

Low input capture Hi-C (liCHi-C) identifies promoter-enhancer interactions at high-resolution

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Long-range interactions between regulatory elements and promoters are key in gene transcriptional control; however, their study requires large amounts of starting material, which is not compatible with clinical scenarios nor the study of rare cell populations. Here we introduce low input capture Hi-C (liCHi-C) as a cost-effective, flexible method to map and robustly compare promoter interactomes at high resolution. As proof of its broad applicability, we implement liCHi-C to study normal and malignant human hematopoietic hierarchy in clinical samples. We demonstrate that the dynamic promoter architecture identifies developmental trajectories and orchestrates transcriptional transitions during cell-state commitment. Moreover, liCHi-C enables the identification of new disease-relevant cell types, genes and pathways potentially deregulated by non-coding alterations at distal regulatory elements. Finally, we show that liCHi-C can be harnessed to uncover genome-wide structural variants, resolve their breakpoints and infer their pathogenic effects. Collectively, our optimized liCHi-C method expands the study of 3D chromatin organization to unique, low-abundance cell populations, and offers an opportunity to uncover novel factors and regulatory networks involved in disease pathogenesis.

Introduction

Enhancers are critical modulators of gene transcription through physical interactions with target promoters that often locate distally in the genome. The physical proximity between enhancers and promoters is ultimately enabled and determined by the three-dimensional folding of the chromatin within the nucleus^{1,2}. Although enhancers can be defined through well-characterized features, predicting their target genes at distal locations remains challenging due to the high complexity of studying enhancer-promoter interactions and the large variability according to cell-type and state. This gap of knowledge is particularly problematic for understanding the molecular mechanisms associated with inherited and *de novo* acquired mutations and epimutations involved in common human diseases, which are all highly enriched at regulatory elements^{3,4}.

To enable the study of genomic regulatory mechanisms underlying disease pathologies at a genome-wide scale, we previously developed the promoter capture Hi-C (PCHi-C) method^{5,6}. This approach allows systematic identification of the promoter interactome (*i.e.*, genomic regions, including distal regulatory regions, in physical proximity with more than 31,000 promoters) independently of the activity status of the interacting regions. This method has allowed us to uncover new aspects of the diversity of transcriptional regulatory factors⁷ and mechanisms in cell differentiation^{8,9} and disease⁵, and it has broadened our capacity to identify hundreds of potential new disease-candidate genes and/or gene pathways potentially deregulated by non-coding disease-associated variants^{5,10,11–18,19–21}. However, PCHi-C relies on the availability of millions of cells, typically ranging between 30–50 million cells per biological replicate, which prohibits the analysis of rare cell populations such as those commonly obtained in clinical settings.

Here, to overcome this limitation, we present liCHi-C, a mini-input method that allows the generation of high-resolution genome-wide promoter interactome maps using very low amounts of starting material. We have validated our new method by benchmarking liCHi-C promoter interactomes against the highest resolution PCHi-C promoter interactomes available to date, demonstrating that the interactomes can be reproducibly interrogated using as low as 50,000 cells of starting material. As a proof of its potential for discovering new insights about gene transcription regulation, we used liCHi-C to study human hematopoiesis *in vivo* and demonstrate its potential for identifying developmental trajectories and providing mechanistic understanding of transcriptional dynamics along *in vivo* cell commitment. Furthermore, we show liCHi-C can be applied to investigate molecular links between disease-associated non-coding alterations at distal regulatory elements with their target genes in rare cell populations that cannot be characterized using PCHi-C. Finally, to support the broad applicability of liCHi-C across disease settings, we analyze primary leukemias and identify patient-specific structural genomic alterations and cancer-specific topological features potentially implicated in gene deregulation and disease etiology. All the computational tools to analyze and integrate liCHi-C data are freely available at <https://github.com/JavierreLab/liCHiC>.

Results

Development and optimization of liCHi-C for low-input samples. In order to enable the detection of the promoter interactome using low-input material, we modified the original PCHi-C to minimize losses during the procedure. Specifically, liCHi-C maximizes library complexity by employing a single-tube, modifying reagent concentration and volume, and eliminating or

modifying the sequence of some steps (Fig. 1a). Additionally, it reduces by half the time spent on the library preparation. For more details, see Methods section and Extended Data Fig. 1a.

To systematically evaluate liCHi-C, we generated libraries from decreasing numbers of human naïve B cells (Supplementary Information Table 1) at controlled ratios and compared these with the most comprehensive PCHi-C data available to date from the exact same cell type that used ~40 million (40M) cells as starting material. Each sample was deep-sequenced and paired-end reads were mapped and filtered using HiCUP pipeline²². Visual inspection of normalized liCHi-C and PCHi-C contact maps showed a high degree of similarity in topological properties (Extended Data Fig. 1b), and for all experimental conditions (*i.e.*, number of starting cells), the percentage of valid reads (Mean = 58.41%; SD = 5.76%) and the capture efficiency (Mean = 61.29%; SD = 9.15%) were similar between both methods, being 23.58% (SD = 1.59%) of those contacts in *trans* (Extended Data Fig. 1c-e and Supplementary Information Table 1). Unsurprisingly, given the higher need for amplification, the PCR duplicates increased with lower amounts of starting cells, ranging from 86% in 50k cell samples, to 12% of the mapped reads in 40M cell samples. Interaction matrices were also highly reproducible for both methods and different amounts of starting material (stratum-adjusted correlation coefficient (SCC) > 0.90) (Fig. 1b and Extended Data Fig. 1f). These data suggest that liCHi-C reliably generates high-quality promoter interactomes, including 31,253 annotated promoters, and can routinely be performed successfully with an input as low as 50k cells. Remarkably, liCHi-C was able to achieve a >10-fold enrichment of read pairs involving promoters when compared with Hi-C using 800 times less of the starting cells (Supplementary Information Table 1).

Comparison of liCHi-C with other C-based methods for profiling promoter interactomes. To formally compare the performance of liCHi-C to detect promoter interactions, we used the CHiCAGO pipeline²³ to call for significant interactions (CHiCAGO score > 5). Distance distribution and nature of interacting fragments were similar across cell number conditions and methods (Extended Data Fig. 2a-c). Specifically, we found a median linear distance between promoters and their interacting regions of 265kb (SD = 30kb), and 89.04% (SD = 3.72%) of these were promoter-to-non-promoter interactions (Extended Data Fig. 2b-c and Supplementary Information Table 1). Principal component analysis (PCA) of CHiCAGO interaction scores across all biological replicates demonstrated that patterns of promoter interactions are highly consistent across biological replicates and group samples according to the number of input cells (Fig. 1c). To further explore the limits of liCHi-C library complexity we performed hierarchical clustering based on their CHiCAGO interaction scores. We observed that promoter interactomes generated from >100k cells reproduce the ones generated with PCHi-C on 40M cells (Extended Data Fig. 2d). Although promoter interactomes with less than 100k cells showed a different clustering profile, potentially reflecting the reduction of significant interactions due the limited library complexity, these retain cell-type specific and invariant topological features (Fig. 1d and Extended Data Fig 2e-g). Collectively, these results demonstrate the high reproducibility of liCHi-C to profile promoter interactomes at a similar resolution as PCHi-C, and underscore the suitability of the approach to investigate these interactions in cell populations present at relatively low abundance within a sample.

In addition, we benchmarked liCHi-C against other existing C-based methods used for detecting the 3D genome topologies of blood lineage, including Low-C²⁴, Hi-C (using a four-cutter²⁵ or a six-cutter⁵ restriction enzyme) and TagHi-C²⁶ (Extended Data Fig 3 and Supplementary Information Table 2). We called chromatin loops in these datasets at a resolution of 5kb, which is similar to the resolution of our liCHi-C data (~4096 bp), using

HICCUPS²⁷, Mustache²⁸ and HiCExplorer²⁹ loop callers with standard parameters (for more details, see Methods section). Despite the fact that these methods have provided fundamental insights about the role and regulation of spatio-temporal genome architecture on rare cell populations, these methods are very limited with respect to the detection of promoter interactions at restriction fragment resolution (Extended Data Fig. 3a-b and Supplementary Information Table 2). Collectively, these results demonstrate that liCHi-C outperforms other existing C-based methods at genome-wide and promoter-wide detection of potential gene regulatory interactions at high resolution using low-cell numbers.

liCHi-C efficiently captures promoter interactomes across different hematopoietic lineages. To assess the capacity of liCHi-C to provide fundamental insight about *in vivo* cell differentiation, we performed high-quality liCHi-C experiments in 9 distinct cellular populations from the human hematopoietic hierarchy (two biological replicates per cell type; 500k cells per replicate), including hematopoietic stem and progenitor cells (HSC), common myeloid progenitors (CMP), common lymphoid B cell progenitors (CLP) and 6 differentiated cell types (Extended Data Fig. 4a-c and Supplementary Information Table 1). As a more comprehensive validation of the liCHi-C method, we first focused on the differentiated cell types for which high quality PCHi-C data is available. Benchmarking of liCHi-C data against PCHi-C data demonstrated high reproducibility between both methods for all profiled cell types (SCC > 0.93) (Fig. 2a and Extended Data Fig. 4c). Moreover, promoter interactomes clearly separated cell types independently of the method used (Extended Data Fig. 4f).

We then analyzed liCHi-C data in detail. Applying CHiCAGO we identified a median of 134,965 high-confidence promoter interactions (CHiCAGO score > 5) per cell type (Extended Data Fig. 4d-e and Supplementary Information Table 1). A PCA of interaction scores demonstrated that promoter interactomes were highly reproducible and cell-type specific (Fig. 2b). While the first principal component separated cells according to their myeloid or lymphoid lineage, the second principal component recapitulated the differentiation potential, allowing altogether to identify both myeloid and lymphoid differentiation trajectories. To decipher these specificities in greater depth we applied Autoclass Bayesian clustering and computed the specificity score of each cluster in each cell type (Fig. 2c-d). Among the 33 clusters, we observed a stem and progenitor-specific cluster (C3), lymphoid-specific clusters (*e.g.*, C10, C13), myeloid-specific clusters (*e.g.*, C25, C27) and cell-type specific clusters (*e.g.*, C4, C7) that contained genes known to be involved in cellular functions important for the given cell types (Fig. 2d-g). These observations were clearly illustrated by the promoter interactomes of *MYB*³⁰, which encodes for a transcription regulator that plays an essential role in the regulation of lymphoid priming and early B cell development (Fig. 2e), *ITGA2B*³¹, which encodes for the megakaryocyte-specific surface marker CD41 (Fig. 2f), and *SARS2*³², a housekeeping gene that encodes for the mitochondrial seryl-tRNA synthetase (Fig. 2g). Collectively, this data demonstrates that promoter interactomes are specific to the differentiation trajectories of cell types, and further suggest that the highly dynamic promoter-centric genome architecture recapitulates the developmental history of hematopoietic cell lineages.

Promoter interactomes reshape transcriptional trajectories during *in vivo* cell commitment. To validate the ability of liCHi-C to uncover mechanistic insights on transcription regulation, we computationally integrated promoter interactome data with RNA-seq and ChIP-seq data from matched cell types (Extended Data Fig. 5a-c and Supplementary Information Table 3). We distinguish between 2 types of promoter-interacting regions (PIRs): non-promoter PIRs (npPIRs), in which the promoter interacting region does not contain any captured gene promoter, and promoter PIRs (pPIRs), in which the promoter interacting region

contains at least a gene promoter. We found high enrichment of histone modifications indicative of active enhancers (e.g., H3K27ac, H3K4me1) and non-coding transcription of regulatory regions (e.g., H3K4me3)³³ at distal npPIRs. These enrichments were positively associated with the expression level of linked genes in a cell-type specific manner (Fig. 3a). Conversely, npPIRs of lowly expressed genes tend to be more enriched in repressive histone marks, such as H3K27me3 and H3K9me3, than more highly expressed ones. These enrichment profiles were highly consistent with the ones obtained with PCHi-C data despite the significant difference in the starting cell number (Extended Data Fig. 5d). Collectively, these results, exemplified by the transcriptional regulation of the T cell-specific gene *GATA3*³⁴ (Fig. 3b), the B cell-specific gene *PAX5*³⁵ (Extended Data Fig. 5e), and the HSC-specific gene *CD34*³⁶ (Extended Data Fig. 5f), demonstrate the capacity of liCHi-C to identify distal regulatory elements for each gene in rare cell types, and suggest that promoter-associated regions are enriched in distal regulatory elements that mirror the cell-type specificity of the interacting gene's expression.

We then applied chromatin assortativity analysis³⁷, which recognizes the preference of a network's nodes to attach to others that have similar features, to test the potential of liCHi-C to discover proteins or chromatin marks mediating genomic contacts within the nucleus (Fig. 3c and Extended Data Fig. 6a). We observed that genomic regions enriched in H3K9me3 histone modification, which have been associated with constitutive heterochromatin and lamina-associated domains, are highly interconnected and may form topological hubs that collaborate with epigenetics to promote gene silencing (Fig. 3d and Extended Data Fig. 6b-d). Collectively, these results illustrate the power of liCHi-C to suggest, after further functional validation, new aspects on the diversity of factors and mechanisms regulating genome architecture.

liCHi-C enables the discovery of disease-relevant cell types and disease-associated genes and pathways. Genetic variation, which frequently affects the non-coding genome, occurs at various levels ranging from single-nucleotide variants, such as single-nucleotide polymorphisms (SNPs), to larger structural variants (SVs). To test liCHi-C's ability to uncover novel associations between non-coding SNPs and disease etiology, we integrated summary statistics from thirty-nine genome-wide association studies (GWAS), including seven autoimmune diseases, eight myeloid cell traits, four lymphoid cell traits, nine blood malignancies and eleven traits non-related to the hematopoietic hierarchy (Supplementary Information Table 4). Using Blockshifter analysis we showed that PIRs called by liCHi-C in a cell type, independently of whether these are shared or not across cell types, are enriched for genetic variants associated with traits or diseases relevant to the cell type (Fig. 4a-b). For instance, variants associated with final maturation of myeloid cells tend to be more enriched at PIRs in mature myeloid cells. Interestingly, similar enrichment profiles were obtained by liCHi-C and PCHi-C despite the dramatic reduction in starting material (Extended Data Fig. 7a). These data demonstrate that liCHi-C can trace the ontogeny of activity of the non-coding genome in association with pathogenic traits and enables the identification of cell types presumably implicated in disease etiology.

We next used the Bayesian prioritization strategy COGS to rank putative disease-associated genes based on GWAS and liCHi-C data. Excluding SNPs at promoter or coding regions, we assigned 24,504 distal non-coding SNPs to potential target genes, which were located at a median genomic distance of 187kb (Fig. 4c). Remarkably, only 19.58% of these were linked to the nearest gene and 38.16% potentially controlled more than one gene. These results highlight the importance of being able to generate data on long-range interactions between

promoters and regulatory elements to avoid misleading associations based on proximity in the context of gene regulation and disease.

