3D Genome Analysis Identifies Enhancer Hijacking Mechanism for High-Risk Factors in Human T-Lineage Acute Lymphoblastic Leukemia

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Recent studies have demonstrated that 3D genome alterations play important roles in tumorigenesis¹⁻³, including the development of hematological malignancies⁴⁻⁷. However, how such alterations may provide key insights into T-lineage acute lymphoblastic leukemia (T-ALL) patients is largely unknown. Here, we report integrated analyses of 3D genome alterations and differentially expressed genes (DEGs) in 18 newly diagnosed T-ALL patients and 4 healthy T cell controls. We found that 3D genome organization at the compartment, topologically associated domains (TAD) and loop levels as well as the gene expression profiles could hierarchically classify different subtypes of T-ALL according to the T cell differentiation trajectory. Alterations in the 3D genome were associated with nearly 45% of the upregulated genes in T-ALL. We also identified 34 previously unrecognized translocations in the noncoding regions of the genome and 44 new loops formed between translocated chromosomes, including translocation-mediated enhancer hijacking of the *HOXA* cluster. Our analysis demonstrated that T-ALLs with *HOXA* cluster overexpression were heterogeneous clinical entities, and ectopic expressions of the *HOXA11-A13* genes, but not other genes in the *HOXA* cluster, were associated with immature phenotypes and poor outcomes. Our findings highlight the potentially important roles of 3D genome alterations in the etiology and prognosis of T-ALL.

Keywords: 3D genome alterations in T-ALL, chromosomal translocation, enhancer hijacking, ectopic *HOXA11-A13* expression, poor prognosis of T-ALL.

48 T-ALL is an aggressive hematological malignancy caused by genetic and epigenetic
49 alterations that affect normal T cell development⁸. Recent whole-exome and RNA
50 sequencing analyses of large T-ALL cohorts are focused on the coding region of the
51 genome and have identified novel driver mutations, dysregulated transcription factors
52 and pathways in T-ALLs⁹⁻¹². To determine whether alterations in the 3D genome
53 organization are associated with malignant transformation of T-ALL, we conducted
54 BL-Hi-C¹³ analysis using purified primary leukemic blasts from 18 newly diagnosed
55 T-ALL patients, including 8 early T-cell precursor ALL (ETP ALL) and 10 non-ETP ALL,
56 two clinical subtypes of T-ALL, as well as normal T cells from 4 healthy volunteers
57 (Supplementary Fig. 1a). The maximum resolutions of the chromatin contact maps for
58 ETP, non-ETP ALL and normal samples were approximately 3.5, 3.5 and 10 kb,
59 respectively (Supplementary Table 1).

60 Principal component analysis (PCA) at the levels of the compartment, TAD and
61 loop structures demonstrated that the T-ALL samples could be separated from the
62 control samples by PC1, while ETP and non-ETP ALL could be separated by PC2 at
63 all three architectural levels (Fig. 1a, upper panels) and be further delineated by
64 hierarchical clustering analysis (Fig. 1a, lower panels). Detailed comparisons of the
65 3D chromosomal organizations of the T-ALL patients and the healthy controls
66 revealed 1.59% of A-to-B and 1.38% of B-to-A T-ALL-associated switches at the
67 compartment level (Supplementary Fig. 1b), 11% (377/3421) T-ALL-specific TAD
68 boundaries (Supplementary Fig. 1c), and 6% (2330/38464) enhanced and 11%
69 (4073/38464) reduced loops (Supplementary Fig. 1d), implying that the global 3D

70 genome alterations could link to the etiology of T-ALL development.

71 RNA-seq analysis was also performed on each sample to investigate the impact
72 of 3D genome alterations on gene expression in the T-ALLs. PCA and hierarchical
73 clustering showed that the transcriptome changes were highly consistent with the
74 corresponding 3D genome structural changes (comparing Fig. 1a and 1b) and hence
75 could serve as a biological readout for the functional impact of 3D genome alterations
76 in T-ALL development.

77 Approximately 29% (996/3392) of the DEGs were associated with 3D genome
78 alterations (Supplementary Table 2). Among them, the genes associated with the
79 B-to-A compartment switches, increased domain scores (D-score) and the
80 corresponding enhanced loops were mostly upregulated (Fig. 1c, red bars and Fig.
81 1d), and were functionally enriched in pathways such as hematopoietic cell lineage,
82 transcriptional misregulation in cancer and cell cycle (Fig. 1e). In contrast, the genes
83 associated with the A-to-B compartment switches, decreased D-scores and reduced
84 loops were mostly downregulated (Fig. 1c, blue bars and Fig. 1d), and these genes
85 were enriched in pathways such as cytokine-cytokine receptor interaction and T cell
86 receptor signaling (Fig. 1e).

