BART3D: Inferring transcriptional regulators associated with differential chromatin interactions from Hi-C data

Zhenjia Wang[1], Yifan Zhang[1,2], Chongzhi Zang[1,2,3]*

[1] Center for Public Health Genomics, [2] Department of Biomedical Engineering, [3] Department of Public Health Sciences, and Department of Biochemistry and Molecular Biology, University of Virginia, Charlottesville, VA 22908, USA

* To whom correspondence should be addressed: zang@virginia.edu

ABSTRACT

Identification of functional transcriptional regulators (TRs) associated with chromatin interactions is an important problem in studies of 3-dimensional genome organization and gene regulation. Direct inference of TR binding has been limited by the resolution of Hi-C data. Here, we present BART3D, a computational method for inferring TRs associated with genome-wide differential chromatin interactions by comparing two Hi-C maps, leveraging public ChIP-seq data for human and mouse. We demonstrate that BART3D can detect target TRs inducing chromatin architecture changes from dynamic Hi-C profiles with TR perturbation. BART3D can be a useful tool in 3D genome data analysis and functional genomics research.

KEYWORDS

Hi-C, transcriptional regulator, ChIP-seq, chromatin architecture, protein-DNA interaction

INTRODUCTION

The three-dimensional (3D) organization of eukaryotic genomes affects transcriptional gene regulation [1-3]. Although topologically associating domains (TADs) appear to be conserved across cell types at the level of cell populations [4-6], chromatin architecture is highly dynamic during development and cell differentiation [7,8], and can be disrupted in disease states [1]. Transcriptional regulators (TRs), including transcription factors and chromatin regulators, are required for the establishment and maintenance of chromosomal architecture [9-11]. Identification of functional TRs associated with chromatin dynamics can help unravel the spatial organization of the genome and the impact of 3D architecture on gene regulation. The 3D organization of the genome can be measured using chromosome conformation capture-based methods such as Hi-C [12] and in situ Hi-C [5]. Chromatin interaction events can be detected by inferring loop structures from signal enrichment in Hi-C contact maps [5,13]. However, limited by the restriction enzyme digestion and ligation procedure in experiments and highly dependent on the sequencing depth, the resolution of Hi-C maps is typically 10^4-10^5 bp, or can be as high as 10^3 bp for ultra-deep *in situ* Hi-C [5]. It is still difficult to reach the sub-nucleosomal resolution of TR binding events (10^1-10^2 bp). HiChIP [14] and PLAC-seq [15] can reach higher resolution for easier association with TR binding but require additional experimental steps to use a preselected protein factor as an anchor, limiting the feasibility for an unbiased TR association analysis. A computational method to identify TR binding directly from lower-resolution Hi-C data is necessary for functional analysis of 3D genome data.

Most computational methods for differential Hi-C data analysis, including diffHic [16], FIND [17], HiCcompare [18], and Selfish [19], focus on detecting changes in chromatin interaction events on the locus-to-locus level by comparing Hi-C data generated in two biological states. Few methods aim to generate a profile of differential interaction across the whole genome to associate with TR binding profiles. TR inference from collected genomic binding profiles is a

more powerful approach than conventional DNA sequence motif search [20-22]. We previously developed BART [20], an algorithm for inferring TRs whose binding profiles associate with a query genomic profile, leveraging over 13,000 human and mouse ChIP-seq datasets collected from the public domain. Here, we present BART3D, a new bioinformatics tool for 3D genome data analysis built upon the BART algorithm. BART3D integrates Hi-C maps with public ChIP-seq data to infer TRs associated with genome-wide changes in chromatin interactions.

RESULTS

Overall design of BART3D

Starting with two Hi-C-type 3D genome contact maps as input, BART3D first generates a genomic differential chromatin interaction (DCI) profile by comparing the two contact maps, then uses the BART algorithm [20] to identify transcriptional regulators (TRs) whose binding sites are associated with either increased or decreased chromatin interactions (Fig. 1). BART3D can accept three formats of unnormalized genomic contact maps as input: 1) raw count matrices from HiC-Pro [23], 2) .hic format files from Juicer [24], and 3) .cool format files [25]. The output is a ranked list of TRs with a series of statistical measurements.