Specifically, using this computational framework on liCHi-C data we prioritized 6,230 candidate genes (with a median of 134 genes per trait/disease at gene-level score > 0.5) and 56 candidate gene pathways according the Reactome Pathway Database³⁸ (Fig. 4d). These genes were highly similar to those prioritized by PCHi-C using 40M cells (Extended Data Fig. 7b-c). For instance, our data suggested that deregulation of *TLE3*, a gene that encodes for a co-repressor protein that negative regulates canonical *WNT* signaling³⁹, could confer susceptibility to lymphocytic leukemia (Fig. 4e). Moreover, it also pointed out “activation of BH3-only proteins” pathway, which is involved in the canonical mitochondrial apoptosis⁴⁰, as being potentially implicated in this type of cancer (Fig. 4d). These and many other examples of expected and unexpected genes and pathways potentially deregulated by non-coding SNPs uncovered in our datasets, warrant follow-up studies to characterize their functional relevance in disease phenotypes. Nonetheless, our results demonstrate the power of liCHi-C to identify potential disease-causative genes and pathways.

liCHi-C can be used to simultaneously diagnose and discover translocations, copy number variations (CNVs) and topological alterations in tumoral samples. After demonstrating the capacity of liCHi-C to prioritize non-coding SNPs with potential functional relevance in clinical settings, we sought to investigate SVs affecting larger genomic regions, including translocations and CNVs. Most of the ligation events detected by proximity-ligation methods, such as liCHi-C, occur between sequences in proximity along the linear genome and the frequency of these events decreases logarithmically with the genomic distance that separates them. However, a translocation alters the linear genome and artificially increases the number of ligation events between the juxtaposed regions. Based on this, we reasoned that liCHi-C could detect genome-wide chromosomal translocations, identify the breakpoints and uncover alterations in gene promoter interactions that could shed light on the pathogenic role of SVs. To test this hypothesis, we generated high quality liCHi-C libraries using primary blasts from two pediatric B cell acute lymphoblastic leukemia (B-ALL) samples (500k cells per library) (Supplementary Information Table 1 and Extended Data Fig. 8b-e) previously analyzed by routine clinical assays (Fig. 5a and Extended Data Fig. 8a). According to FISH and karyotyping analysis, B-ALL sample 1 carried a balanced translocation between chromosomes 8 and 14, which appeared as ‘butterfly’ blocks of interactions between the translocated chromosomes on the liCHi-C interaction matrix (Fig. 5a top). A closer examination of the interaction directionalities identified the restriction fragments affected by the breakpoints and allowed the reconstruction of the resulting chromosome where the promoter of *MYC* becomes rearranged next to the “constant” gene cluster of the IgH locus (Fig. 5b). Through this focused analysis of promoter interactions, our data suggested that *MYC* expression may be simultaneously controlled by the “*MYC* blood enhancer cluster” (BENC)⁴¹, located 2Mb downstream, and the IgH-specific enhancer 3'-regulatory region (3'-RR)⁴², rearranged 300kb upstream (Fig. 5c). B-ALL sample 2 carried an unbalanced translocation between the same chromosomes that generated single blocks of contacts on the normalized liCHi-C interaction matrix (Fig. 5a bottom). Consequently, the *CEBPD* gene⁴³ becomes juxtaposed to the IgH locus, which potentially alters its regulatory landscape (Extended Data Fig. 8f-g). Collectively, these results demonstrate liCHi-C’s capacity to generate high-quality chromatin conformation maps and regulatory landscapes directly from primary patient tissue samples to detect any type of translocation and surmise the pathogenic effects at a genome-wide scale.

In addition to chromosome rearrangements, we tested whether liCHi-C can be used to detect CNVs. Gains or losses of genetic material imply an increase or decrease in the ligation events within the altered regions, respectively. Therefore, we reasoned that CNVs should appear when comparing liCHi-C normalized interaction matrices of cells carrying the CNV against control cells. To test this hypothesis, we focused on the B-ALL sample 2 that, according to the karyotyping analysis, carried a trisomy of chromosome 21 and a partial trisomy affecting the translocated region of the q arm of chromosome 8. As shown in Fig. 5a bottom, both trisomies were identified, demonstrating that liCHi-C can be used for genome-wide detection of CNVs and to scan breakpoints from primary patient tissues without the need for a reference.

Finally, we tested the ability of liCHi-C to identify disease-specific regulatory 3D chromatin landscapes that may be implicated in disease etiology. To do so, we applied Autoclass Bayesian clustering of liCHi-C significant interactions (CHiCAGO score > 5) called either on B-ALL samples or on the postulated healthy cells of origin of these hematological malignancies (*i.e.*, HSC, CLP and naïve B cells) (Fig. 5d). Specificity score analysis of each cluster in each cell type identified promoter interactions specifically acquired (C5-9) or lost (C10-19) in one or both B-ALL samples (Fig. 5e), which included key transcription factors involved in B cell differentiation and function (*e.g.*, *PAX5*⁴⁴, *ARID5B*⁴⁵), well-known tumor suppressor genes (*e.g.*, *BTG1*⁴⁴, *IKZF1*⁴⁴) and protooncogenes (*e.g.*, *MYC*⁴⁶). For instance, several *HOXA* genes have been associated with normal hematopoiesis and blood malignancies^{47,48}. According to liCHi-C data, these genes lose connectivity with their enhancers, which could link their transcriptional deregulation with malignant transformation (Fig. 5f). Collectively, these results support the broad applicability of liCHi-C to uncover factors and mechanisms involved in disease etiology through simultaneously identifying disease-specific promoter-centered genome topologies and detecting translocations, CNVs, breakpoints and their effects on transcriptional deregulation.

liCHi-C can be customized to improve its resolution. liCHi-C resolution is determined by the restriction enzyme used for the library generation and defines the range of significant interactions to be detected. To demonstrate the adaptability of our new method to interrogate promoter interactions at different resolutions we used primary blasts from a third B-ALL sample to generate two high-quality liCHi-C libraries using a six-cutter restriction enzyme (HindIII) and a four-cutter restriction enzyme (MboI) respectively (~250k cells per library) (Supplementary Information Table 1 and Extended Data Fig. 8h-k). Patient 3 carried a monosomy of chromosome 7, which was clearly identified as a reduction of reads on the contact matrix generated by both restriction enzymes (Fig. 6a-b).

liCHi-C libraries generated with MboI detected 1.78 times more significant interactions (Extended Data Fig. 8j), which were characterized by having half of the median linear distance between promoters and their interacting regions (Fig. 6c). Indeed, although the shortest significant interaction was similar for both libraries (2574bp for HindIII and 1939 for MboI), the highest frequency of interactions was found at a distance 2.15 times larger for HindIII restriction (Fig. 6c). This data, illustrated by the promoter interactome of the *DDX41* (Fig. 6d), a DEAD box RNA helicase associated with B-ALL and other blood malignancies⁴⁹, demonstrates that the use of a four-cutter restriction enzyme increases the power to detect short-range interactions and compromises the detection of the long-range ones. Taken together, our results demonstrate liCHi-C capability to provide fundamental and clinical insights about gene-regulatory interactions at different levels of resolution.

Discussion

High-throughput chromatin conformation capture methodologies such as Hi-C⁵⁰ have revolutionized our understanding of long-range gene transcriptional control. However, many aspects of its dynamics along *in vivo* differentiation and stimulation, as well as its alteration in disease, remain largely unexplored due to the lack of genome-wide methodologies to study the promoter-centric genome architecture at high resolution with low-input material. Whereas single-cell^{51,52} or low-input^{24,26,53,54} approaches exist, these generate sparse contact maps with low resolution that do not allow the study of specific chromatin interactomes. More recently, HiChIP⁵⁵ and HiCuT⁵⁶ methods have been developed to study long-range chromatin interactions mediated by a specific protein. Although both technologies are compatible with low-input cell numbers, these rely on the availability of high-quality antibodies that recognize the target protein. Besides, these methods cannot be used to compare chromatin interactomes between conditions in which the binding of the target protein is different, which is very common due to the inherent dynamic nature of chromatin.

To overcome these limitations, we have developed liCHi-C, a new mini-input cost-effective method to robustly map and compare promoter interactomes at high resolution in rare cell populations previously unmeasurable. Up to 12 liCHi-C libraries can be generated in 6 days with a total cost of 1500 euros per library (including sequencing cost). Unlike methods that depend on enrichment based on the use of antibodies^{56,55,57,58}, liCHi-C only relies on biotinylated RNAs designed to hybridize against the annotated promoters to ultimately enrich for promoter interactions from a Hi-C library. Thus, it is able to identify long-range contacts of both active and inactive promoters and robustly compare interactomes between any condition. In addition, this capture strategy provides high versatility since any customized capture system from a wide range of coverage can be designed according to the interactome to be studied. For instance, liCHi-C can be easily coupled with capture systems designed to study the interactome of a collection of non-coding alterations or the enhancer interactome. Indeed, it can even be adapted to the study of the interactome of just a few loci as other 3C-based capture methods do^{59,60}.

liCHi-C significantly broadens the capacity for studying organism developments, *in vivo* cell commitment and cellular response to a wide range of external stimuli. As a proof of concept, we have used liCHi-C to study, for the first time, human hematopoietic hierarchy. Our data demonstrates that the promoter interactome can identify the differentiation trajectory. In addition, this suggests a massive dynamic rewiring of the three-dimensional epigenetic landscape parallel to transcriptional decisions during *in vivo* cell commitment.

liCHi-C enables the study of primary samples, thus addressing a key limitation of PCHi-C to apply this type of analysis to clinical samples. This new method is especially relevant as most inherited and acquired mutations and epimutations for common human diseases, which largely remain unexplored, are all highly enriched at regulatory elements and cannot be readily modeled in *in vitro* systems. Genetic and epigenetic alterations at distal regulatory elements have the potential to alter the regulatory properties and ultimately lead to quantitative changes in expression of distal target genes with pathological outcomes. As distal regulatory elements and their topological properties are highly dynamic, cell-type specific and state-dependent, it is critical to identify the relevant human cell types for each disease and profile their full repertoire of regulatory elements and target genes. Here, we demonstrate that liCHi-C is able to fulfill this need. Focused on GWAS data we have demonstrated that liCHi-C identifies unexpected etiological associations and exposes new disease-associated genes and pathways.

Although we have mostly focused on inherited risk factors, our computational framework can be adapted to study acquired mutations and epimutations at any type of distal regulatory elements (e.g., silencer or primed enhancer), since liCHi-C identifies long-range contacts promoters independently of their activity.

How chromatin organization contributes to disease pathogenesis remains largely unexplored. As we have shown, liCHi-C has the potential to contribute to filling this gap of knowledge. Taking malignant neoplasms as a model, we have shown that liCHi-C can be used to discover disease-specific topological alterations in clinical samples and generate hypotheses about genetic factors underlying disease mechanisms. Simultaneously, liCHi-C can uncover SVs at a genomic scale, resolve the positions of their breakpoints and predict their functional effects, including the formation of new regulatory landscapes, in an agnostic manner. Although we focused on the analysis of translocations and duplications associated with cancer, liCHi-C is also able to identify and characterize other types of SVs, such as inversions and deletions in any disease context. In addition, the liCHi-C method holds high potential for disease diagnosis equivalent to other chromosome conformation capture technologies^{61,62}, since SVs are hallmarks of mental retardation, infertility, developmental disorders and cancer.

Despite the broad applicability of our method, there are still several factors to take into consideration. One inherent limitation of liCHi-C is that its resolution is determined by the restriction enzyme used for the library generation. However, as we have demonstrated, it can be increased by replacing the enzyme by another one with greater resolution or exchange it for micrococcal nuclease. In addition, the interpretation of the biological meaning of non-coding alterations purely based on distal chromatin interactions can be challenging. Nevertheless, an integrative analysis of liCHi-C data, gene expression and chromatin states might be indicative of causal relationships, which should be validated with functional assays. Finally, the identification of exact genomic coordinates of the SVs' breakpoints is not possible unless these map near restriction sites. However, long-range sequencing approaches coupled with liCHi-C can allow to map the SVs at the nucleotide resolution. Besides, this combinatorial approach increases the mapability efficiency of the chromatin contacts at repetitive sequences, which can be target of SVs and other mutations and epimutations. Despite these considerations, our data proves that it is feasible to generate high quality genome-wide promoter interaction maps from low amounts of primary patient material. We anticipate that the robustness and the inherent flexibility for customization make liCHi-C an attractive option that will allow the analysis of spatial genome architecture within reach of personalized clinical diagnostics and development biology.

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Methods

Cell isolation

Naïve B cells (nB), naïve CD4⁺ cells (nCD4), naïve CD8⁺ cells (nCD8), megakaryocytes (MK), erythroblasts (Ery) and monocytes (Mon) were isolated as described in⁵. Hematopoietic stem and progenitor cells (HSC; CD34⁺, CD38⁻) common myeloid progenitors (CMP; CD34⁺, CD38⁺, CD33⁺) and common lymphoid B cell progenitors (CLP; CD34⁺, CD38⁺, CD19⁺) were purified by CD34 positive selection (Miltenyi cat. #130-046-703) and fluorescence-activated cell sorting (FACS) from 4 donations (2 per biological replicate) of 15-to 22-week-old human fetal liver and fetal bone marrow as previously described⁶³. Briefly, after CD34⁺ selection, positive cells were stained for flow cytometry with the following fluorophore-conjugated monoclonal antibodies, all from BD Biosciences: CD34 PE/Cy7 (cat. #348811), CD38 FITC (cat. #555459), CD19 BV421 (cat. #562440), CD33 APC (cat. #551378) and CD10 PE (cat. #555375). FACS was performed using a BD FACSAria Fusion. B-ALL samples were isolated from bone marrow of pediatric patients after CD19⁺ by FACS sorter. All samples were obtained from consent donations of volunteers after approval by the Ethics committee.

Fixation and cell quantification

Cells were quantified, resuspended in 1ml of RPMI 1640 culture medium containing 10% FBS and 2% of methanol-free formaldehyde (Thermo Fisher cat. #28908) and incubated in a rocker for 10 minutes at room temperature. Formaldehyde was quenched with glycine to a final concentration of 0.125M. Then, cells were washed with cold 1X PBS. Specific cell numbers were sorted into low-retention 1.5ml tubes (BD FACSJazz sorter, 1.0 Drop Pure sorting mode), pelleted, flash-frozen in dry ice and stored at -80 °C.

liCHi-C method

During the whole protocol, low-retention tips and tubes were used to minimize cell loss. Pelleted cells were softly resuspended in 500µl ice cold lysis buffer (10mM Tris-HCl pH 8.0, 10mM NaCl, 0.2% IGEPAL CA-630, 1x cOmplete EDTA-free protease inhibitor cocktail (Merck cat. #11873580001)) and incubated 30 minutes on ice to extract the nuclei. Soft inversions of the tube were performed during the incubation every 5 minutes. Nuclei were centrifuged (1000g and 4°C for 10 min) and 450µl of supernatant was discarded (50µl of supernatant was left in the tube to avoid cell losses). The cell pellet was resuspended in 500µl ice cold 1.25x NEB2 buffer (New England Biolabs cat. #B7002S). After centrifugation (1000g and 4°C for 10 min), 500µl of supernatant was removed.

Afterwards, 129ul of 1.25x NEBuffer 2 were added to the low-retention tube to obtain a final volume of 179ul. Then, 5.5µl of 10% SDS (AppliChem cat. #A0676,0250) were laid on the

wall of the tube and mixed by inversion. After incubation at 37°C and 950 rpm for 30 minutes, 37.5 μ l 10% Triton X-100 (AppliChem cat. #A4975,0100) were laid on the wall of the tube, mixed by inversion and incubated at 37°C and 950 rpm for 30 minutes. Chromatin within the nuclei was overnight digested at 37°C and 950 rpm after adding 7.5 μ l of HindIII restriction enzyme at 100U/ μ l or 37.5 μ l of MboI restriction enzyme at 25U/ μ l (New England Biolabs cat. #R0104T or #R0147M). The following day, an extra digestion during one more hour was performed after adding 2.5 μ l of the HindII enzyme or 12.5 μ l of the MboI enzyme.