87 Among the upregulated DEGs, *CDK6* is a potential target for T-ALL treatment¹⁴.
88 The *CDK6* locus exhibited a strong intra-TAD interaction, and its expression was
89 upregulated in all T-ALL samples (Fig. 1f; Supplementary Fig. 1e). Similarly, several
90 upregulated oncogenic driver genes and T-ALL-associated transcription factors, such
91 as *MYB*, *MYCN*, *BCL11A*, *SOX4* and *WT1*, also had increased D-scores

92 (Supplementary Fig. 1f).

93 While the majority of the DEGs were associated with structural changes at 1 or 2
94 levels, transcription factor *SOX4*, *WT1* and *TFDP2* had structural alterations at all 3
95 levels (Fig. 1g, Supplementary Fig. 1g and Supplementary Table 2). Importantly,
96 when RcisTarget¹⁵ was used to predict the key transcription factors that might control
97 the upregulated genes in the T-ALLs, *SOX4*, *WT1* and *TFDP2* were among the
98 top-ranking candidates that potentially regulated approximately 28% of the
99 upregulated genes (Supplementary Table 3). After eliminating the overlapping
100 upregulated DEGs, we estimated that 3D genome alterations could associate with
101 approximately 45% of the upregulated genes in T-ALL, of which 56% could be directly
102 related to structural alterations, while the rest could be due to structure
103 alteration-mediated dysregulated transcription factors.

104 ETP ALL is a subclass of T-ALL stalled at the early T progenitor stage, while
105 non-ETP ALLs are blocked at the later T cell differentiation stages¹⁶. Our 3D genome
106 landscape analyses could separate the ETP ALL samples from the non-ETP ALL
107 samples (Fig. 1a), suggesting that the chromosomal organizations of T-ALL may
108 represent different “frozen stages” of T cell development¹⁷. To test this hypothesis, we
109 projected the T-ALL samples onto the T cell developmental trajectory defined by
110 RNA-seq analysis¹⁸. PCA revealed that most of the ETP ALL samples were arrested
111 at the immature stage, corresponding to the LMPP to Thy1 stages, while the non-ETP
112 samples were arrested at the Thy2 to Thy4 stages (Fig. 2a). Since the ETP and
113 non-ETP ALLs can be better separated at the loop level (Fig. 1a), we further analyzed

114 the differences in loop structures between ETP and non-ETP ALL samples and
115 identified 1820 enhanced and 831 reduced loops in ETP (Fig. 2b). When plotting gene
116 expression changes between ETP and non-ETP ALL against the combined p-value of
117 the loop strength and D-score changes, we found a strong positive correlation
118 (Pearson's correlation coefficient 0.685; Fig. 2c). Approximately 20% and 16% of the
119 upregulated genes in ETP and non-ETP ALL, respectively, harbored chromatin
120 structure changes, including key transcription factors or oncogenes, such as *CEBPA*,
121 *MYCN* and *LYL1* for ETP and *LEF1*, *TCF12* and *PAX9* for non-ETP ALL (Fig. 2c and
122 Supplementary Table 4).

123 Gene ontology analysis further revealed that genes associated with the ETP ALL
124 enhanced loops were enriched in immune response-activating signal transduction,
125 myeloid cell differentiation and regulation of B cell activation, consistent with the
126 definition of ETP ALL (Fig. 2d). Genes associated with the non-ETP ALL enhanced
127 loops were enriched in terms such as positive regulation of RNA metabolism,
128 transcription, and TCR V(D)J recombination (Fig. 2d). The lack of TCR rearrangement
129 in most of the ETP ALL samples (Supplementary Fig. 2a) and different
130 rearrangements in individual non-ETP samples (Supplementary Fig. 2b) could be
131 easily observed from the Hi-C maps (Fig. 2e). Sample 093 was a unique case as it fell
132 between ETP and non-ETP ALL (Fig. 1a and 1b; Fig. 2a) and had significant TCR
133 rearrangement (Supplementary Fig. 2a). We also observed a lack of *RAG1* and
134 *PTCRA* expression in most of the ETP ALL samples, which are essential for TCR
135 V(D)J rearrangements (Fig. 2f).

136 The 3D genome alteration analysis also provided a potential explanation for ETP
137 and non-ETP ALL-specific transcription factor expressions. For example, we detected
138 subtype-specific loops and expression patterns in the *MEF2C* locus in ETP and the
139 *PAX9* locus in the non-ETP ALL samples, respectively, which were associated with
140 H3K27ac and CTCF marks in the ETP cell line KE37 and non-ETP cell line Jurkat (Fig.
141 2g and 2h).

