaluation was performed using paired two-sided t test between all per bin-interactions between DMSO and γSI (n = 2 replicates). b) Representation of TAD boundary alteration events (red dots; none identified). Plots depict pair-wise comparisons for TAD boundary losses of adjacent CUTLL1 (untreated, left) TADs and for TAD boundary gains of adjacent CUTLL1 (γSI treated, right) TADs. Dotted line represents outlier threshold as in Fig. 3c and d). c) Virtual 4C of H3K27ac HiChIP in CUTLL1, using MYC promoter as viewpoint (chr8: 128,747,680), showing edgeR-normalized CPM. H3K27ac ChIP-seq track for MYC locus shown as fold-enrichment over input. Detected significant loops as arc-representation (below) from mango pipeline utilizing two-sided binomial test per matrix-diagonal followed by multiple testing correction (FDR < 0.1; CPM > 5). Number replicates: CUTLL1 H3K27ac HiChIP n = 1; CUTLL1 H3K27ac ChIP-seq n = 2. d) H3K27ac signal (enrichment over input) (left), chromatin interaction of the highest peak by 4C-seq (center) for the interaction of LUNAR1 promoter with its upstream enhancer and LUNAR1 expression (right). All quantifications are normalized to the respective average T cell signal, shown in percent. Significance of differences was calculated using diffBind with edgeR-method (for H3K27ac ChIP-seq, FDR) and edgeR (for 4C-seq interactions and GRO-seq as P value and FDR respectively). Error bars indicate s.d.; center value indicates mean. Number replicates: CUTLL1 DMSO H3K27ac n = 2; CUTLL1 γSI H3K27ac n = 2; CUTLL1 DMSO 4 C n = 2; CUTLL1 γSI 4 C n = 2; CUTLL1 DMSO GRO-seq n = 2; CUTLL1 γSI GRO-seq n = 2. e) H3K27ac signal (left), chromatin interaction of the highest peak by 4C-seq (center) for the interaction of APCDD1 enhancer with the downstream APCDD1 promoter and APCDD1 expression (right). All quantifications are normalized to the respective average T cell signal, shown in percent. Significance of differences was calculated using diffBind with edgeR-method (for H3K27ac ChIP-seq, FDR) and edgeR (for 4C-seq interactions and GRO-seq as P value and FDR respectively). Error bars indicate s.d.; center value indicates mean. Number replicates: CUTLL1 DMSO H3K27ac n = 2; CUTLL1 γSI H3K27ac n = 2; CUTLL1 DMSO 4 C n = 2; CUTLL1 γSI 4 C n = 2; CUTLL1 DMSO GRO-seq n = 2; CUTLL1 γSI GRO-seq n = 2. f) Schematic of γSI sensitive and insensitive enhancer. g) Peak width of stable (black; n = 111) or decreased H3K27ac signal (green, n = 76) as defined in Fig. 5a. Significant difference between the distributions is estimated by a two-sided Wilcoxon test. Number replicates: CUTLL1 DMSO H3K27ac n = 2; CUTLL1 γSI H3K27ac n = 2.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Treatment with γSI does not alter all NOTCH1 dynamic enhancers. 

a) 4C-seq using MYC promoter as viewpoint. Positive y-axis shows interactions with viewpoint as normalized read counts, negative y-axis shows significance of differential interactions as log10(P value) calculated using edgeR function glmQLFTest (CUTLL1 DMSO n = 5; CUTLL1 γSI n = 3). Tracks below show H3K27ac, NOTCH1 ChIP-seq and GRO-seq (positive strand only) as fold-enrichment where applicable, and counts-per-million reads otherwise. Grey areas indicate MYC super-enhancer elements. 

b) Quantification of H3K27ac signal (enrichment over input), chromatin interactions by 4C-seq for the interactions of MYC promoter and MYC expression. Interaction changes are measured by centering the 40 kb bin on highest peaks within N-Me/NDME, CEE or BDME/BECE elements. MYC expression was measured by qPCR. All quantifications are normalized to CUTLL1 DMSO, shown in percent. Error bars indicate s.d.; center value indicates mean. Significance is shown as false-discovery rate (FDR) for H3K72ac signal change (R package DiffBind with edgeR-method), P value for chromatin interaction change (edgeR function glmQLFTest) or one-tailed t test for qPCR changes. 