After digestion, MboI enzyme was washed with NEBuffer 2 by centrifuging the sample and removing the supernatant to avoid possible re-digestion of ligated fragments afterwards. Cohesive restriction fragment ends were filled in during 75 minutes at 37°C. To do so, 30 μ l of master mix composed by 5 μ l of 5U/ μ l Klenow polymerase (New England Biolabs cat. #M0210L), 0.75 μ l of each 10mM dCTP, dGTP and dTTP, and 18.75 μ l of 0.4mM biotin-14-dATP (Invitrogen #19524-016) were added.

In-nucleus ligation of DNA fragments was carried out during 4 hours at 16°C after adding 12.5 μ l of 1U/ μ l T4 DNA ligase (Thermo Fisher cat. #15224025), 50 μ l of 10x ligation buffer (NEB #B0202S), 5 μ l of 10mg/ml BSA (NEB # B9001S) and 170.5ml of water. Afterwards, DNA ligation products were decrosslinked by adding 30 μ l of Proteinase K 10mg/ml (Merck cat. #3115879001) and incubating overnight at 65°C. The following day, an extra decrosslink during two more hours was performed after adding 15 μ l of the Proteinase K enzyme.

To purify the decrosslinked DNA ligation products, a single phenol-chloroform-isoamyl alcohol (25:24:1 v/v) purification was carried out followed by ethanol precipitation for 1 hour at -80°C in presence of 30 μ g Glycoblue (Thermo Fisher cat. #AM9515) as a coprecipitant. DNA ligation products were resuspended in 130 μ l of nuclease free water and concentration was assessed by fluorimetric quantification using the Qubit dsDNA HS Assay Kit (Thermo Fisher cat. #Q32851).

Optional 3C controls assessing the detection of cell-type invariant interactions in the HindIII liCHi-C libraries can be performed by amplifying 50-100ng of the DNA with 37 cycles of PCR amplification (see Supplementary Information Table 5 for primer information) and running the reactions in a 1.6% agarose gel. Correct fill-in and ligation can also be tested by reamplifying 2.5 μ l of PCR products 5 more cycles, differentially digesting the product with either HindIII, NheI (Thermo Fisher cat. #ER0975), both enzymes or none and running the product on a 1.6% agarose gel.

Biotin removal of the non-ligated ends was skipped. DNA ligation products were sonicated using Covaris M220 focused-ultrasonic (20% duty factor, 50 peak incident power, 200 cycles per burst, 65 seconds) in 130 μ l tubes (Covaris cat. #520077). After shearing, DNA ends were repaired by adding 6.5 μ l of T4 DNA polymerase 3U/ μ l (New England Biolabs cat. #M0203L), 6.5 μ l of T4 polynucleotide kinase 10U/ μ l (New England Biolabs cat. #M0201L) 1.3 μ l of Klenow polymerase 5U/ μ l (New England Biolabs cat. #M0210L), 18 μ l of dNTP mix 2.5mM each and 18 μ l of 10x ligation buffer (New England Biolabs cat. #B0202S) and incubating for 30 minutes at 20°C.

Biotinylated informative DNA ligation products were pulled down using Dynabeads MyOne streptavidin C1 paramagnetic beads (Thermo Fisher cat. #65001). After thorough washing of the ligation products-beads complex and having the sample in 35.7 μ l of volume, blunt DNA fragments on the beads were adenine-tailed by adding 7 μ l of Klenow 3'→5' exo- polymerase

5U/μl (New England Biolabs cat. #M0212L), 2.3μl of dATP 10mM and 5 μl NEB2 of 10x NEBuffer 2 and incubating the mixture 30 minutes at 37°C and a further 10 minutes at 65°C to inactivate the enzyme.

After thorough washing of the ligation products-beads complex and having the sample in 50μl of 1x ligation buffer, PE Illumina adapters (Supplementary Information Table 5) were ligated to the adenine-tailed DNA fragments by adding 1μl of T4 DNA ligase 2000U/μl (New England Biolabs cat. #M0202T) and 4μl of preannealed adaptor mix 15μM and incubating the mixture 2h at room temperature.

The bead-bound ligation products were amplified 8-13 cycles by PCR (Supplementary Information Table 5 for cycle recommendations according to starting cell number) using Phusion high-fidelity PCR master mix with HF buffer (New England Biolabs cat. #M0531L)

After recovering the amplified library from the supernatant, size distribution was tailored to 300-800bp by double-sided size selection and purified using CleanNGS SPRI beads (0.4-1 volumes; CleanNA cat. #CNGS-0050). DNA concentration was quantified on an Agilent Tapestation platform using high sensitivity D1000 ScreenTape system and samples were stored at -20°C.

Enrichment of promoter-containing ligation products was performed using SureSelectXT Target Enrichment System for the Illumina Platform (Agilent Technologies) as instructed by the manufacturer, and the library was amplified 4 cycles by PCR using Phusion high-fidelity PCR master mix with HF buffer (New England Biolabs cat. #M0531L). Finally, the end product was purified using CleanNGS SPRI beads (0.9 volumes; CleanNA cat. #CNGS-0050) and paired-end sequenced.

The fully detailed protocol will be uploaded to Protocol Exchange platform or similar upon acceptance of the manuscript.

Sequencing

B-ALL liCHi-C libraries were sequenced by Macrogen Inc using HiseqX 150+150PE platform. The rest of liCHi-C libraries were sequenced by BGI Genomics using DNBseq 100+100PE platform.

B-ALL cytogenetic and FISH analysis

Cytogenetic analyses of B-ALL samples were carried out on G-banded chromosomes obtained from 24-hour unstimulated culture. FISH analyses were performed on fixed cell suspensions of the bone marrow using the LSI MYC probe (Metasystems, XL MYC BA) and the LSI IGH probe (Metasystems, XL IGH BA), respectively. Between 200 to 400 interphase nuclei were scored.

liCHi-C processing

Paired-end reads were processed using HiCUP (0.8.2)²². First, the genome was computationally digested using the target sequence of the restriction enzyme. Then, the different steps of the HiCUP pipeline were applied to map the reads to the human genome (GRCh38.p13), filter out all the experimental artifacts and remove the duplicated reads and retain only the valid unique paired reads. To assess the capture efficiency, we filtered out those paired reads for which any end overlaps with a captured restriction fragment, retaining only the unique captured valid

reads for further analysis. Library statistics for all samples are presented in Supplementary Information Table 1.

liCHi-C interaction calling

Interaction confidence scores were computed using the CHiCAGO R package as described in^{23,64}. In summary, this pipeline implements a statistical model with two components (biological and technical background), together with normalization and multiple testing methods for capture Hi-C data. CHiCAGO analysis was performed in merged samples to increase the sensitivity, after assessing for reproducibility between biological replicates using: i) the stratum adjusted correlation coefficient according to⁶⁵, averaged over chromosomes, ii) principal component analysis, and iii) hierarchical clustering. The reproducibility-score was also used to compare PCHi-C and liCHi-C libraries of the same cell types. Significant interactions with a CHiCAGO score ≥ 5 were considered as high confidence interactions. Interaction statistics for all samples are presented in Supplementary Information Table 1.

Loop calling using Hi-C, Low-C and TagHi-C

We processed Hi-C, TagHi-C and Low-C data with HiCUP²² as detailed above, using the human (GRCh38.p13) and mouse reference genomes (GRCm39). Loop calling was performed with three different methods: HICCUPS²⁷ (1.22.01), Mustache²⁸ (1.2.7) and HiCExplorer²⁹ (3.7.2). All loop callers were used with default parameters on Knight-Ruiz normalized matrices at 5 kb resolution and a maximum loop distance of 8 Mb. Specific parameters for HICCUPS were: -cpu--ignore_sparsity; for Mustache, the p value threshold was set to 0.05.

Clustering of promoter interactions

Interactions were clustered using the Autoclass algorithm and for each cluster a specificity score was computed as described in⁵ using the asinh-transformed CHiCAGO scores. Clustering of cell types was performed using a hierarchical method with average linkage based on Euclidean distances, and principal component analysis was performed using the pcomp function in R. For interaction data handling we used an in-house R package which allows us to compute distance distributions, filter interactions by the presence of histones marks, or generate virtual 4C of specific genes.

ChIP-seq processing

Paired-end reads were processed following ENCODE standards. Reads were trimmed using Trim Galore (0.6.5) to remove sequencing adapters, and then mapped using bowtie2 (2.3.2) to the reference genome at PRS (GRCh38.p13) with –very-sensitive parameter. We filtered out low quality reads, reads overlapping the ENCODE blacklist and duplicated reads. Peak calling was performed using macs2 (2.2.7.1) in broad and narrow mode depending on the histone mark using an input sample as control, with default parameters. Bigwig files were generated using the function bamCoverage from deepTools (3.2.1) and scaled based on the background normalization of the samples as described in⁶⁶ before merging the biological replicates together for visualization purposes. All ChIP-seq data analyzed in this article is presented in Supplementary Information Table 3.

Histones mark enrichment at PIRs

Enrichment of the different histones mark ChIP-seq in both promoters and npPIRs was performed using a permutation test implemented in GenoMatriXeR⁶⁷, with 5000 randomizations using the randomizeRegions option. Promoter interactions were classified based on the RPKM expression quartiles of their genes, and the promoters and npPIRs analyzed

separately, generating four groups of either promoters or npPIRs from lower expression to higher expression.

Chromatin assortativity

Chromatin assortativity was computed for the promoter-pPIR and promoter-npPIR subnetworks separately using ChAseR R package (<https://bitbucket.org/eraineri/chaser>) as described in³⁷. We validated our results against a set of 1000 randomizations preserving the genomic distances between nodes and the chromosomes distribution of interactions. The abundance of each histone mark on the nodes of the networks was also computed.

Gene ontology and pathways enrichment analysis

Gene ontology (GO) enrichment analysis was performed using clusterProfiler R package for the 3 ontologies: molecular functions, biological processes and cellular components. The pathway analysis was done using ReactomePA⁶⁸. We used the prioritized protein-coding genes by COGS (gene score ≥ 0.5) to compute enrichment in genes in Reactome pathways, adjusting p values by false discovery rate. In both analyses we used as universe all the genes in the capture design.

Data visualization

To visualize the contact matrices, the bam files, containing unique captured valid reads, were transformed into pair files using bam2pairs. Then they were converted into matrices in cool format with a resolution of 1Mb, 250kb and 100kb using cooler⁶⁹ (0.8.11). The visualization of the matrices was done using HiCEexplorer⁷⁰. For the visualization of significant interactions generated by liCHi-C we used the WashU Epigenome Brower⁷¹ and karyoploteR R package⁷².

GWAS summary statistics and imputation

GWAS summary data was obtained from the NHGRI-EBI GWAS Catalog⁷³ and from the UK Biobank - Neale Lab (UK Biobank, *n.d.*; <http://www.nealelab.is/uk-biobank>), as reported in Supplementary Information Table 4. Those datasets that were not in GRCh38 coordinates were converted to it using *liftOver*. To avoid spuriously strong association statistics, we filtered out SNPs with p value $< 5 \cdot 10^{-8}$ for which there were no SNPs in LD ($r^2 > 0.6$ using 1000 genomes EUR cohort as a reference genotype set⁷⁴ or within 50kb with p value $< 10^5$. To increase the power of the GWAS we applied the Poor Man's Imputation as described in⁵ to the summary statistics described above using as reference genotype set the 1000 Genomes EUR cohort. We used the GRCh38 HapMap Phase II genetic map lifted from GRCh37 coordinates⁷⁵ to define regions with 1cM recombination frequency to be used for the imputation. The MHC region (GRCh38:6:25-35Mb) was excluded from the analysis. Manhattan plots were visualized using the qqman package (0.1.8)⁷⁶.

GWAS enrichment at PIRs

Enrichment of SNPs in PIRs was performed using Blockshifter as described in⁵ which considers the correlation between GWAS and PIRs. Blockshifter computes a z-score using a competitive test for each trait and cell type set.

liCHi-C GWAS prioritizing genes

Prioritization of relevant genes for each GWAS trait and cell type was performed using the COGS algorithm as described in⁵. Briefly, this method considers linkage disequilibrium to estimate the posterior probability of each SNP being causal for each trait. Then, these SNPs were used to compute a gene-score for all the genes involved in liCHi-C significant interactions

in at least one cell type. This gene-score is composed of 3 components: coding SNPs annotated by VEP (104)⁷⁷; SNPs located in promoter regions; and SNPs overlapping Other-Ends.

SVs analysis

Translocations of B-ALL samples were identified using PLIER⁶¹. Briefly it compares the genome-wide interactions of a region against a set of random permutations computing a z-score. To detect CNVs, we applied BIC-seq2⁷⁸, an algorithm that uses Bayesian information criterion-based segmentation on normalized data using naïve B samples as control genome. Visual inspection of both translocations and CNVs was performed by computing the log2ratio between the contact matrices of B-ALL and CLP samples.

Data availability

All the liCHi-C datasets generated in the present article will be available in EGA. *Accession number will be provided after completing the data upload.*

Code availability

All the analyses done in the present article are accessible in the following GitHub repository: <https://github.com/JavierreLab/liCHiC>

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Clinical Research Ethics Committee of University Hospital Germans Trias i Pujol (REF.CEI: PI-18-205).

Author contributions

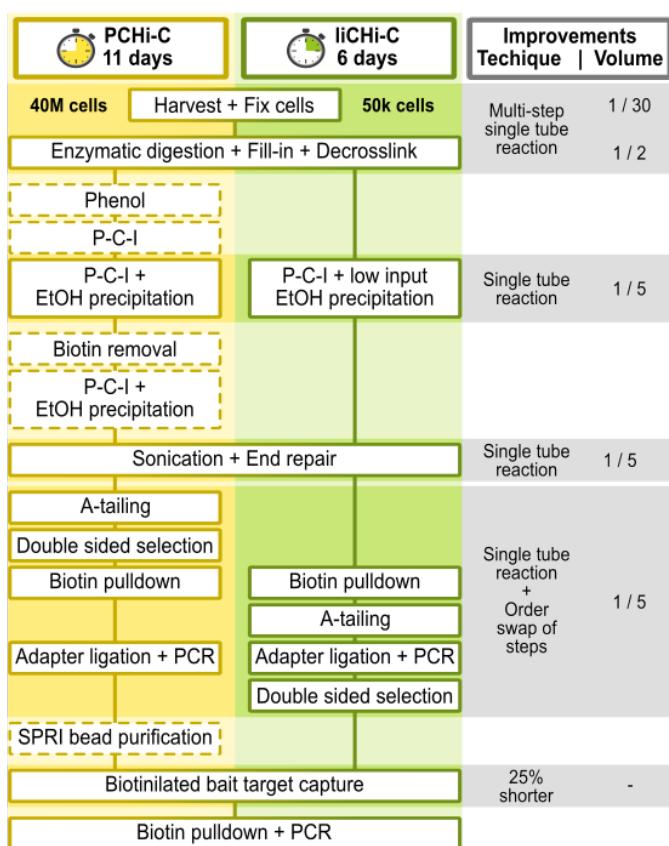
BMJ designed research; OM, CG, EC, DC, MC, CB and PM contributed on sample isolation; LR, APR, RM and BMJ performed research; LT-D, PL-M, AN-A, FS analyzed data; BMJ wrote the paper with support of LT-D, LR and PL-M; and all authors provided feedback on the manuscript.