142 Chromosomal translocation is one of the major driving forces for tumorigenesis¹⁹,
143 especially for leukemia⁸. By adapting hic_breakfinder²⁰, we identified 46
144 translocations in 14/18 T-ALL samples (Supplementary Table 5), of which 34 were
145 novel events and 26 were interchromosomal events (Fig. 3a, red lines). Among 78
146 unique breakpoints identified, 47% were located in noncoding regions, and 66% were
147 located in the stable A compartment (Supplementary Fig. 3a). These newly identified
148 translocations not only influenced the expression of the nearest genes (Fig. 3a) but
149 also resulted in the formation of 44 new loops across the translocated chromosomes,
150 which we named translocation-mediated loops (Supplementary Fig. 3b;
151 Supplementary Table 6). Interestingly, the ends of these translocation-mediated loops
152 tend to anchor at the pre-existing loop anchors and CTCF binding sites (Fig. 3b).
153 Importantly, nearly 78% of the translocation-mediated loops with CTCF motifs were
154 linked to pairs of convergently orientated CTCF motifs (Fig. 3c), indicating that these
155 loops may be mediated by loop extrusion mechanism²¹⁻²³.

156 We then investigated the potential mechanisms underlying
157 translocation-mediated T-ALL classification and gene activation. Clinically, non-ETP

158 ALL can be further classified into the HOXA, TLX and TAL subtypes according to their
159 gene expression profiles²⁴. Notably, there was a complete match between loop-based
160 hierarchical clustering and T-ALL subtypes, which were signified by chromosomal
161 translocation-mediated dysregulation of T-ALL-associated transcription factors (Fig.
162 3d).

163 Translocations can activate T-ALL-associated transcription factors via either “cis”
164 or “trans” mechanisms. The “cis” mechanism involved translocation-mediated
165 enhancer hijacking in the ETP, TLX and TAL subtypes (Fig. 3d and Supplementary Fig.
166 3b), of which the ectopically expressed genes, such as *TLX3*, hijacked the enhancers
167 from the translocated *BCL11B* gene via translocation-mediated loops (Fig. 3e and
168 Supplementary Fig. 3c-d). The “cis” mechanism included *BCL11B-TLX3*, *TRB-TAL2*,
169 and 3 novel *HOXA* translocations identified in this study (Supplementary Fig. 3b and
170 Supplementary Table 6; see Fig. 5). Interestingly, most of the hijacked enhancers are
171 from genes that are normally expressed during T cell development, such as *BCL11B*
172 and *TRB*, which lead to ectopic expression of T-ALL-associated transcription factors
173 in the T lineage and block normal differentiation (Supplementary Fig. 3d). The “trans”
174 mechanism involves translocation-mediated gene fusions, such as the *PSIP1-NUP98*,
175 *SET-NUP214*, and *MLL* (*KMT2A-MLLT1*, *PICALM-MLLT10*, and *DDX3X-MLLT10*)
176 gene fusion events, which could epigenetically activate *HOXA* cluster gene
177 expressions²⁵⁻²⁸. These results suggest that translocation-mediated enhancer
178 hijacking or fusion events may drive ectopic transcription factor activation, leading to
179 specific pathogenic gene expression profiles of the T-ALL subgroups.

180 The dysregulated *HOXA* cluster, which contains 11 genes, is a common feature
 181 of T-ALL²⁴. However, whether *HOXA*-positive T-ALLs represent a homogeneous
 182 clinical entity has not been systematically studied. We conducted unsupervised
 183 hierarchical clustering based on *HOXA* gene expressions, which separated 15 T-ALL
 184 samples without *HOXA* translocation into *HOXA*-negative (*HOXA*⁻) and
 185 *HOXA*-positive expression (*HOXA*⁺) groups. Translocation-mediated fusion events
 186 could be detected in 5/7 *HOXA*⁺ samples. The *HOXA*⁺ T-ALLs could be further
 187 separated into 2 subgroups: the 3'*HOXA*⁺ or 5'*HOXA*⁺ subgroups, with respect to the
 188 location of the *HOXA* genes within the *HOXA* cluster (Fig. 4a). The expression
 189 patterns of the three *HOXA* translocation cases (*HOXA*-T, breakpoints shown in Fig.
 190 4b) were closer to those in the 5'*HOXA*⁺ subgroup, characterized by ectopic
 191 *HOXA11-A13* expressions (Fig. 4a). Interestingly, the 5'*HOXA*⁺ and *HOXA*-T cases in
 192 our Hi-C study are associated with double negative (DN) and ETP phenotypes (Fig.
 193 4a), suggesting that T-ALLs with ectopic *HOXA* cluster gene expression are
 194 heterogeneous entities.

195 The *HOXA* genes are transcriptionally repressed in normal T cells but can be
 196 transactivated in T-ALLs by fusion proteins that recruit histone methyltransferase
 197 DOT1L to the *HOXA* locus^{25,29}. Although this mechanism uncovered how the *HOXA*
 198 cluster is activated, it cannot explain the diverse *HOXA* expression patterns
 199 associated with different fusion proteins. By integrating Hi-C maps with *HOXA* gene
 200 expression patterns, we found that the differential *HOXA* gene expressions were
 201 associated with different 3D genome organizations in samples without *HOXA*

translocation. Hi-C maps and CTCF motif orientations showed that the 11 *HOXA* genes were partitioned between 2 TADs (Fig. 4b and Supplementary Fig. 4a): the CTCF binding site C11/13 was used as the 3' boundary of the 5' TAD in all samples, while the 5' boundaries of the 3' TADs varied among different samples: C7/9 was used by most of the *HOXA*⁻ (6/8) and all 5'*HOXA*⁺ samples (2/2), while C10/11 was used by most of the 3'*HOXA*⁺ samples (4/5) (red and blue arrows/lines, respectively; Fig. 4b and Supplementary Fig. 4a).