We employ an innovative approach to quantify the difference between two Hi-C contact matrices under different conditions, e.g., treatment and control. To account for the negative correlation between intra-chromosomal interaction frequency and genomic distance (Supplementary Fig. S1) [12] and to extract chromatin architecture information, we first normalize the contact matrix of each chromosome using a distance-based approach, where the read count in each bin pair is normalized by the average read count across all bin pairs at the same genomic distance (Supplementary Fig. S2). For any region in a chromosome, we consider the intra-chromosomal interactions between this region and its flanking regions within a certain genomic distance, e.g., 500 kb, quantified by an array of contact scores in a column/row of the

contact matrix, represented by the dashed, blue, 45-degree boxes in Fig. 1a. We assess the DCI for this region by comparing the arrays of contact scores from the two conditions, e.g., treatment and control (Fig. 1a). The DCI score is calculated as the paired t-test statistic between the two sets of contact scores:

$$DCI = \frac{\bar{d}}{s_d/\sqrt{n}}$$

where $\bar{d}$ and s_d are the mean and the standard deviation, respectively, of $\{d_i\}, i = 1, \ldots, n$, defined as the difference in contact scores between treatment and control, calculated by subtracting the paired elements from the two arrays, and *n* is the length of the contact score arrays. Positive or negative DCI scores represent increased or decreased chromatin interactions, respectively, from control to treatment. We generate a genome-wide DCI profile by scanning all chromosomes to calculate DCI scores for every non-overlapping bin across each chromosome (Fig. 1b).

We then infer TRs whose genome-wide binding profiles are associated with the DCI profile derived from Hi-C contact matrices (Fig. 1c,d). We map the genomic DCI profile to the union DNaseI hypersensitive sites (UDHS), a curated dataset representing all putative cis-regulatory elements (CREs) in the genome [26], and generate a cis-regulatory profile in which the score for each candidate CRE is set to equal the DCI score of the genomic region in which the CRE is located. We use the BART algorithm [20] to infer TRs that preferentially bind at CREs with a high score, representing increased chromatin interactions. Then we flip the DCI profile and perform BART analysis again to infer TRs whose binding are associated with decreased chromatin interactions (Fig. 1e).

Performance of distance-based normalization

We first evaluated the feasibility and performance of the distance-based normalization method used in BART3D. By targeting a specific factor of interest, HiChIP signals are enriched at the target-bound loci [14]. With appropriate normalization, the HiChIP target factor should be inferred from a static HiChIP dataset. Using this measure, we collected 84 HiChIP datasets targeting different TRs and tested how different normalization methods affect the inference of the HiChIP target factor. For each HiChIP dataset, we generated genomic contact maps at 5kb resolution without normalization, with ICE normalization [23], and with distance-based normalization. We then generated a genomic profile from each contact map, in which each 5kb bin across the genome is scored as the sum of interaction signals between this bin and all of its flanking bins within 500 kb. We used BART to infer TRs associated with this genomic interaction profile. As a control, we also ran BART analysis on the HiChIP sequence read pile-up profile and used the rank of the target factor as a reference. Overall, the distance-based normalization yielded high ranks of the HiChIP factors comparable to the HiChIP profile control, higher than either unnormalized or ICE normalized approaches (Fig. 2). This result indicates that the distance-based normalization is an optimal approach for quantifying Hi-C matrices for BART3D analysis.

BART3D can identify perturbed TRs from dynamic Hi-C data

To demonstrate the performance of BART3D, we calculated DCI profiles and inferred TRs for several published Hi-C experiments comparing wild type (WT) with DNA-associating factor knockout (KO) models in mouse cells. The KO targets include transcription factor Ctcf [27] and cohesin complex component Rad21 [28], known to function cooperatively to induce DNA looping and maintain TAD structures [29]; as well as Nr1d1 [30], and Smchd1 [31], both of which have repressive effects on transcriptional regulation and chromatin architecture. As expected, genomic regions containing binding sites of CTCF or RAD21 exhibit decreased chromatin interaction levels after KO (Fig. 3a,b), while those containing NR1D1 or SMCHD1

sites associate with increased chromatin interactions after KO in their corresponding samples (Fig. 3c,d). This result indicates that the DCI profile can connect perturbed protein binding sites with differential chromatin interaction. Indeed, the BART3D results show that the KO factors are always among the top-ranked TRs inferred to be associated with the corresponding decreased or increased chromatin interactions for all four cases (Fig. 4, labeled in red). These results show that BART3D can successfully infer TRs that induce chromatin interaction changes from Hi-C data.