Competing interest

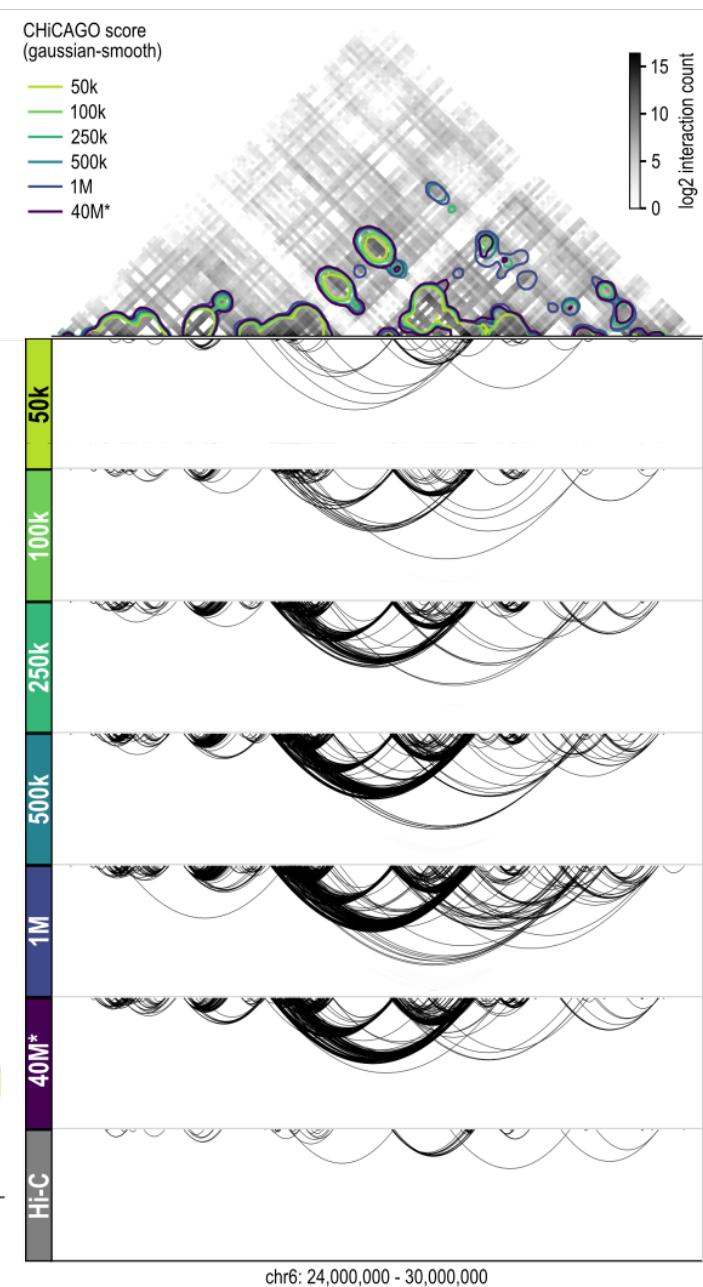
No competing interest

Figures and Legends

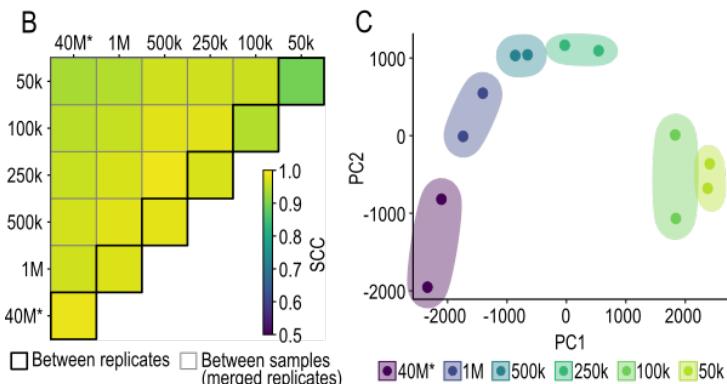
A



D



B



C

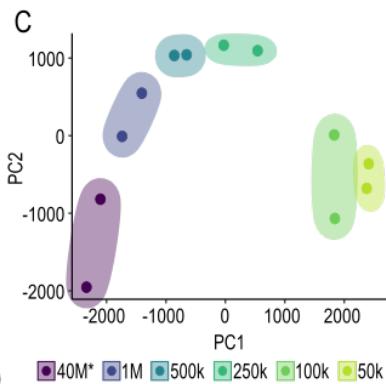


Fig. 1 liCHi-C is a robust and reproducible method to study the promoter interactome in low-abundance cell populations.

- a.** Schematic comparison of the PCHi-C vs liCHi-C workflow. Dash-bordered boxes denote removed steps from the original PCHi-C protocol.
- b.** Heatmap displaying stratum adjusted correlation coefficient (SCC) between promoter interactomes of human naïve B cells obtained by liCHi-C and PCHi-C (*) using different cell numbers. Reproducibility between biological replicates and between pairs of merged biological replicates are surrounded by black and grey lines respectively. 1000 (k), million (M).
- c.** Principal Component Analysis of CHiCAGO significant interactions (CHiCAGO scores > 5) of biological replicates detected by liCHi-C and PCHi-C (*) using different cell numbers.
- d.** Top: Interaction matrix at 50kb resolution generated with PCHi-C and 40 million cells. Colored contour plot over the interaction matrix represents gaussian smoothing (alpha=1.2) of the significant CHICAGO interactions detected by liCHi-C data using different numbers of input cells. Bottom: Significant interactions (arcs) detected with liCHi-C and PCHi-C (*) and Hi-C using different numbers of input cells

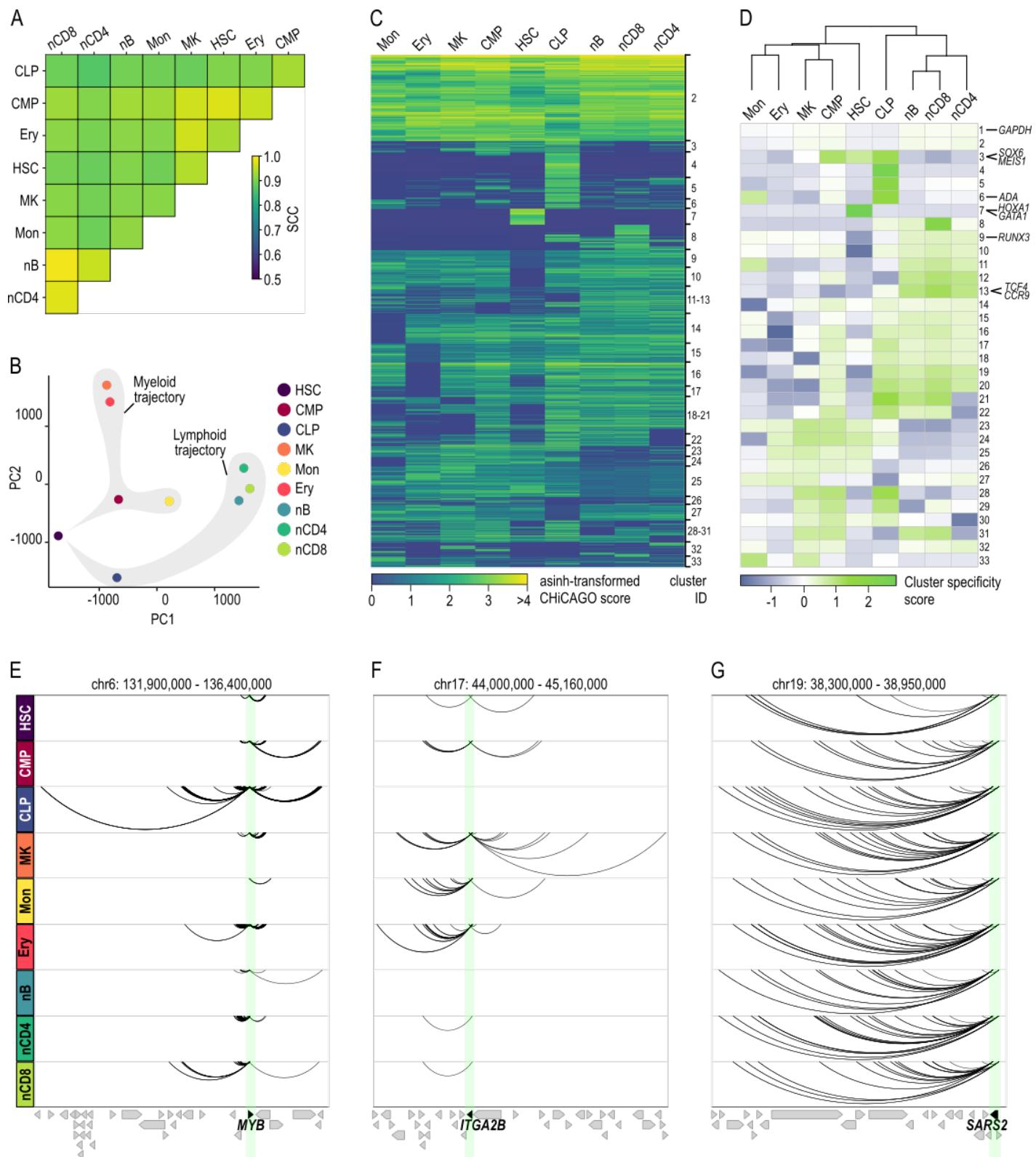


Fig. 2 liCHi-C enables the study of *in vivo* human hematopoiesis.

a. Heatmap displaying stratum adjusted correlation coefficient (SCC) between promoter interactomes of merged biological replicates. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblast (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8).

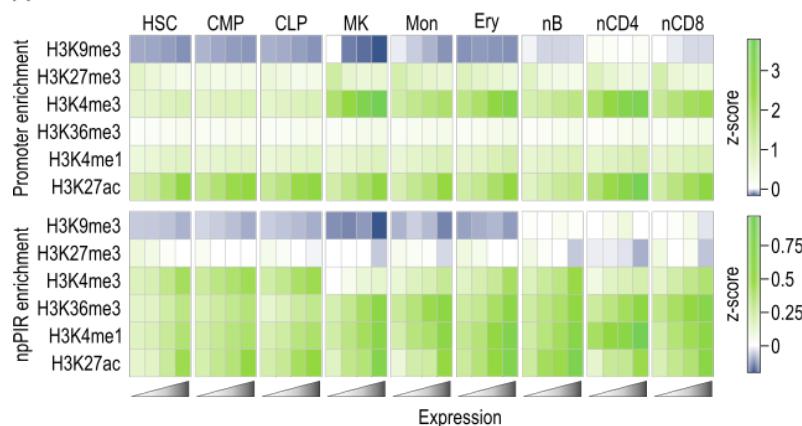
b. Principal Component Analysis of liCHi-C significant interactions (CHiCAGO scores > 5) from merged biological replicates. Shaded in grey are the predicted differentiation trajectories for both lymphoid and myeloid lineages.

c. Heatmap of asinh-transformed CHiCAGO score of significant interactions in at least one cell type clustered using Autoclass algorithm.

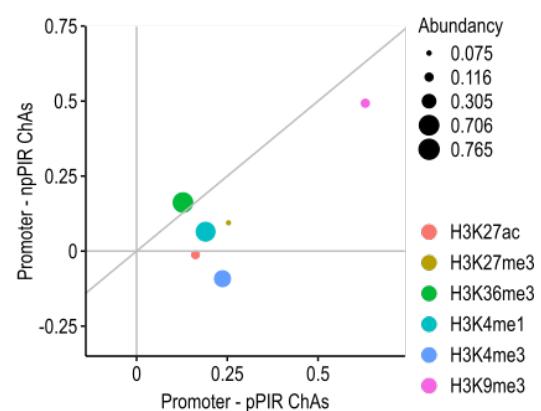
d. Top: Dendrogram of hierarchical clustering with average linkage based on Euclidean distances of CHiCAGO significant interactions of merged samples. Bottom: Heatmap of cluster specificity score of each Autoclass cluster.

MYB (e.) **ITGA2B (f.)** and **SARS2 (g.)** promoter-centered interactions (arcs) according to liCHi-C data. Arrows symbolize gene placement and orientation along the genomic window. Green shade depicts the gene promoter.

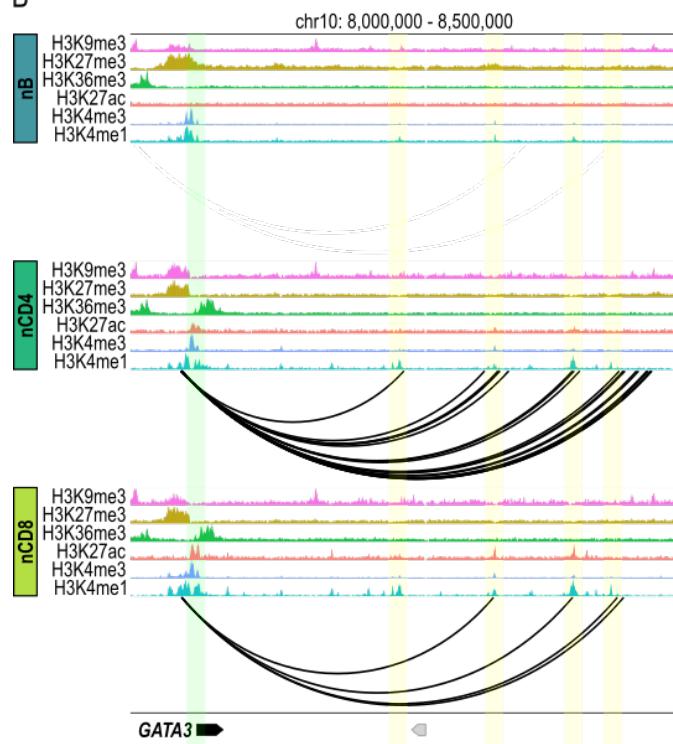
A



C



B



D

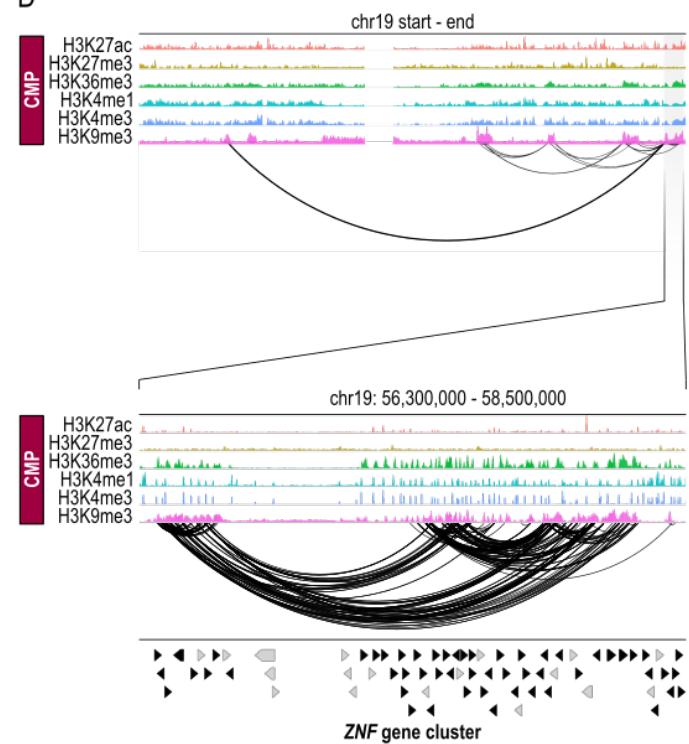


Fig. 3 liCHi-C identifies enhancer-promoter pairs and proteins involved in chromatin organization.

a. Heatmap representing enrichment (expressed in terms of z-scores) of promoters (top) and non-promoter promoter-interacting regions (npPIRs) (bottom) for histone marks. PIRs and promoters were classified by the quartiles level of FPKM expression of the associated gene. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblast (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8).

b. *GATA3* regulatory landscape in naïve B (top), naïve CD4⁺ T (middle) and naïve CD8⁺ T (bottom) cells according to histone modifications and liCHi-C data. Green shade depicts the gene promoter, while yellow shades depict putative enhancer regions for that gene in any of these cell types. Arrows symbolize gene placement and orientation along the genomic window.

c. Scatterplot plot of chromatin assortativity (ChAs) of 6 histone marks computed for the promoter-pPIR sub-network (x axis) against the promoter-npPIR (y axis) for common myeloid progenitor cells. Dot size depends on the abundancy of each histone mark.

d. H3K9me3-centred interaction network (arcs) for common myeloid progenitor cells. Top graph depicts whole chromosome 19, while bottom graph shows a zoom centered on a *ZNF* gene cluster. Arrows symbolize gene placement and orientation along the genomic window.