We further identified 6 enhancer regions in each TAD, labeled E1 to E12, which could interact with the *HOXA* cluster (Fig. 4c). Using *HOXA*⁻ cases as common denominators (Fig. 4c, top panel), we calculated the overall differential interaction intensities. Although there was no significant difference between the healthy controls and the *HOXA*⁻ cases in the 12 interaction regions, we found significantly enhanced interactions between E2-E6 and genes in the 3'*HOXA* subgroup, as well as between E8, 9, 11, 12 and genes in the 5'*HOXA* subgroups, either as a group average (Fig. 4c) or individually (Supplementary Fig. 4b-c). ChIP-seq analysis of the *HOXA*⁺ Loucy cell line indicated that these increased interactions may be correlated with gains in the H3K27ac histone mark (Fig. 4c). The KMT2A-MLLT1, PICALM-MLLT10, DDX3X-MLLT10 and SET-NUP214 fusions were associated with interaction dynamics and *HOXA*1-A10 expression in the 3'*HOXA* subgroup, while the PSIP1-NUP98 fusion event was associated with the 5'*HOXA* subgroup (Fig. 4d). These results suggest that different translocation-mediated fusion events may epigenetically and preferentially

223 alter the 3D genome interactome within the *HOXA* cluster and control a specific set of
224 *HOXA* gene expression.

225 To explain the gene expression patterns seen in the three *HOXA*-T samples, we
226 mapped the breakpoints and found that all the breakpoints were located within the 5'
227 TAD, upstream of the *HOXA13* gene. The translocation partner breakpoints lie in the
228 gene bodies of the *BCL11B* and *CDK6* genes on chromosome 14 and chromosome 7,
229 respectively, as well as upstream of the *ERG* gene on chromosome 21 (Fig. 5 and
230 Supplementary Fig. 5). By examining the TAD structures associated with the
231 translocations, using *HOXA*⁺ samples as controls, we found that these translocations
232 mediated new loop formations between the 5' of the *HOXA* cluster and active
233 enhancers associated with *BCL11B* and *ERG* genes, leading to ectopic expression of
234 *HOXA13* in the case of 076 and *HOXA9-A13* in the case of 077 (blue circles for new
235 loops and green bar graphs for gene expressions; Fig. 5a and b; Supplementary Fig.
236 3b). For the case of 108, the inter-TAD inversion led to the adoption of active *CDK6*
237 enhancers and new loop formation (Fig. 5c), causing ectopic expression of
238 *HOXA11-A13* (Fig. 5c). These results suggest that translocations and inversion
239 associated with the 5' TAD of the *HOXA* cluster lead to dysregulated 5' *HOXA* gene
240 expressions through new loop formation-mediated enhancer hijacking. Again, the
241 existence of the CTCF binding sites C11/13 in the case of 076, C7/9 in the case of 077,
242 and C10/11 in the case of 108 (Fig. 5d) could insulate genes located in the 3' TAD
243 from the influence of the hijacked enhancers, leading to 5'*HOXA*-specific expression
244 patterns (Fig. 4b).

245 We next investigated whether ectopic *HOXA* cluster expression was associated
246 with poor prognosis. For this, we analyzed a cohort of T-ALL patients with outcome
247 information (see our companion paper). We found that *HOXA11* or *HOXA13* positivity,
248 alone or in combination, but not the expression of other *HOXA* genes, such as the
249 previously used biomarker *HOXA9*, was associated with poor overall and event-free
250 survivals in young adult and pediatric T-ALLs. In contrast, T-ALLs with *TAL1/2*
251 positivity, which was mutually exclusive from that with *HOXA* positivity, were
252 associated with better overall survival, while *TLX* positivity had no association with the
253 outcome (Fig. 6a and Supplementary Fig. 6a-b). Compared to the *HOXA1-A10*⁺, *TAL*⁺
254 and *TLX*⁺ samples, the *HOXA11-A13*⁺ cases were characterized by DN and ETP
255 phenotypes (Fig. 6b and Supplementary Fig. 6c), as well as a higher rate of
256 JAK-STAT pathway gene mutations^{9,30} (Fig. 6c). In multivariate analysis, *HOXA13*⁺
257 status could serve as an independent predictor for the overall survival of pediatric and
258 young adult T-ALLs (Fig. 6d).