In addition to the KO factor itself, we also found other TRs highly ranked in the BART3D results from the KO/WT Hi-C comparisons (Fig. 4). For Ctcf or Rad21 KO (Fig. 4a,b), several top inferred TRs, including SMC1A, SMC3, and STAG1 are all components of the cohesin complex [32]. For Nr1d1 KO (Fig. 4c), other top-ranked TRs are either co-regulators (PPARA, HNF4A, and FOXA2) or a target (ARNTL) of NR1D1, which regulates metabolic and circadian pathways [33-36]. For Smchd1 KO (Fig. 4d), SUZ12, EZH2, BMI1, and RNF2 are all related to polycomb group (PcG) factors, which have been shown to interact with SMCHD1 [37] and have repressive effects on transcription and chromatin state. These results indicate that BART3D can help identify novel TRs with biological relevance by mining Hi-C data.

DISCUSSION

We developed BART3D for differential analysis of Hi-C data and to infer functional TRs associated with changes in chromatin interactions. BART3D overcomes the relatively low resolution of Hi-C data and connects chromatin interactions on the multi-kb to Mb level to cis-regulatory events on the nucleosomal or base-pair level by accounting for statistical differences in Hi-C signals within a large distance range and using a predefined genomic CRE set. BART3D uses a distance-based normalization approach, which can remove cross-sample biases (Supplementary Fig. S2) and outperforms ICE normalization [23] in detecting local chromatin

interactions (Fig. 2). We use dynamic Hi-C datasets from TR KO experiments to show that BART3D can accurately infer TRs inducing chromatin architecture changes as well as other TRs with biological relevance.

In the framework of BART3D, we assume that differential chromatin interaction is mainly caused by changes in genomic binding of transcriptional regulator proteins, which act primarily in cis. Other events that can also result in pattern changes on Hi-C maps such as structural variations in the genome are not considered in BART3D. Under this assumption, we focus on intra-chromosomal interactions within a certain range of chromosomal distance and completely ignore inter-chromosomal interactions. The default genomic distance is set as 200 kb, but users can adjust this parameter in exploratory studies for optimizing discovery power, as different TRs may associate with chromatin interactions at different genomic ranges (Fig. 5). There are not many other tunable parameters. The bin size should be consistent with the Hi-C contact maps under interrogation and is restricted to the Hi-C data resolution. Replicates of Hi-C data are not accounted for in the DCI calculation but can be used to generate a background control for TR inference, i.e., TRs inferred from comparing replicates of Hi-C data from the same biological condition are likely due to technical variations and can be considered as false positive. Such TRs should be discarded if they also appear in results from cross-condition Hi-C comparisons.

Although developed for Hi-C data analysis, BART3D can also be applied to other 3D-genome data, such as ChIA-PET [38], HiChIP [14], and PLAC-seq [15]. When analyzing HiChIP or PLAC-seq data using BART3D, one may notice that the ChIP factor tends to appear on the top of the inferred TR list. Because HiChIP/PLAC-seq signals are always enriched at genomic binding sites of the ChIP factor regardless of chromatin interaction changes, the inference of a ChIP factor and its known co-factors should be considered false positives and removed for result interpretation. We plan to account for this effect and develop an extended version for

analyzing HiChIP/PLAC-seq data in the future. In addition, TR inference in BART3D is limited to collected ChIP-seq data, which currently include 918 human TRs and 565 mouse TRs but still grow rapidly and require regular updates and maintenance. Nevertheless, BART3D provides a framework for accurate inference of TRs associated with differential chromatin interactions and has broad applications in making biologically meaningful inferences and generating hypotheses from 3D genome data.

CONCLUSIONS

In conclusion, our results demonstrate that BART3D can infer functional TRs associated with dynamic chromatin interactions from differential Hi-C data, despite the resolution of Hi-C data is lower than most TR binding patterns. BART3D is a useful tool for differential analysis of Hi-C data and can help provide biological insights from 3D genome profiles.

METHODS

Data collection

Hi-C, HiChIP, and ChIP-seq data were collected from NCBI GEO [39] in fastq format. Detailed information including accession numbers of all samples used in this work can be found in Supplementary Tables S1-S3.

Data processing

Hi-C (Supplementary Table S1) and HiChIP (Supplementary Table S3) sequence reads were aligned to the human (hg38) or mouse (mm10) reference genomes and processed using HiC-Pro [23]. Contact matrices were generated at a resolution of 5kb and normalized as described in *Normalization of contact matrices*. ChIP-seq (Supplementary Table S2) reads were aligned to the mouse reference genome (mm10) using BWA [40]. Sam files were then converted into bam files using samtools [41]. MACS2 [42] was used to call peaks under the FDR threshold of 0.05.