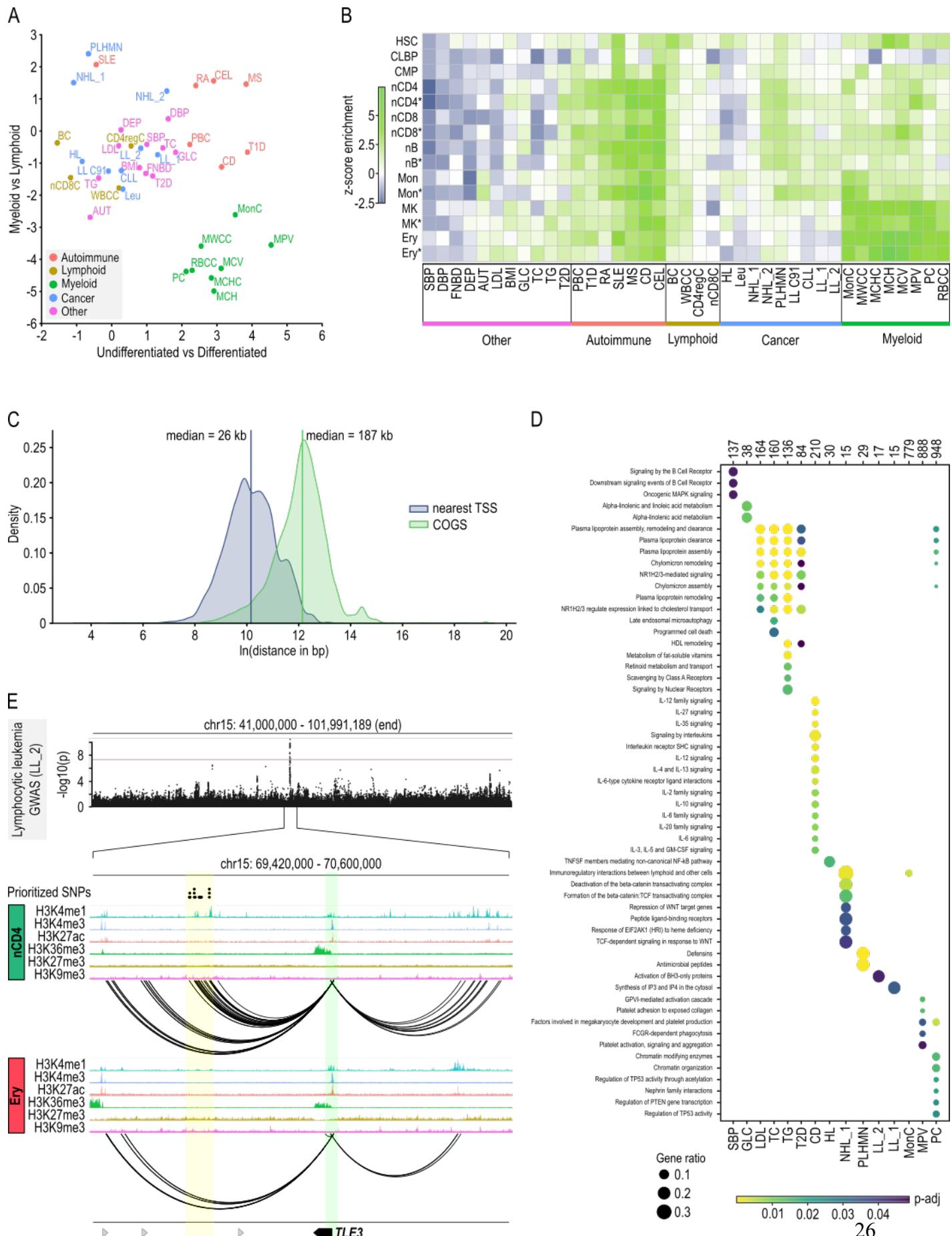


Fig. 4 liCHi-C enables the discovery of new disease-relevant cell types and disease-associated genes and gene pathways.

a. Enrichment of GWAS summary statistics at promoter-interacting regions (PIRs) by cell type. Axes reflect Blockshifter z-scores for two different tissue group comparisons: myeloid versus lymphoid (y axis) and undifferentiated against differentiated cells (x axis). Traits are labeled and colored by category: autoimmune disorder (red), lymphoid trait (yellow), myeloid trait (green), blood cancer (blue) and other (purple). Systolic blood pressure (SBP), diastolic blood pressure (DBP), femoral neck bone mineral density (FNBD), depression (DEP) autism (AUT), low density lipoprotein (LDL), body mass index (BMI), glucose sensitivity (GLC), total cholesterol (TC), triglycerides (TG), type II diabetes (T2D), primary biliary cirrhosis (PBC), type I diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), Crohn's disease (CD), celiac disease (CD), B cell absolute count (BC), white blood cell count (WBCC), CD4 regulatory T cell absolute count (CD4regC), Naïve CD8⁺ T cell absolute count (nCD8C), Hodgkins lymphoma (HL), leukemia (Leu), non-Hodgkins lymphoma_1 (NHL_1), non-Hodgkins lymphoma_2 (NHL_2), primary lymphoid and hematopoietic malignant neoplasms (PLHMN), lymphoid leukemia (LLC91), chronic lymphocytic leukemia (CLL), lymphoid leukaemia_1 (LL_1), lymphoid leukaemia_2 (LL_2), monocyte count (MC), myeloid white cell count (MWCC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean platelet volume (MPV), platelet count (PC), red blood cell count (RBCC).

b. Heatmap of Blockshifter enrichment z-scores of GWAS summary statistics at promoter-interacting regions (PIRs) by individual cell types using endothelial cells as a control. For each trait, comparisons have been made between each individual cell type against the control endothelial precursors. Green indicates enrichment in the labeled tissue; blue indicates enrichment in the endothelial cell control. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblasts (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8).

c. Density distribution of the distance between selected SNPs and the transcription start site (TSS) of the nearest protein-coding gene (blue) and the TSS of the gene prioritized by COGS (green). For each GWAS trait, we selected all the significantly associated SNPs (p value $< 5 \cdot 10^{-8}$) interacting with at least one protein-coding gene prioritized by COGS. Coding SNPs or non-coding SNPs overlapping promoters were excluded. Vertical lines represent median distance.

d. Bubble plot of traits with significant enrichment ($p\text{-adj} < 0.05$) in one or more pathways from the Reactome database. Top numbers indicate the total number of genes analyzed for each trait (gene-score > 0.5), bubble size indicates the ratio of test genes to those in the pathway, and blue to yellow corresponds to decreasing adjusted p value for enrichment.

e. Example of prioritized gene (*TLE3*) for the trait lymphocytic leukemia (LL_2) in naïve CD4⁺ cells. Top: Manhattan plot; grey line indicates the significance cut-off ($5 \cdot 10^{-8}$). Middle: prioritized SNPs (black dots) at naïve CD4⁺ (nCD4) specific enhancer. Bottom: *TLE3* regulatory landscape nCD4 and erythroblasts (Ery). Green shade depicts the gene promoter, while yellow shades depict putative enhancer region for that gene enriched in prioritized non-coding SNPs. Arrows symbolize gene placement and orientation along the genomic window.

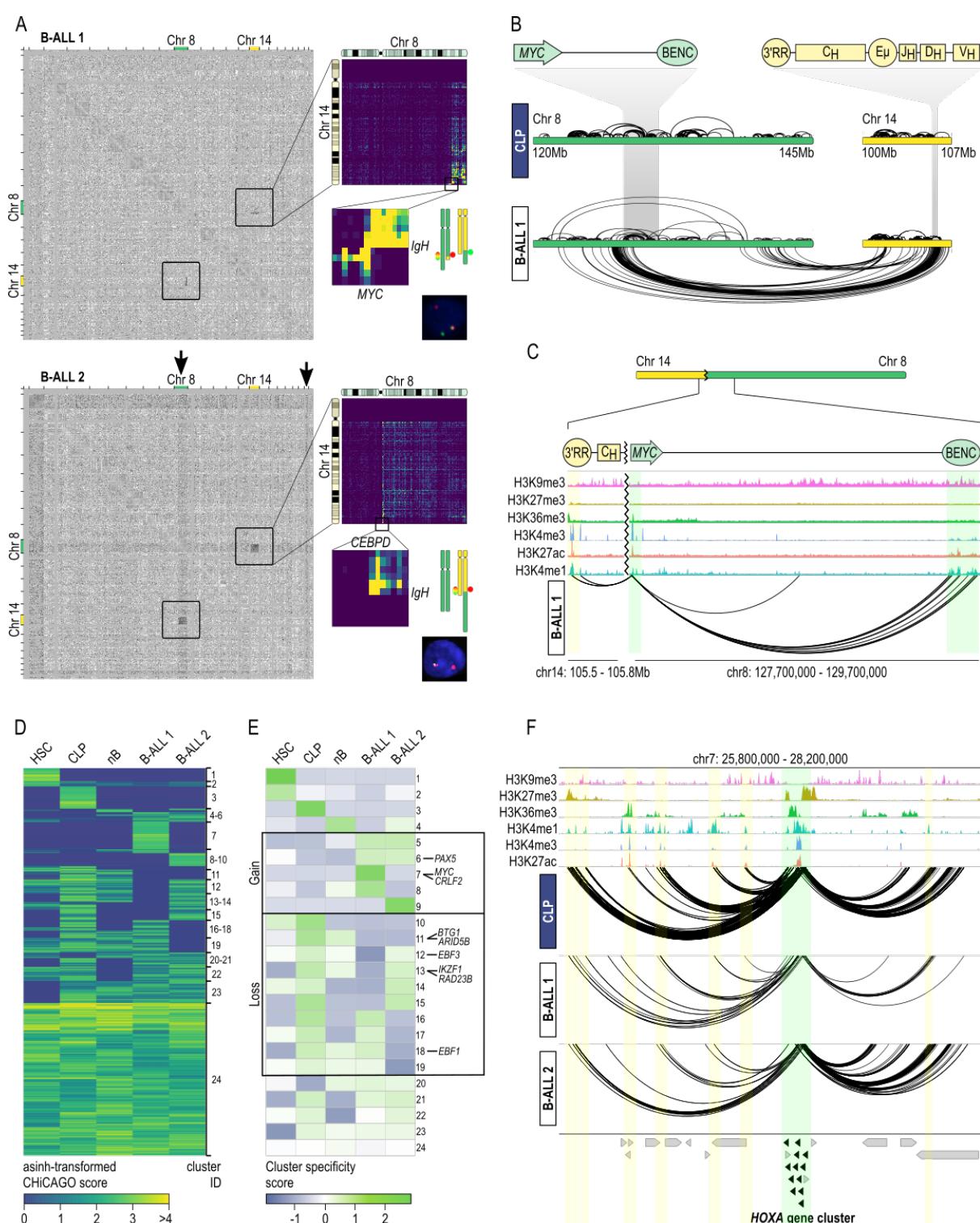


Fig. 5 liCHiC simultaneously detects translocations, copy number variations and topological alterations in tumor samples

a. Detection of structural variants using liCHiC data for B cell precursor acute lymphoblastic leukemia (B-ALL) 1 (top) and B-ALL 2 (bottom). Grey matrices on the left represent the log₂ ratio between B-ALL and CLP contact matrices at 1Mb resolution across the genome. Black

arrows indicate the location of copy number gains. On the top right: matrices at a 250kb resolution of the chromosomes involved in the translocations. On the middle right: zoom-ins of the breakpoint regions and schematic representation of the translocated chromosomes and the location of the FISH probes. On the bottom right: FISH images displaying the translocation.

b. Top: Schematic representation of *MYC* and *IgH* genes loci. Bottom: interaction landscape of chromosomes 8 and 14 in common B cell lymphoid progenitors (CLP) and B-ALL 1 sample. Interactions within and between chromosomes are represented over and below the chromosomes respectively.

c. Reconstruction of *MYC* promoter interaction landscape on the derivative chromosome in B-ALL 1. Green shades depict the *MYC* promoter and BENC enhancer from chromosome 8, and the yellow shadow the 3' RR enhancer from chromosome 14.

d. Heatmap of asinh-transformed CHiCAGO score of significant interactions in at least one cell type clustered using Autoclass algorithm. Hematopoietic stem cell (HSC), naïve B cell (nB).

e. Heatmap of cluster specificity score of each Autoclass cluster. Clusters containing interactions specific to B-ALL or interactions specifically lost in B-ALL are boxed.

f. *HOXA* gene promoter-centered interaction landscape (arcs) in CLP, B-ALL 1 and B-ALL 2 samples. Green shade depicts the *HOXA* gene cluster, while yellow shades depict putative enhancer regions for that gene in any of these cell types. Arrows symbolize gene placement and orientation along the genomic window.