259 Taken together, our results imply that 3D genome alterations, in addition to
260 previously identified oncogenic driver mutations and dysregulated pathways, may
261 play critical roles in rewiring T-ALL transcriptome and regulating T-ALL development.
262 The newly identified translocations and enhancer hijacking mechanism may provide a
263 potential explanation for how T-ALL-associated transcription factors are ectopically
264 activated and block differentiation at certain T cell developmental stages. Finally, we
265 identified the associations of ectopic *HOXA11-A13* expression and poor survival of
266 T-ALL, corresponding to the subtype currently incurable by standard treatments, and

267 suggested that anti-JAK-STAT inhibitors may benefit this group of patients.

268

269 **Author contributions**

270 LY, HZ and HW conceived the project; YC designed the Hi-C and RNA-seq
271 experiments; LY, HZ, MS and WW performed the Hi-C and RNA-seq experiments; FC
272 designed the bioinformatic pipelines and performed the Hi-C and RNA-seq integrated
273 analyses, while BD conducted the survival analysis. QJ, LZ and XH contributed the
274 clinical samples and data. LY, FC and BD generated the figures and tables. LY, FC
275 and HW wrote the manuscript with help from all authors. XH was in charge of the
276 clinical study; MZ and YC oversaw the bioinformatics analyses, and HW supervised
277 the entire project.

278

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289

290 **Conflicts of Interest**

291 The authors declare no competing financial interests.

292

Materials and Methods

Patients and samples. Eighteen T-ALL patient samples were collected under the protocol approved by the ethics committee of the Institute of Hematology at Peking University. The patient characteristics are described in Supplementary Table 7. ETP status was defined as previously published¹⁶. Leukemia blast cells were prepared by density-gradient centrifugation of bone marrow samples, and CD19⁻CD14⁻CD235⁻CD45⁺CD7⁺ cells were further purified by FACS analysis using anti-human antibodies for RNA-seq and Hi-C library preparations. Peripheral blood samples were obtained from four healthy donors under the approval of the ethics committee of Peking University. T cells were purified using the EasySep™ Direct Human T Cell Isolation Kit (StemCell Technology #19661).

RNA-seq library preparation, data processing, and differential gene expression

analysis. RNA-seq libraries were prepared with TruSeq RNA Library Prep Kit v2 (Illumina). Paired-end RNA-seq reads of the 18 patients and 4 healthy controls were generated with an average depth of 15 million read pairs. Reads were aligned to the hg19 genome with TopHat (v2.1.0) using default settings³¹. Duplicates were removed, and aligned reads were calculated for each protein-coding gene using HTSeq³², followed by FPKM transformation by normalizing gene exon length and sequencing depth. Raw RNA-seq data for Loucy and Jurkat were downloaded from the GEO database and analyzed as described above.

DESeq2³³ was applied to identify the differentially expressed genes with FDR <0.01 and fold change >2. Genes with fewer than 5 reads in 20% of the samples or with mean reads fewer than 2 were excluded. Signal tracks were generated by using BEDTools³⁴ genomeCoveragebed to produce bedGraph files scaled to 1 million reads per data set. Then, the UCSC Genome Browser utility³⁵ bedGraphToBigWig was used with default parameters to generate bigwig files.

ChIP-seq, ATAC-seq data processing and motif analysis.

ChIP-seq reads were mapped to the hg19 genome with Bowtie2³⁶ (v2.3.5) using default parameters, while

ATAC-seq reads were mapped with Bowtie2 using parameter -X 2000 --no-mixed --no-discordant --no-unal. Aligned reads were filtered for a minimum MAPQ of 20, and duplicates were removed using SAMtools³⁷. Signal tracks and peaks were generated by using the -SPMR option in MACS2³⁸. Then, the UCSC Genome Browser utility bedGraphToBigWig was used with default parameters to transform the bedgraph files to bigwig files. FIMO³⁹ was used to detect the 20 bp CTCF motif from the Homer motif database in Loucy and Jurkat CTCF peaks with default parameters.

330

Hi-C and Hi-C data processing. Hi-C was performed on one million cells/sample, according to the BL-Hi-C protocol¹³. Raw BL-Hi-C reads were processed by the in-house HiCpipe framework, which integrated several Hi-C analysis methods for to generate multiple features of the Hi-C data. In particular, ChIA-PET2⁴⁰ was used to trim the bridge linkers and HiC-Pro⁴¹ to align reads, filter artifact fragments, and remove duplicates; Juicer⁴² was applied to the resulting uniquely mapped contacts to generate individual or merged Hi-C files that could be deposited as contact matrices with multiple resolutions. Knight-Ruiz⁴³ (KR)-normalized matrices were used in the compartment and TAD analyses.

340

Compartment and TAD analysis. The compartment was calculated with the eigenvector command of Juicer under 100 kb resolution KR normalized Hi-C matrices. For every 100 kb bin, A or B compartments were defined by the over 70% sample majority rule.