c) Cropped western blot images immunoblotted with MYC antibody. Unprocessed western blots can be found as Source Data. Experiment was repeated twice with similar results. 

d) CTCF ChIP-qPCR of lost MYC boundary upon γSI in CUTLL1 (n = 3). Error bars indicate s.d.; center value indicates mean. Significance was calculated using unpaired two-sided t test. 

e) 4C-seq analysis using IKZF2 promoter as viewpoint after γSI treatment. Positive y-axis shows normalized read counts, negative y-axis shows significance of differential interactions as log10(P value) calculated using edgeR function glmQLFTest (CUTLL1 DMSO n = 3; CUTLL1 γSI n = 3). Tracks below show H3K27ac, NOTCH1 ChIP-seq and GRO-seq (negative strand only) as fold-enrichment over input where applicable, and counts-per-million reads otherwise. Grey area indicates IKZF2 enhancer. 

f) H3K27ac signal is specific for enhancer highlighted in d). Interaction changes are measured by centering the 40 kb bin on the highest enhancer peak. IKZF2 expression after γSI treatment was measured by GRO-seq. All quantifications are normalized to the average T cell signal, shown in percent. Error bars indicate s.d.; center value indicates mean. Significance is shown as false-discovery rate (FDR) for H3K72ac signal (R package DiffBind with edgeR-method), P value for chromatin interaction (edgeR function glmQLFTest) or one-tailed t test for qPCR expression.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Treatment of T-ALL with THZ1 reduces also γSI insensitive promoter-enhancer interactions. a) H3K27ac signal is specific for N-Me/NDME, CEE and BDME/BENC. Interaction changes are measured by centering the 40 kb bin on highest peaks within N-Me/NDME, CEE or BDME/BENC elements. MYC expression after THZ1 treatment was measured by qPCR. All quantifications are normalized to the average CUTLL1 DMSO signal, shown in percent. Error bars indicate s.d.; center value indicates mean. Significance is shown as false-discovery rate (FDR) for H3K72ac signal (R package DiffBind with edgeR-method), P value for chromatin interaction (edgeR function glmQLFTest) or two-sided t test for qPCR expression. b) Cropped western blot images immunoblotted with MYC antibody. Unprocessed western blots can be found as Source Data. Experiment was repeated twice with similar results. c) CTCF ChIP-qPCR, shown as enrichment over input, of CTCF site in lost boundary in MYC locus (n = 3). Error bars indicate s.d.; center value indicates mean. Significance was calculated using unpaired two-sided t test. d) Inter-probe distance between MYC promoter and MYC-CCE measured by DNA-FISH analysis. Statistical difference between distributions of probe distances was calculated using two-sample one-sided Kolmogorov Smirnov test. Error bars indicate s.d.; center value indicates median. Probe-pairs CUTLL1 DMSO = 2001. Probe-pairs CUTLL1 THZ1 = 1308. Median distance CUTLL1 DMSO = 264.28µm. Median distance CUTLL1 THZ1 = 321.69µm. e) 4C-seq using MYC promoter as viewpoint in Jurkat cells. Positive y-axis shows normalized interaction strength with the viewpoint, negative y-axis shows significance of differential interactions as log10(P value) calculated using edgeR function glmQLFTest (n = 3). Grey areas indicate MYC super-enhancer elements. f) Interaction changes are measured by centering the 40 kb bin on N-Me/NDME, CEE or the BDME/BENC. Error bars indicate s.d.; center value indicates mean. Significance is shown as P value for chromatin interaction changes (edgeR function glmQLFTest). g) Quantification of changes in H3K27ac signal (enrichment over input) and chromatin interactions of IKZF2 enhancer in CUTLL1. All quantifications are normalized to the average CUTLL1 DMSO signal, shown in percent. Error bars indicate s.d.; center value indicates mean. Significance is shown as false-discovery rate (FDR) for H3K72ac signal change (R package DiffBind with edgeR-method), P value for chromatin interaction change (edgeR function glmQLFTest).
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**Software and code**

**Policy information about availability of computer code**

**Data collection**

- All NCBI GEO datasets listed in Supplementary Table 2 were downloaded using sra toolkit version 2.8.0.
- RNA-Seq data from Chen et al. (see Supplementary Table 2) was downloaded via FTP from data owner upon request.