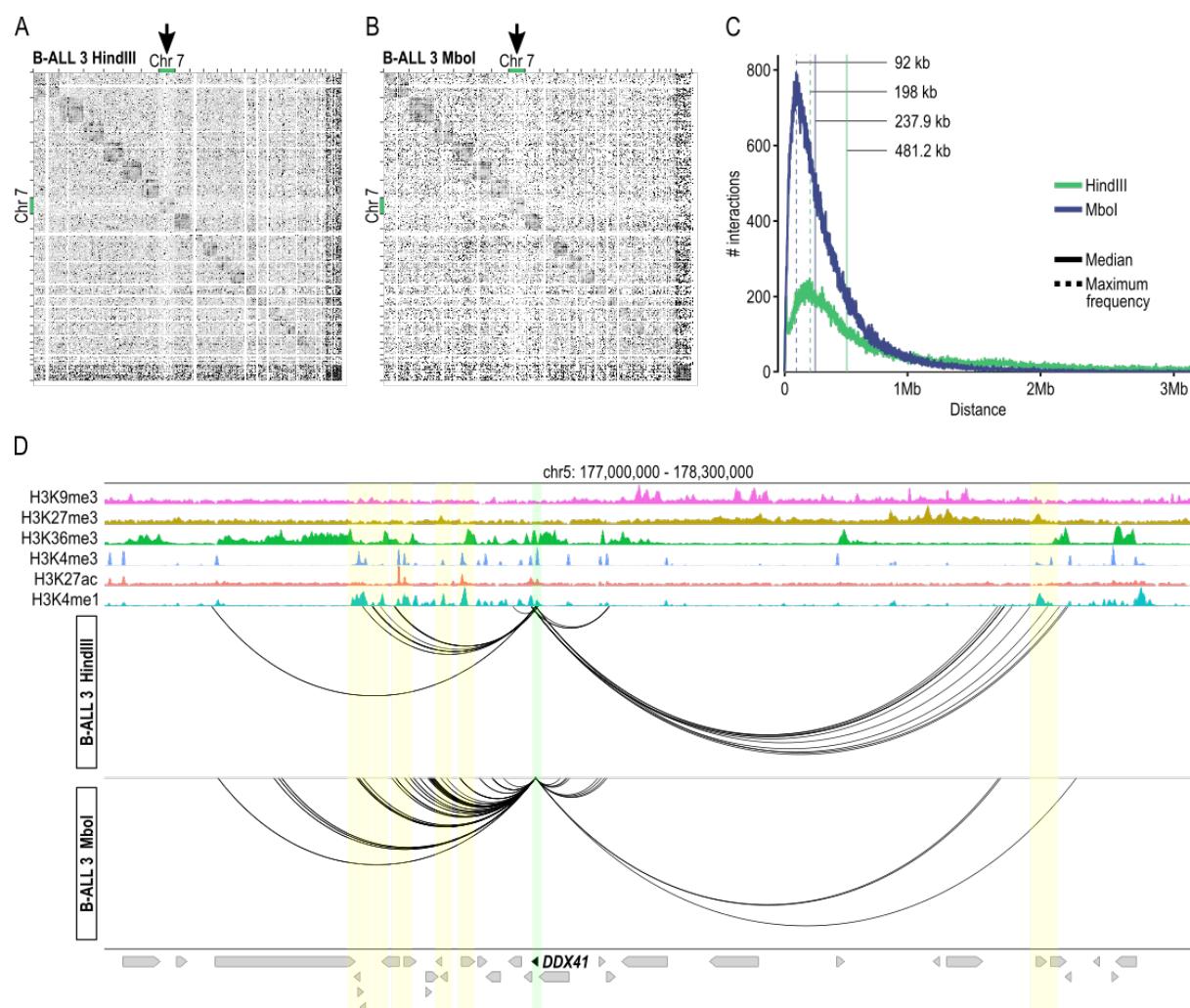
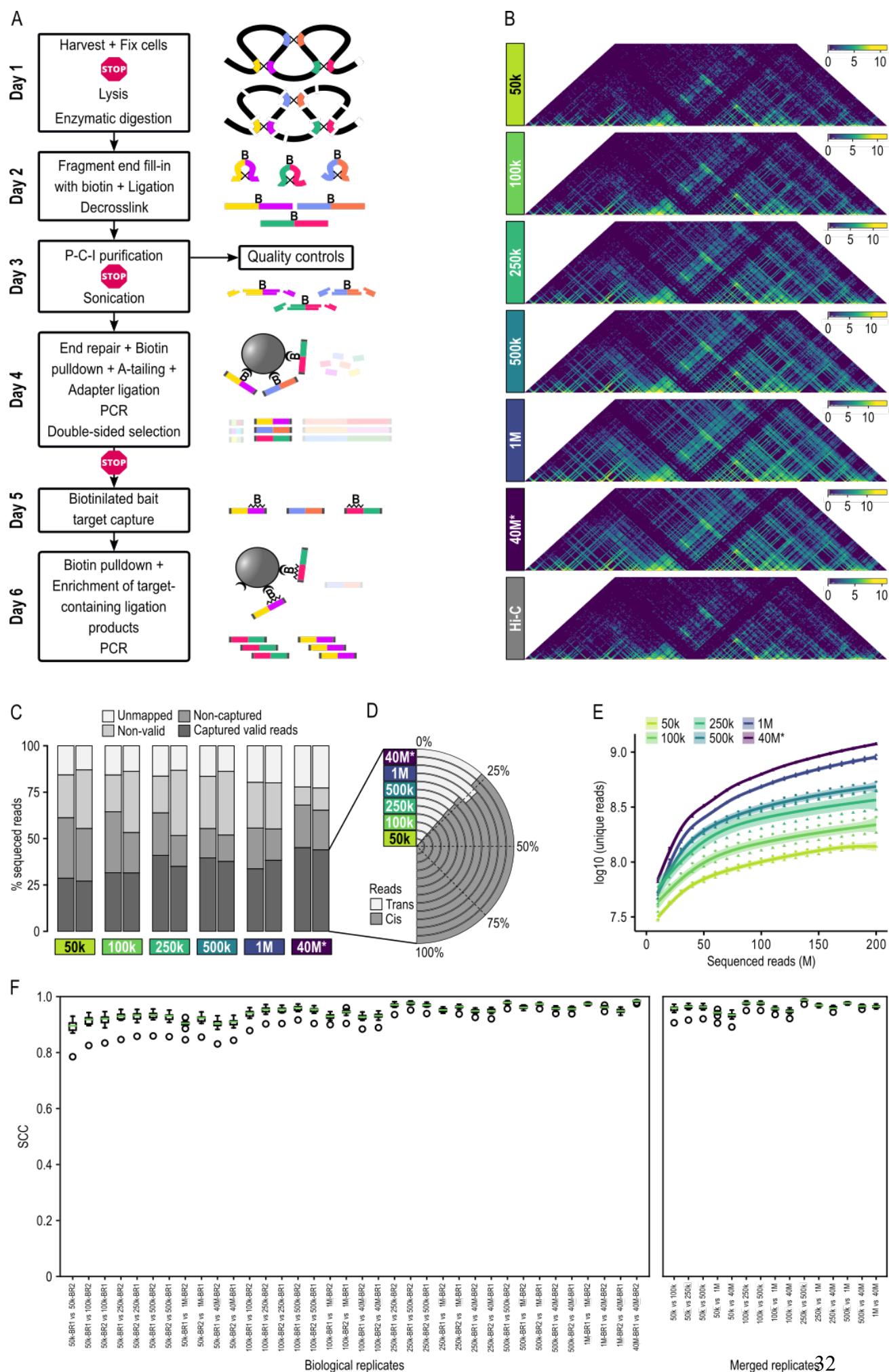


Fig. 6 Comparison of liCHi-C libraries at different resolutions.

Detection of structural variants using liCHi-C data for B cell precursor acute lymphoblastic leukemia (B-ALL) 3 generated using a six-cutter restriction enzyme (HindIII) (**a**) and a four-cutter restriction enzyme (MboI) (**b**). Grey matrices represent the log₂ ratio between B-ALL and CLP contact matrices at 1Mb resolution across the genome. Black arrows indicate the location of copy number losses.

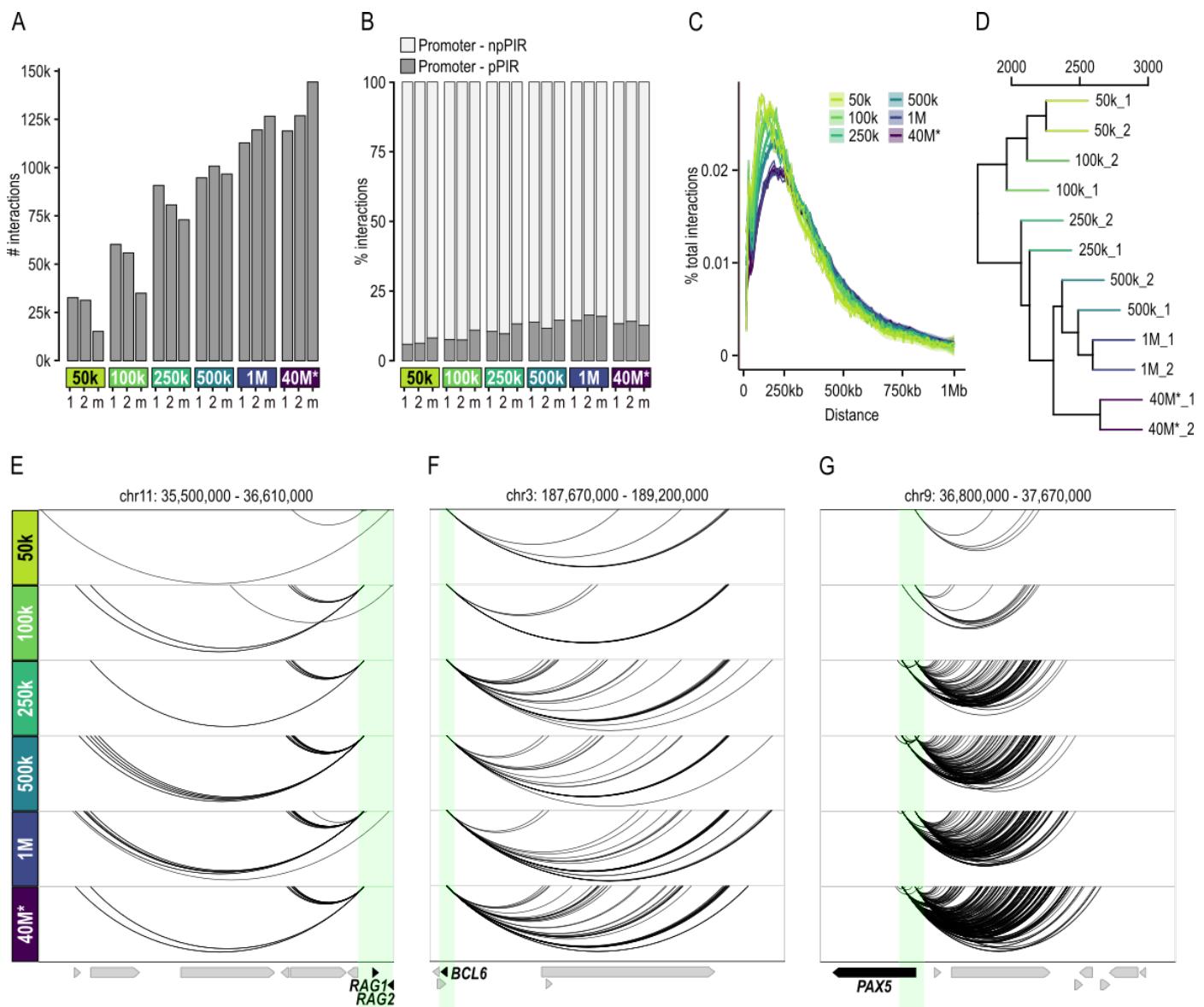
c. Absolute frequency of interaction according to the genomic distance between interacting regions for liCHi-C libraries generated with MboI (blue) and HindIII (green) digestion respectively. Median and highest absolute frequency of interactions are represented by solid and dashed lines respectively.

d. *DDX41* gene promoter-centered interaction landscape (arcs) generated with HindIII (top) and MboI (bottom) respectively. Green shade depicts the *DDX41* gene promoter, while yellow shades depict putative enhancer regions for it. Arrows symbolize gene placement and orientation along the genomic window.



Supplementary Data Fig. 1

- a.** In-depth overview of the liCHi-C workflow. “STOP” icons symbolize points in which the protocol may be safely stopped for up to several weeks by freezing the material. Optional quality controls of the first part of the protocol before sonicating may be performed at the expense of an extra day.
- b.** Interaction matrices of liCHi-C, PCHi-C (*) and Hi-C binned at 50kb resolution of experiments with different numbers of input cells. 1000 (k), million (M).
- c.** Proportions of reads passed through the different steps of HiCUP.
- d.** Cis-trans interaction ratio of valid captured reads. Cis and trans mean interactions within and between chromosome respectively.
- e.** Saturation plot representing unique reads respect to total number of sequenced reads.
- f.** Boxplots of the stratum adjusted correlation coefficient (SCC) between promoter interactomes of human naïve B cells obtained by PCHi-C (*) and liCHi-C using different cell numbers. Medians are represented by green lines. Each boxplot corresponds to the comparison between biological replicates (left) or merged replicates of experiments of different numbers of input cells (right).



Supplementary Data Fig. 2

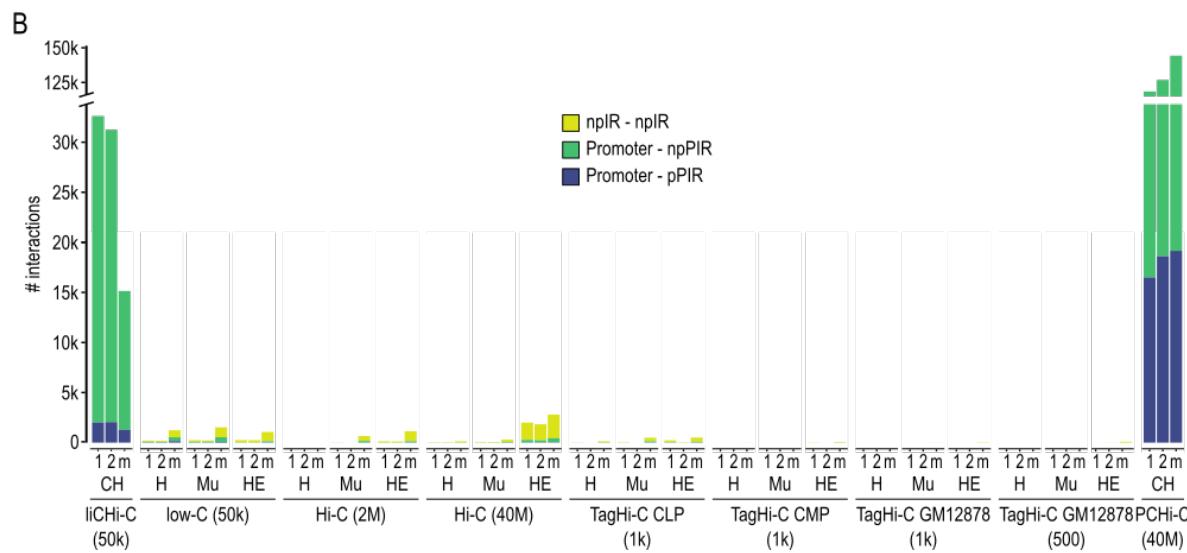
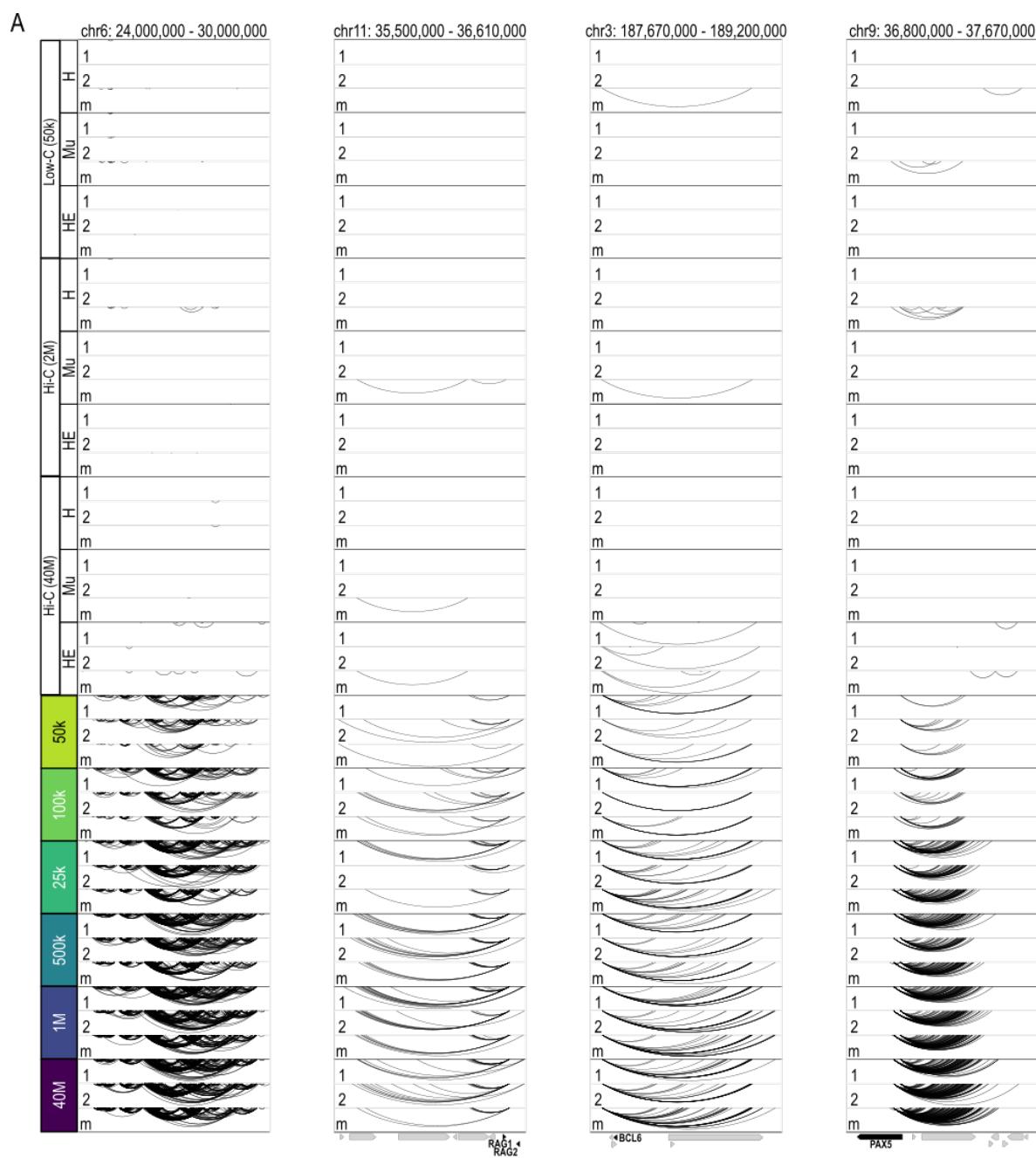
a. Total number of CHiCAGO significant interactions (score >5) of biological replicates (1 and 2) and merged samples (m) obtained by PCHi-C (*) and liCHi-C using different numbers of naïve B cells as input material. 1000 (k), million (M).