TAD boundaries were calculated by the Insulation score method⁴⁴ (with parameters: -is 1000000 -ids 200000 -im mean -nt 0.1) on pooled 40 kb Hi-C matrices of the healthy T cell controls, ETP and non-ETP samples. The resulting TAD boundaries were merged and assigned with relative insulation scores of all samples calculated from HiCDB⁴⁵. Differential TAD boundaries were defined with a t-test FDR<0.01 and a difference between cases and controls higher than 50% quantile of the overall difference.

A TAD was defined when its boundaries were detected in at least two conditions

among normal T cells, ETPs and non-ETPs. The domain score⁴⁶ was calculated in each sample by dividing the intra-TAD interactions with all interactions connected to the corresponding TAD. Differential domain scores were calculated with a t-test FDR<0.01 and fold change higher than 70% quantile of the overall fold change.

Loop detection and differential loop calling. Loops were called by HiCCUPS⁴² at 5 kb and 10 kb resolutions with default parameters (except -d 15000,20000) for pooled Hi-C matrices of the healthy T cell controls, ETP and non-ETP samples, respectively. The differential loop detection method was adapted from Douglas *et al.*⁴⁷. Loops were split into two distance ranges (> or < 150 kb) to minimize potential bias (Rubin *et al.*⁴⁸). Differential loops were called within each range (FDR < 0.1) and then combined.

Loop aggregation and functional analysis. Aggregate peak analysis (APA) plots were generated to assess the quality of loop detection and explore the characteristics of different loop classes by the Juicer APA command⁴² under 5 kb resolution. Its output matrix was normalized by the loop number that contributed to the matrix generation. For analysis of the function of dynamic loops between non-ETP and ETP, the loop anchors were analyzed by GREAT⁴⁹ (v3.0.0) using the nearest gene within 100 kb to generate the enriched biological process. In other sections, genes related to loops were determined if their promoter (5 kb around TSS) overlapped the loop anchors, and DAVID⁵⁰ 6.8 was used for KEGG pathway enrichment analysis.

Visualization and V4C plot generation. Tracks of Hi-C maps and ChIP-seq were generated by pyGenomeTracks⁵¹. Hi-C maps of each condition were normalized by its cis interaction pairs. A visual 4C (V4C) plot for specific loci was generated as the interaction s related to the corresponding viewpoint under 10 kb resolution.

Translocation and translocation-mediated loop detection with Hi-C. hic_breakfinder²⁰ was adapted to detect translocations in 18 T-ALL patient samples. After we filtered the “translocations” also detected in normal controls, the remaining

translocations were manually assessed, and the precise breakpoints were determined. As the average depth of patient Hi-C samples is 486 million read pairs and the read length is 150 bp, Hi-C raw data were treated as single ends to refine the breakpoint locations to single base-pair resolution. Any single ends that could be mapped to two different chromatins without the BL-Hi-C bridge linker in between were chimeric reads. The chimeric reads detected from the BL-Hi-C data overlapped with the aforementioned translocations at 5~20 kb resolution. For each translocation, the exact locations (single base-pair resolution) supported by more than 3 chimeric reads were identified as the actual breakpoints and are reported in Supplementary Table 5.

As translocation-mediated loops were hard to identify by loop detection tools designed for intrachromosomal loop detection and easy to capture by visualization, their locations were manually recorded on interchromosome Hi-C maps with the help of Juicebox⁵², which is an interactive visualization software.

Translocation, translocation-mediated loop annotation and visualization. The nearest genes to translocation breakpoints were determined by BEDTools. Known translocations were collected from references 2 and 4 and ChimerPub⁵³. A translocation was considered novel if any of the breakpoints was not near any known breakpoint within a 100 kb distance. For translocation-mediated loops, the genes with a promoter or gene body overlapping the loop anchors were annotated as the associated genes. A gene near a breakpoint was considered upregulated if its FPKM was >1- and 2-fold higher than the control samples without nearby breakpoints. Hi-C heatmaps and Visual 4C plots of the reassembled chromatin were generated by MATLAB code.

Statistics. Specific statistical analyses are described in each section. In general, the Wilcoxon rank sum test was employed in R for comparisons of distributions. Survival analysis was performed by a Cox regression model using overall and event-free survival as outcomes. Overall survival was defined as the time from diagnosis to death from any cause. Event-free survival was defined as the time from diagnosis to treatment

413 failure, relapse, or death from any cause. The proportional hazard assumption was
 414 tested. Variables tested in the multivariable Cox regression model were sex, age
 415 (pediatric vs. adult), white blood cell counts, hemoglobin levels, platelet counts,
 416 hepatosplenomegaly, percentage of blasts in the bone marrow and MRD status.
 417 Eighty-six patient samples with RNA-seq data were used for DN- and ETP-
 418 enrichment analyses, of which 38 samples under the age of 40 with outcome and 63
 419 samples with whole exon sequencing (WES) data were used for survival and mutation
 420 analyses, respectively.

421

422 **Code availability.** Custom scripts described in the Online Methods will be made
 423 available upon request.