**Data analysis**

- bowtie2 version 2.3.1. Hi-C bench. genomic-tools. R version 3.3.0. ICE-normalization according to Imakeav et al.. TAD calling by hic-ratio.
- MACS2 version 2.0.1. bedtools version 2.27.1. diffBind version 2.2.12. IGV version 2.3.83. PWMScan. deepTools version 2.3.3. STAIR-aligner version 2.5.0c. ngsutils version 0.5.7. edgeR version 3.14.0. bowtie version 1.0.0. ROSE version 2015. picard-tools version XX. HiCnv.

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**Data**

**Policy information about availability of data**

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All sequencing data created within this study was uploaded to NCBI GEO (https://www.ncbi.nlm.nih.gov/geo/) and is available under the accession GSE115896.
Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No prior sample size determination was conducted. All experiments were conducted in at least 2 biological replicates. Statistical testing ensured significant findings. |
| Data exclusions | No replicates were excluded, and all attempts to replicate were successful. |
| Replication | All experiments were conducted in at least 2 biological replicates. For all sequencing data-types, successful replication has been confirmed with Principal Component Analysis. |
| Randomization | Randomization was relevant to the study, because the difference between healthy and disease was assessed. |
| Blinding | The investigators were not blinded to sample group allocation, because the difference between healthy and disease was assessed. Sample group assignments were further ensured using Principal Component Analysis on all relevant sequencing data. |

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| [ ] [ ] Palaeontology            | [x] MRI-based neuroimaging |
| [ ] [x] Animals and other organisms |       |
| [x] [x] Human research participants |     |
| [x] [ ] Clinical data            |       |

Antibodies

Antibodies used

CTCF (D31H2; Cell Signaling Catalog no: 3418; lot 3 &4); H3K27ac (Active motif; Catalog no: 39133, Lot no: 01518010); c-MYC (D84C12; Cell Signaling; Catalog no: 5605, dilution 1:500) Lot no: 15; Actin (Millipore, clone C4, Catalog no: MAB1501R, Lot no: 2819194, dilution 1:3000)

Validation

Validation of antibodies is ensured by commercial manufacture for the application used. For CTCF antibody from Cell Signaling, the datasheet for validation is available at https://media.cellsignal.com/pdf/3418.pdf For H3K27ac antibody from Active motif, the datasheet for validation is available at https://www.activemotif.com/documents/tds/39133.pdf For C-MYC, the validation is available in https://media.cellsignal.com/pdf/14819.pdf

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
The CUTLL1 and Jurkat cell lines were a gift from Adolfo Ferrando’s lab at Columbia.

Authentication

Cell lines have been authenticated by PCR detection of originally described translocations, detection of intra-nuclear NOTCH1 and sensitivity to originally described drugs

Mycoplasma contamination

Cell lines were tested negative for mycoplasma.
Commonly misidentified lines
(See ICLAC register)
The cell lines used in this study are not listed on the ICLAC list of commonly misidentified cell lines.

Animals and other organisms

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

Laboratory animals
NOD-SCID-Ii2rg−/− (NSG) mice between 4 to 8 weeks age

Wild animals
This study did not include wild animals.

Field-collected samples
This study did not include field-collected samples.

Ethics oversight
All animal experiments were performed in accordance with protocols approved by the New York University Institutional Animal Care and Use Committee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics
Additional co-variates (age, gender, diagnosis, mutational status) are listed in Supplementary Information.

Recruitment
Healthy T cells have been ordered commercially. Leukemia samples have been selected for two specific sub-types but potential biases are discussed in Figure 1 and Supplementary Information combining mutation status, expression and chromatin interaction information.

Ethics oversight
Samples were collected by Columbia Presbyterian Hospital or Weill Cornell Medical College with informed consent and approved and analyzed under the supervision of the Columbia University Medical Center Institutional Review Board or Weill Cornell Medical College Institutional Review Board.