b. Proportions of promoter-npPIR and promoter-pPIR of CHiCAGO significant interactions of biological replicates.

c. Distance distribution of CHiCAGO significant interactions of merged samples.

d. Dendrogram of hierarchical clustering with average linkage based on Euclidean distances of CHiCAGO significant interactions of biological replicates.

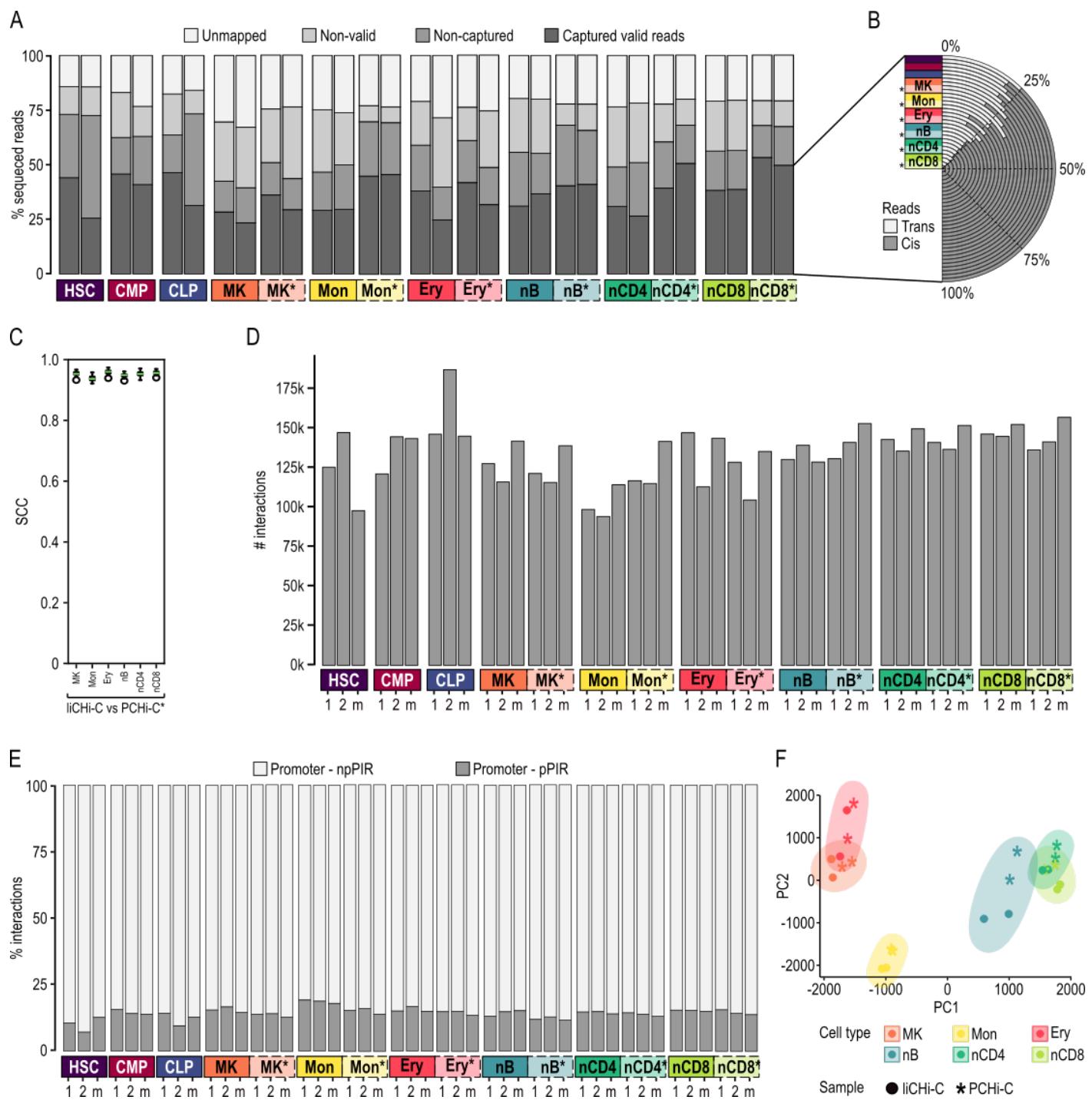
RAG1/2 (e.) *BCL6* (f.) and *PAX5* (g.) promoter-centered interactions (arcs) according to liCHi-C data. Green shade depicts gene promoters, while yellow shades depict putative enhancer regions for that gene. Arrows symbolize gene placement and orientation along the genomic window.



Supplementary Data Fig. 3

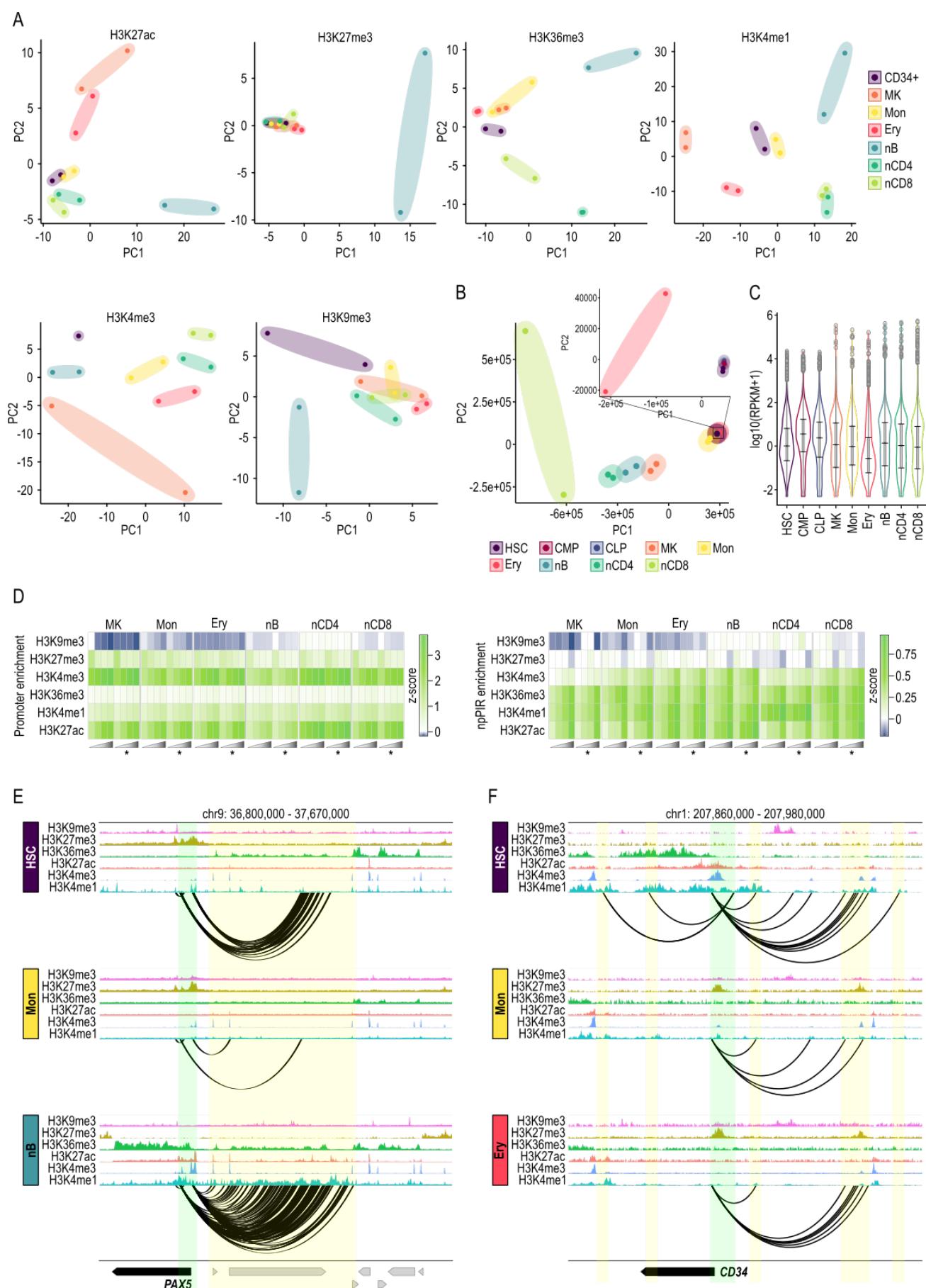
a. Side-by-side comparisons of significant interactions at selected loci from Fig. 1D and Extended Data Fig. 2e-g detected in human naïve B cells using liCHi-C, PCHi-C, Low-C and Hi-C methods. Significant loops were called in each biological replicate (1 and 2) and at the merged level (m) using standard parameters and different loop callers. Specifically, liCHi-C and PCHi-C data was analyzed by CHiCAGO (CH) at the HindIII restriction fragment resolution. Hi-C and Low-C data was analyzed by HiCCUPS (H), Mustache (Mu) and HiCExplorer (HE) at the 5kb resolution. Cell numbers per biological replicate are indicated in each case. 1000 (k), million (M).

b. Number of significant interactions detected by different 3C-based methods and starting material. Significant loops were called in each biological replicate (1 and 2) and at the merged level (m). Loops from liCHi-C and PCHi-C were called by CHiCAGO (CH) at the HindIII restriction fragment resolution (~4096 bp). Interactions from Low-C and Hi-C were called by HiCCUPS (H), Mustache (Mu) and HiCExplorer (HE) at the 5kb resolution using standard parameters. Interactions were classified as interactions between two promoters (Promoter-pPIR; blue), a promoter and a non-promoter region (Promoter-npPIR; green) and two non-promoter interacting regions (npPIR- npPIR; yellow). Only loops that engage at least one gene promoter at one node (green and blue categories) have the potential to control gene transcription.



Supplementary Data Fig. 4

- a.** Proportions of liCHi-C reads passed through the different steps of HiCUP. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblast (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8).
- b.** Cis-trans interaction ratio of liCHi-C valid captured reads. Cis and trans mean interactions within and between chromosome respectively.
- c.** Boxplots of the stratum adjusted correlation coefficient (SCC) between promoter interactomes obtained by PCHi-C and liCHi-C. Medians are represented by green lines
- d.** Total number of CHiCAGO significant interactions (score > 5) called in biological replicates (1 and 2) and merged samples (m).
- e.** Proportions of promoter-npPIR and promoter-pPIR of significant interactions of biological replicates (1 and 2) and merged samples (m) detected by liCHi-C and PCHi-C.
- f.** Principal component analysis of CHiCAGO significant interactions called in each biological replicate of liCHi-C and PCHi-C.