424

425 **Data availability.** All sequencing data are available through the Gene Expression
 426 Omnibus (GEO) via accession GSE. Accession codes of the published data used in
 427 this study are as follows: CTCF ChIP-seq of CD4+ T cell and Jurkat cell line,
 428 GSE12889; CTCF ChIP-seq of Loucy cell line, GSE123214; ATAC-seq of CD4+ T cell,
 429 GSE87254; ATAC-seq of Jurkat cell line, GSE115438; H3K27ac ChIP-seq of CD4+ T
 430 cell, GSE122826; H3K27ac ChIP-seq of Jurkat cell line, GSE68978; H3K27ac
 431 ChIP-seq of Loucy cell line, GSE74311; RNA-Seq of Loucy cell line, GSE100694;
 432 RNA-seq of T cell development, GSE69239. The raw sequence data reported in this
 433 paper have been deposited in the Genome Sequence Archive⁵⁴ in BIG Data Center⁵⁵,
 434 Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession
 435 number HRA000113 that is publicly accessible at <https://bigd.big.ac.cn/gsa>.

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Fig.1 Global 3D genome architectures in T-ALLs. (a) PCA (upper) and unsupervised hierarchical clustering analysis (lower) of compartment, domain score (D-score) and loop in normal T cells and T-ALLs. (b) PCA (upper) and unsupervised hierarchical clustering analysis (lower) of gene expression profiles in normal T cells and T-ALLs. (c) Association of DEGs and genomic alterations at the levels of compartment, TAD and loop. Red, upregulation; blue, downregulated. The p values were calculated using Wilcoxon rank sum test. (d) A summary of DEGs and their corresponding chromatin structure changes. (e) KEGG analysis for enriched pathways in T-ALLs and normal T cells based on DEGs that are associated with chromatin structural changes. (f) and (g) Top, heatmaps show the compartment scores for each sample across the genomic regions of the *CDK6* and *SOX4* loci, respectively. Bar plots on the right show the gene expression level of *CDK6* and *SOX4* for each sample. Bottom, Hi-C contact maps of the *CDK6* and *SOX4* loci in normal T cell and T-ALL; blue circles: enhanced loops in T-ALL. ATAC-seq tracks and ChIP-seq tracks of CTCF and H3K27ac of normal T cells and T-ALL Jurket cells are also included.

Fig. 2 ETP and non-ETP ALLs have different loop structures. (a) PCA shows the association between ETP and non-ETP ALL gene expression profiles and T cell developmental trajectory. (b) APA plots for loops that are enhanced (top) or reduced (bottom) in ETP compared with non-ETP ALL. (c) Scatterplot shows the correlation between gene expression changes and structural changes. The structural change was defined by the combined p-value of D-score and loop strength change. Top ETP and non-ETP ALL-associated transcription factors with structural changes are highlighted in red and green, respectively. (d) GO terms for genes adjacent to enhanced loop anchors in ETP (top) and non-ETP (bottom). (e) Hi-C contact maps for the TCR genomic regions in ETP and non-ETP ALLs. (f) *RAG1* and *PTCRA* expression levels. (g) and (h) Hi-C contact maps for TADs enclosing the genomic loci of ETP expressed *MEF2C* (g) and non-ETP expressed *PAX9* (h). Anchors of differential loops are labeled with dashed lines.

a

b

c

d

e

case 093
t(5;14) (q35;q32)

case 117
t(5;14) (q35;q32)

Fig. 3 Chromosomal rearrangements in T-ALLs. (a) The genomic landscape of translocations discovered by Hi-C. The novel translocation partners are connected by red lines and known translocations are shown by black lines. Breakpoints nearest genes with increased or decreased expressions are highlighted by red and blue, respectively. Recurrent breakpoints nearest genes are labeled in bold. The genes closest to the breakpoint in noncoding regions are marked by star symbols. (b) Majority of the translocation-mediated loop anchors are pre-existing loop anchors and contains CTCF binding sites. (c) Majority of translocation-mediated loops have convergent CTCF motif orientation. (d) The loop-based clustering overlaps with leukemogenic transcription factor- or translocations-based clustering in T-ALLs. *STIL-TAL1* fusions were detected by RT-qPCR, the other translocations were detected by Hi-C. (e) Upper panels: Hi-C heatmaps for cases with *BCL11B-TLX3* translocation (top), visual 4C plots generated from Hi-C contact maps using *TLX3* promoter as the viewpoint (middle) and averaged Hi-C heatmaps for cases without *BCL11B-TLX3* translocation as controls (bottom). Breakpoints are marked by the red lines and yellow lightning bolts. Translocation-mediated loops are highlighted by blue circles, corresponding loop locations at controls are highlighted by dotted line circles. Lower panels: a schematic illustration for the consequence of the *BCL11B-TLX3* translocation.