Normalization of contact matrices

Given a Hi-C contact matrix $A = \{a_{ij}\}$, the observed read count a_{ij} represents the interaction frequency between a pair of genomic bins i and j. To account for the negative correlation between the intra-chromosomal interaction frequency and the genomic distance between the bin pair [12], we normalized the contact matrix of each chromosome as follows: for any given genomic distance $d_k = k * r$, where r is the bin size (data resolution), we employed a normalization factor $\bar{S}_{d_k}$ as the average read count across all bin pairs with the same genomic distance d_k in this chromosome, i.e., $\bar{S}_{d_k} = (\sum_{j-i=k} a_{ij})/n$, where *n* is the total number of bin pairs with distance d_k. The read count a_{ij} of the bin pair with distance d_k was normalized by $\bar{S}_{d_k}$ as $a'_{ij} = a_{ij}/\bar{S}_{d_k}$. The matrix A was normalized into $A' = \{a'_{ij}\}$ for each chromosome.

Detection of differential chromatin interactions

We denoted the normalized Hi-C contact matrices as $T = \{t_{ij}\}$ for the Treatment condition and $C = \{c_{ij}\}$ for the Control condition, respectively, and $\mathcal{B} = \{1, 2, \ldots, \lfloor l/r \rfloor\}$ representing all equal-sized non-overlapping bins within a chromosome, where l is the length of the chromosome and r is the bin size. For a given genomic region $x \in \mathcal{B}$ and a pre-defined range of genomic distance L, the interaction frequencies between x and its flanking regions with genomic distance up to L were collected, as $IT = \{t_{xk}\}$ from T and $IC = \{c_{xk}\}$ from C, respectively, where $k \in \mathcal{B}, x - L/r \leq k \leq x + L/r$. The differential chromatin interaction (DCI) score at x was calculated as the paired-sample *t*-test statistic between the two arrays of interaction frequencies IT and IC as follows:

$$d_{xk} = t_{xk} - c_{xk},$$

$$DCI = \frac{\bar{d}}{s_d/\sqrt{n}}$$

where d_{xk} is the difference in interactions between each paired element in IT and IC; $\bar{d}$ and s_d are the mean and standard deviation of $\{d_{xk}\}$, respectively; n is the length of each array. Here we have $n = 2L/r + 1$.

Inference of TRs associated with differential chromatin interactions

We used previously curated union DNaseI hypersensitive sites (UDHS), which include 2,723,010 unique non-overlapping DNase-seq peaks for human and 1,529,448 for mouse, to represent all putative cis-regulatory elements (CREs) in the genome [26]. A genome-wide DCI profile was generated by calculating the DCI score of every bin across each chromosome. The DCI profile was mapped to UDHS such that the score for each candidate CRE is set to be equal to the DCI score of the genomic bin where the CRE is located.

We used the BART algorithm to infer TRs associated with differential chromatin interactions [20]. The analysis was done twice, for inferring TRs associated with increased and decreased chromatin interactions separately. For increased chromatin interactions, we ranked all CREs decreasingly by their scores, i.e., CREs with high positive scores would be ranked at the front. We calculated an association score between the CRE profile and each TR binding profile for all ChIP-seq datasets. The association score is defined as the area under the ROC curve (AUC) using the DCI score on CRE as the predictor for TR binding, set as a binary value indicating whether the CRE is overlapped with a peak of that TR from the ChIP-seq dataset. To account for multiple ChIP-seq datasets for the same TR, the Wilcoxon rank-sum test was then applied to assess each TR's significance by comparing the association scores from all ChIP-seq data for this TR with those from all other ChIP-seq datasets, and a background model was used to detect the specificity of each TR. A series of quantification scores with statistical assessments were included for a final ranked list of inferred TRs. For decreased chromatin interactions, the CRE profile was flipped, so that the lowest DCI scores representing CREs the most decreased

chromatin interactions are ranked at the front, and the BART analysis was then performed in the same way.