Supplementary Data Fig. 5

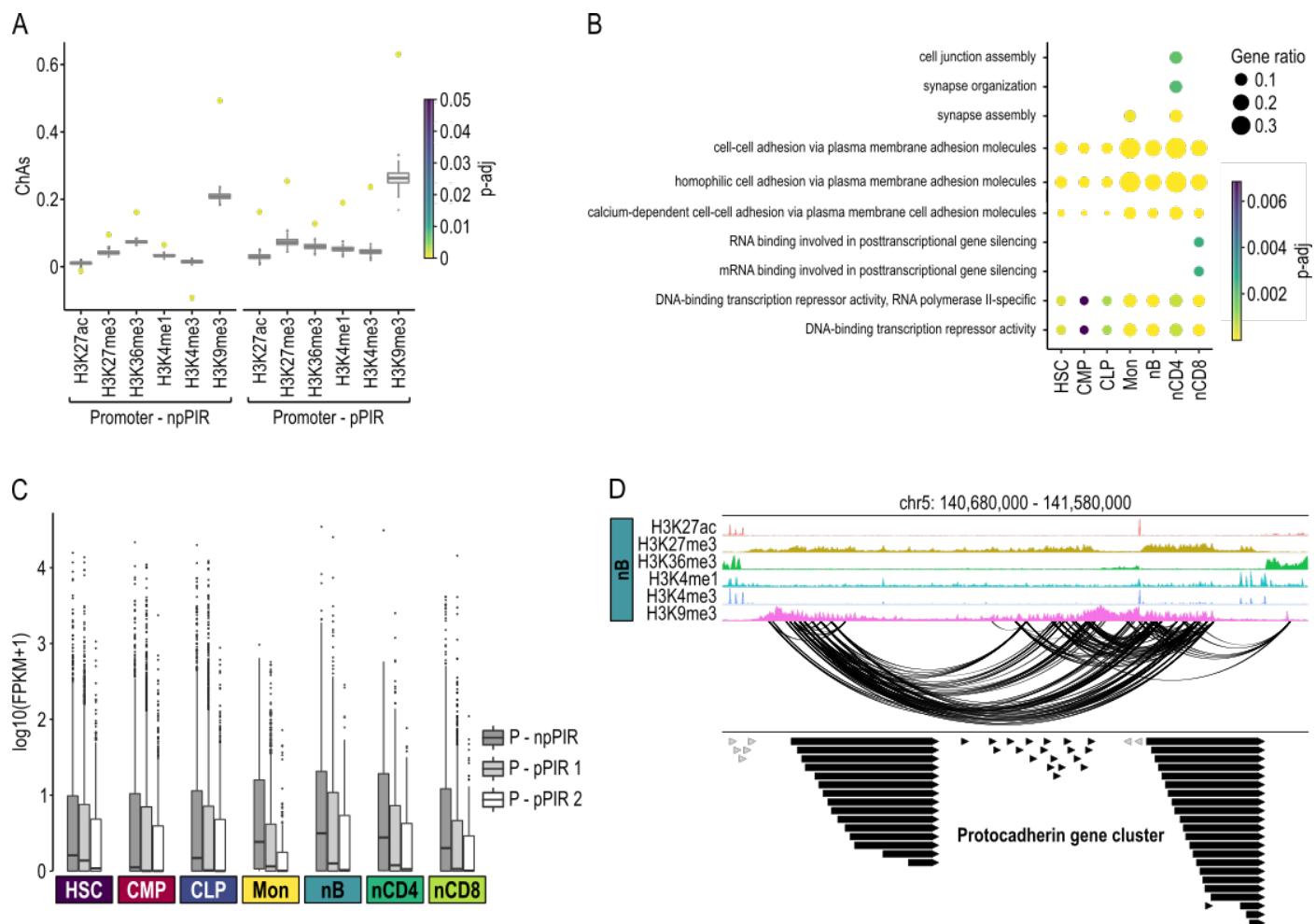
Principal component analysis plot of the significant ChIP-seq peaks from 6 different histone marks (**a.**) and FPKM expression values (**b.**). Zoom-in of some samples are shown for clear visualization. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblast (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8).

c. Violin plot representing FPKM expression values of merged RNA-seq samples, highlighting the quartiles cut-offs used to classify significant interactions by the expression of its genes.

d. Heatmap representing enrichment (expressed in terms of z-scores) of promoters (left) and non-promoter promoter-interacting regions (npPIRs) (right) for histone marks. Promoter-interacting regions (PIRs) and promoters were identified by PCHi-C using 40 million cells and classified by the quartiles level of FPKM expression of the associated gene.

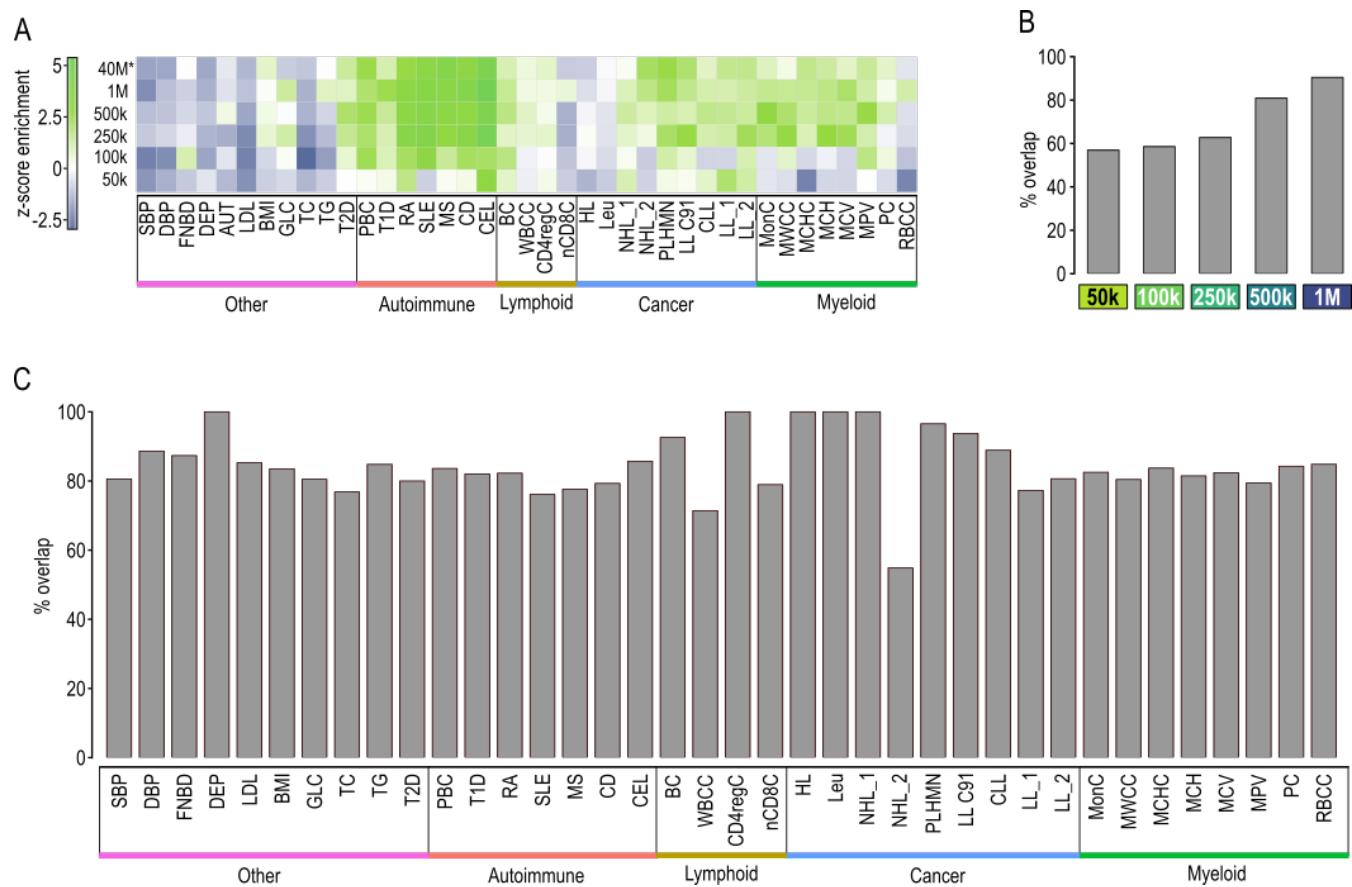
e. *PAX5* promoter-centered interaction landscape (arcs) in hematopoietic stem cells (top), monocytes (middle) and naïve B cells (bottom). Green shade depicts the gene promoter, while yellow shades depict putative enhancer regions for that gene in any of these cell types.

f. *CD34* promoter-centered interaction landscape (arcs) in hematopoietic stem cells (top), monocytes (middle) and erythrocytes (bottom). Green shade depicts the gene promoter, while yellow shades depict putative enhancer regions for that gene in any of these cell types.



Supplementary Data Fig. 6

- a.** Boxplot displaying chromatin assortativity (ChAs) values of 1000 distance-preserved randomized networks. ChAs values of the liCHi-C promoter-npPIRs subnetwork (left) and promoter-pPIR subnetwork (right) for each histone mark in common myeloid progenitor cells are represented with colored dots. Color represents adjusted p-value.
- b.** Gene Ontology terms enrichment for genes involved in H3K9me3-centred interaction networks based on liCHi-C data. Hematopoietic stem cell (HSC), common myeloid progenitor (CMP), common B cell lymphoid progenitor (CLP), megakaryocytes (MK), monocytes (Mon), erythroblast (Ery), naïve B cell (nB), naïve CD4⁺ cells (nCD4) and naïve CD8⁺ cells (nCD8). Color and point size represent adjusted p-value and gene ratio respectively.
- c.** Boxplots of log10 FPKM expression values of the genes involved in H3K9me3-mediated promoter interaction networks. Interactions with peak of H3K9me3 in the npPIRs interacting with a promoter (P-npPIR), interactions with a H3K9me3 peak in one of the two interacting promoters (P-pPIR 1), interactions with H3K9me3 peaks in both interacting promoters (P-pPIR 2).
- d.** H3K9me3-mediated promoter interaction network centered on the protocadherin genes cluster in naïve B cells. Arrows symbolize gene placement and orientation along the genomic window.

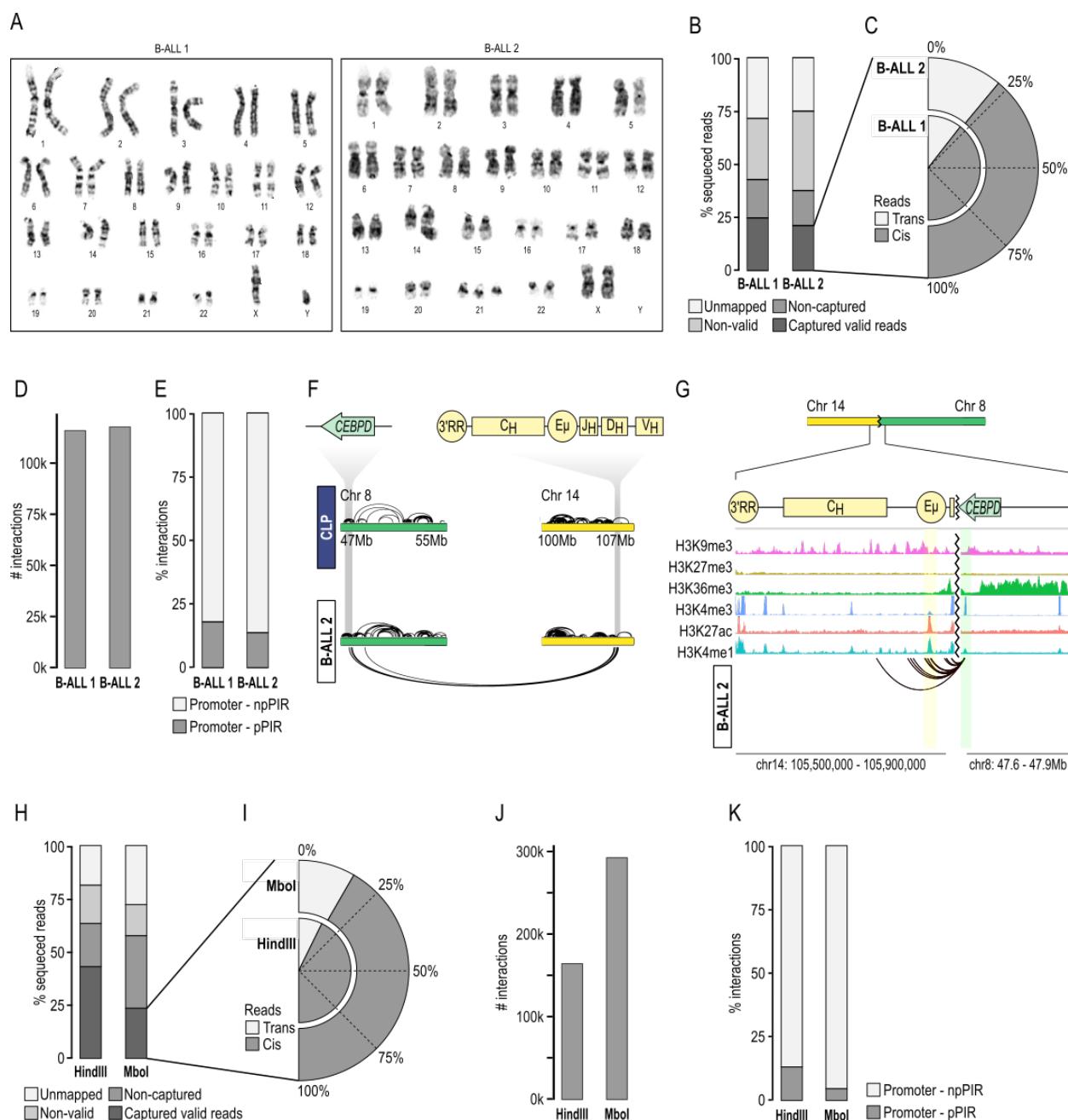


Supplementary Data Fig. 7

a. Heatmap of Blockshifter z-scores reflecting the enrichment of SNPs at promoter-interacting regions (PIRs) called by liCHi-C. For each trait, comparisons have been made between each naïve B cell titration against the control endothelial precursors. Green indicates enrichment in naïve B cells; blue indicates enrichment in the endothelial cell control. Traits are labeled and colored by category: autoimmune disorder (red), lymphoid trait (yellow), myeloid trait (green), blood cancer (blue) and other (purple). Systolic blood pressure (SBP), diastolic blood pressure (DBP), femoral neck bone mineral density (FNBD), depression (DEP) autism (AUT), low density lipoprotein (LDL), body mass index (BMI), glucose sensitivity (GLC), total cholesterol (TC), triglycerides (TG), type II diabetes (T2D), primary biliary cirrhosis (PBC), type I diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), Crohn's disease (CD), celiac disease (CD), B cell absolute count (BC), white blood cell count (WBCC), CD4 regulatory T cell absolute count (CD4regC), Naïve CD8⁺ T cell absolute count (nCD8C), Hodgkins lymphoma (HL), leukemia (Leu), non-Hodgkins lymphoma_1 (NHL_1), non-Hodgkins lymphoma_2 (NHL_2), primary lymphoid and hematopoietic malignant neoplasms (PLHMN), lymphoid leukemia (LLC91), chronic lymphocytic leukemia (CLL), lymphoid leukaemia_1 (LL_1), lymphoid leukaemia_2 (LL_2), monocyte count (MC), myeloid white cell count (MWCC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean platelet volume (MPV), platelet count (PC), red blood cell count (RBCC), 1000 (k), million (M).

b. Percentage of overlap between the set of genes prioritized by COGS between naïve B cells liCHi-C against the set of genes prioritized with PCHi-C using 40M sample for lymphoid traits.

c. Percentage of overlap between the set of genes prioritized by COGS with liCHi-C against the set of genes prioritized with PCHi-C. Unlike in **b**, the 6 cell types and all GWAS datasets were included in the analysis.



Supplementary Data Fig. 8

- a.** G-band karyotype of pediatric B cell precursor acute lymphoblastic leukemia (B-ALL) 1 and B-ALL 2 samples.
- b.** Proportions of liCHi-C reads passed through the different steps of HiCUP.
- c.** Cis-trans interaction ratio of liCHi-C valid captured reads. Cis and trans mean interactions within and between chromosome respectively.
- d.** Total number of liCHi-C significant interactions.
- e.** Proportions of promoter-npPIR and promoter-pPIR of liCHi-C significant interactions.
- f.** Top: Schematic representation of *CEBPD* and *IgH* gene loci. Bottom: interaction landscape of chromosomes 8 and 14 in common B cell lymphoid progenitors (CLP) and B-ALL 2 sample. Interactions within and between chromosomes are represented over and below the chromosomes respectively.
- g.** Reconstruction of *CEBPD* promoter interaction landscape on the derivative chromosome in B-ALL 2. Green shade depicts the *CEBPD* promoter and the yellow shadow the enhancer μ (E μ) of the *IgH* loci.
- h.** Proportions of liCHi-C reads from B-ALL patient 3 passed through the different steps of HiCUP.
- i.** Cis-trans interaction ratio of liCHi-C valid captured reads from B-ALL patient 3 generated with HindIII and MboI restriction enzyme. Cis and trans mean interactions within and between chromosome respectively.
- j.** Total number of liCHi-C significant interactions from B-ALL patient 3.
- k.** Proportions of promoter-npPIR and promoter-pPIR of liCHi-C significant interactions detected using HindIII and MboI digestion respectively.