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

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.
Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

ChIP-Seq data was deposited at NCBI GEO under accession GSE115893. Token: ujmpiasozxstlwx
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Files in database submission

T cell CTCF rep1
T cell CTCF rep2
T-ALL1 CTCF rep1
T-ALL1 CTCF rep2
T-ALL2 CTCF rep1
T-ALL2 input
Jurkat CTCF rep1
Jurkat CTCF rep2
Jurkat input
CUTLL1 CTCF rep1
CUTLL1 CTCF rep2
CUTLL1 CTCF rep3
CUTLL1 CTCF rep4
CUTLL1 CTCF rep5
CUTLL1 H3K27ac rep1
CUTLL1 H3K27ac rep2
CUTLL1 H3K27ac rep3
CUTLL1 H3K27ac rep4
CUTLL1 g51 H3K27ac rep1
CUTLL1 g51 H3K27ac rep2
CUTLL1 THZ1 H3K27ac rep1
CUTLL1 THZ1 H3K27ac rep2

Files in database submission

T cell CTCF rep1
T cell CTCF rep2
T-ALL1 CTCF rep1
T-ALL1 CTCF rep2
T-ALL2 CTCF rep1
T-ALL2 input
Jurkat CTCF rep1
Jurkat CTCF rep2
Jurkat input
CUTLL1 CTCF rep1
CUTLL1 CTCF rep2
CUTLL1 CTCF rep3
CUTLL1 CTCF rep4
CUTLL1 CTCF rep5
CUTLL1 H3K27ac rep1
CUTLL1 H3K27ac rep2
CUTLL1 H3K27ac rep3
CUTLL1 H3K27ac rep4
CUTLL1 g51 H3K27ac rep1
CUTLL1 g51 H3K27ac rep2
CUTLL1 THZ1 H3K27ac rep1
CUTLL1 THZ1 H3K27ac rep2
Methodology

Replicates

CTCF:
- T cells 2 replicates + 1 input
- T-ALL 1 as 2 replicates + 1 input
- T-ALL 2 as 1 replicates + 1 input
- CUTLL1 DMSO as 5 replicates + 1 input
- CUTLL1 gSI as 3 replicates + 1 input
- Jurkat as 2 replicates + 1 input

H3K27ac:
- CUTLL1 DMSO as 2 replicates + 1 input
- CUTLL1 gSI as 2 replicates + 1 input
- CUTLL1 THZ1 as 2 replicates + 1 input

Sequencing depth

Sequencing depth is detailed in Supplementary Table 1.

Antibodies

CTCF (D31H2; Catalog no: 3418, lot 3 and 4); Lot no:; 10 ug antibody used per IP
H3K27ac (Active motif; Catalog no: 39133) ; Lot no: Lot no: 01518010, 5ug antibody used per IP

Peak calling parameters

MACS2 parameters for CTCF: --nomodel --extsize=200 --qvalue 0.05
MACS2 parameters for H3K27ac: --broad --nomodel --extsize=200 --qvalue 0.05 --broad-cutoff 0.05

using -c option to specify input samples

Data quality

Based on merged peaks and peak strength, we performed Principal Component Analysis to ensure replication.

Total number of peaks detected with above peak-calling approach:
- T cells CTCF: 34443
- T-ALL 1 CTCF: 28730
- T-ALL 2 CTCF: 64059
- CUTLL1 CTCF: 25213
- CUTLL1 gSI CTCF: 18111
- Jurkat CTCF: 15196
- CUTLL1 H3K27ac: 30726
- CUTLL1 gSI H3K27ac: 25309
- CUTLL1 THZ1 H3K27ac: 30542

Software

Read alignment: Reads were aligned against the reference sequence hg19 with bowtie2 (version 2.3.1) with standard parameters and only uniquely mapped reads were kept with MAPQ > 20.

Deduplication: Aligned reads were filtered for duplicated reads using picard-tools version 2.6.0.

Peak-calling: Peak calling for CTCF and H3K27ac was performed using MACS2 (version 2.0.1) using narrow (CTCF) and broad (--broad; H3K27ac) option (special parameters: --no-model).

Differential binding: To identify differentially bound peaks, we performed diffBind (version 2.2.12) analysis, using the normalization option DBA_EDGER.

Bigwig: For visualization purposes, we generated fold-enrichment bigwig files by applying MACS2 (version 2.0.1) bdgcmp over input (-m FE)