Fig. 4 Chromatin interaction profile and expression patterns of the HOXA cluster

in T-ALLs. (a) T-ALL subtypes with different *HOXA* gene expression patterns. *HOXA*-translocation (*HOXA*-T) negative cases are grouped by unsupervised hierarchical clustering based on the *HOXA* gene expressions. (b) ChIP-seq tracks of CTCF and H3K27ac and ATAC-seq tracks in T cell, *HOXA*⁻ Jurkat and *HOXA*⁺ Loucy T-ALL cells in the genomic region corresponding to the pink bar of Fig. 4c. CTCF motif orientations, 5'TAD and 3'TAD boundaries and *HOXA*-T breakpoints are also included. (c) Top: a Hi-C heatmap shows the average interaction intensity of *HOXA*⁻ cases in chr7: 25,750,000-28,250,000 (hg19), which includes *HOXA* gene cluster and its 3' and 5' TADs. Middle left: from top to bottom, Hi-C heatmaps show differential interaction intensities between normal T cell vs. *HOXA*⁻, 3' *HOXA*⁺ vs. *HOXA*⁻ and 5' *HOXA*⁺ vs. *HOXA*⁻ cases using visual 4C plot. Each line represents interactome of a *HOXA* gene shown on the left. *HOXA5* and *HOXA6* share one line as they located in the same bin. The main enhancers are highlighted with orange in the 3' TAD and blue in the 5' TAD. Middle right: Top right of each heatmap represents the averaged interaction intensities of normal T cell, 3' *HOXA*⁺ cases and 5' *HOXA*⁺ cases, respectively; bottom left of each heatmap represents the differential interaction intensities between each group and *HOXA*⁻ cases. Bottom: ChIP-seq tracks for CTCF and H3K27ac and ATAC-seq tracks in T cell, *HOXA*⁻ Jurkat and *HOXA*⁺ Loucy T-ALL cells. (d) Schematic illustrations of the associations among 3D genomic interactions, *HOXA* cluster expressions and associated fusion events in 3' *HOXA* cases (left) and 5' *HOXA* cases (right).

Fig. 5. Translocation-mediated enhancer hijacking and ectopic *HOXA* gene expressions in T-ALLs. (a-c) Individual Hi-C heatmaps for three *HOXA*-T cases (top) and averaged Hi-C heatmaps of *HOXA*⁻ control cases (bottom). Middle panels are differential visual 4C plots between *HOXA*-T cases and *HOXA*⁻ using *HOXA1-A13* genes (from top to bottom) as the viewpoint. The green bar graphs on the right show the expression level of each *HOXA* gene. Breakpoints are marked by the red lines and yellow lightning bolts. Translocation-mediated loops are highlighted by blue circles, corresponding locations in control samples are marked by dotted circles. (d) Schematic illustrations of the consequences of *HOXA*-related translocations (upper) and inversion (lower).

a

b

c

d

	Univariate			Multivariate (HOXA13)			Multivariate (HOXA11-13)		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
HOXA13 expression (FPKM) ≥ 1 vs. < 1	4.675	1.39-15.69	0.013	38.25	1.37-1071	0.032			
HOXA11-13 expression (FPKM) ≥ 1 vs. < 1	5.872	1.84-18.74	0.003				8.84	0.82-95.24	0.072
Gender Males vs. Female	1.13	0.42-3.01	0.806	0.959	0.05-17.78	0.978	0.89	0.066-12.015	0.93
Age Pediatric vs. Adult	0.21	0.07-0.64	0.006	0.154	0.006-3.684	0.248	0.34	0.014-8.444	0.512
WBC Low vs. High	1.46	0.62-3.43	0.381	73.52	1.59-3390	0.028	31.95	1.5-680.5	0.026
Hemoglobin	1	0.99-1.02	0.571	1.02	0.94-1.11	0.574	1.008	0.94-1.077	0.813
Platelet	1	0.99-1	0.952	0.986	0.968-1.004	0.127	0.987	0.971-1.004	0.139
Hepatosplenomegaly Yes vs. No	1.47	0.34-6.33	0.605	0.24	0.004-15.882	0.504	1.67	0.022-128.414	0.817
Blasts in BM	1.01	0.98-1.03	0.621	1.107	0.906-1.352	0.319	1.08	0.9-1.294	0.407

Fig. 6 Ectopic *HOXA11-A13* expressions are correlated with poor outcomes in pediatric and young adult T-ALLs. (a) Kaplan-Meier overall survival curves for patients with (red) and without (blue) ectopic *HOXA13* expression (left), *HOXA11-A13* expressions (middle) and *TAL1/TAL2* expressions (right) in pediatric and young adult patients. (b) The associations between ectopic *HOXA11-A13* expression and DN and ETP phenotypes. *P*-values are calculated by Fisher exact test (**: $p < 0.01$). (c) The proportion of cases with JAK-STAT pathway mutations in each T-ALL subgroup, *P*-values are calculated by Fisher exact test (**: $p < 0.01$). (d) Univariable and multivariable analysis of overall survival. HR, hazard ratio; CI, confidence interval.