Determination of default genomic distance parameter

Using different genomic distance range parameters might lead to different TR inference results, because the acting range of different TRs vary a lot. In practice, users may try different distance parameters for exploratory studies. To set an appropriate default value for this parameter, we applied BART3D on a series of Hi-C datasets (Supplementary Table S1) comparing the wide type with perturbation (deletion or activation) of different TRs using different genomic distance ranges, i.e., 50 kb, 100 kb, 200 kb, 500 kb, and 1Mb. We compared the rank of the perturbation target factor in the BART3D results across different genomic distance ranges, and found that 200 kb is where most perturbation factors were ranked on top (Fig. 5). Therefore, we set 200 kb as the default value for the genomic distance range parameter.

Availability of data and materials

Implemented in Python, BART3D package with source code is freely available at https://github.com/zanglab/bart3d

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Authors' contributions

C.Z. conceived the project and designed the method. Z.W. and Y.Z. implemented the method and performed the analysis. Z.W. and C.Z. wrote the manuscript.

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Figure 1

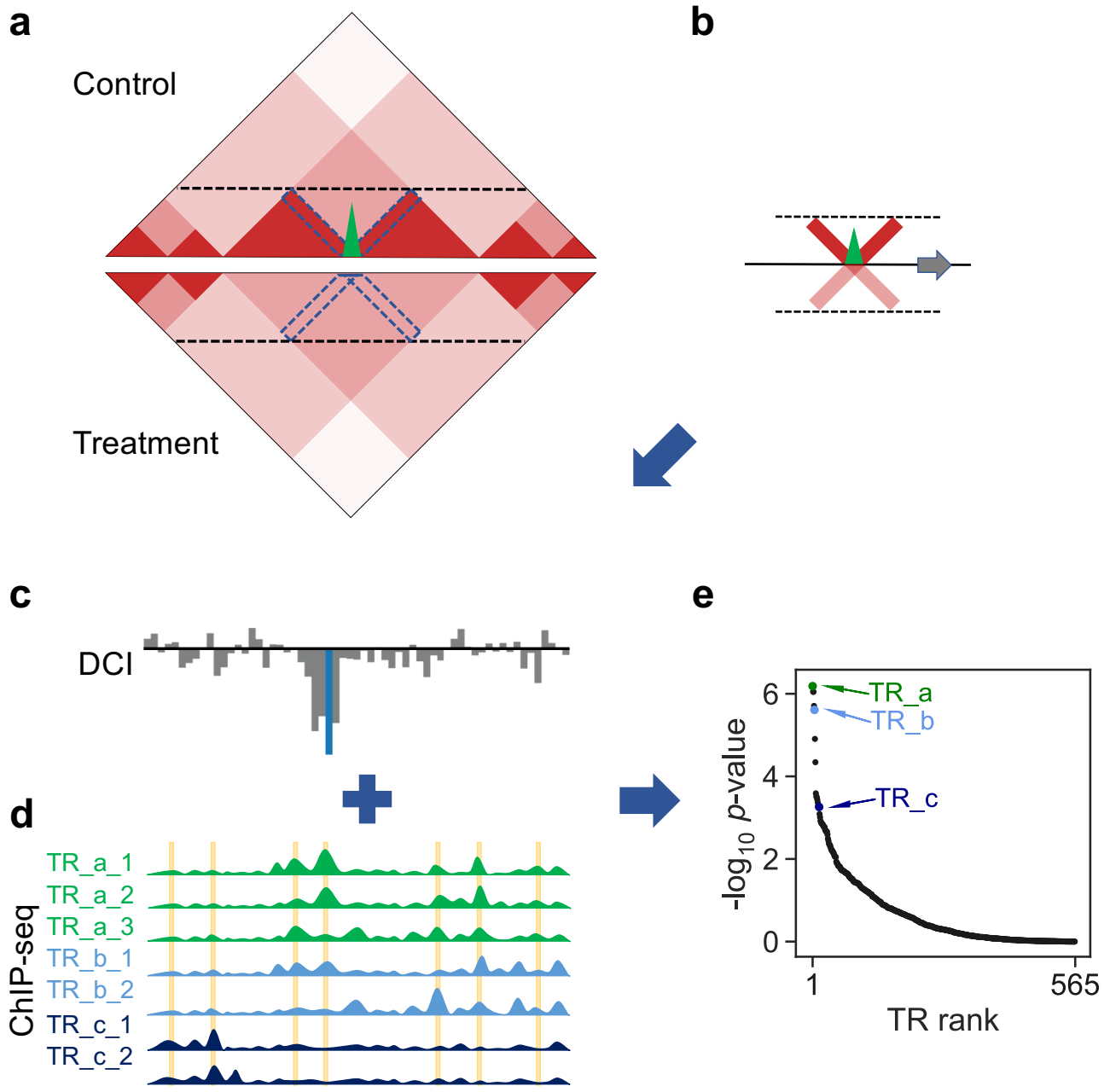


Figure 1. BART3D workflow. BART3D takes as input two Hi-C contact matrices (Control and Treatment) (**a**), scans each chromosome to calculate the DCI score at every bin by comparing the interaction counts (blue dashed boxes at 45-degree angle) within a certain distance boundary (black horizontal dashed lines) between the two conditions (**b**), and derives a DCI profile (**c**). The BART algorithm is then applied to associate the DCI profile with a large collection of public TR ChIP-seq data (**d**) for TR inference analysis. BART3D output is two ranked lists of all TRs associated with increase or decrease of chromatin interactions (**e**).

Figure 2

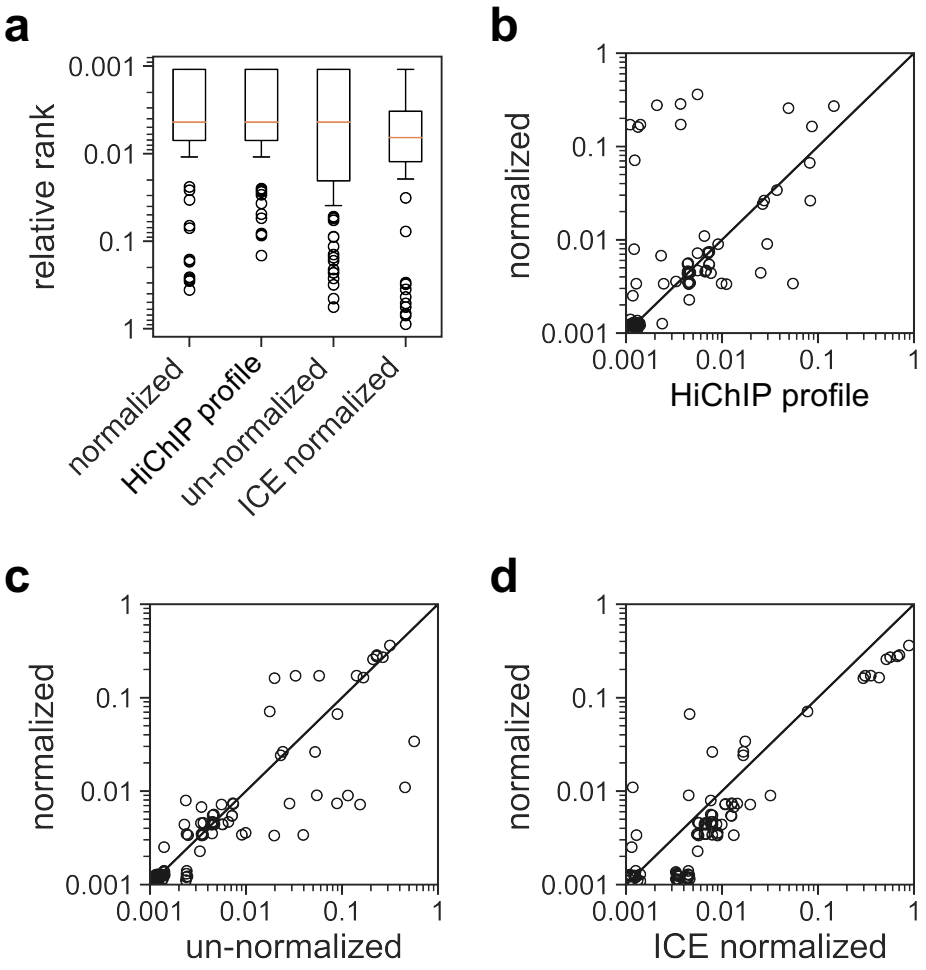


Figure 2. Comparison of different normalization methods.

(**a**) BART results of target TRs from 84 HiChIP datasets using different normalization methods. For distance-based normalized (labeled as "normalized"), unnormalized, and ICE normalized, BART was applied to a genomic region profile scored by summarizing the interaction frequencies of each 5kb bin to its flanking bins within 500kb. For the HiChIP profile, BART was applied to the HiChIP sequence read bam file (as positive control). Relative rank represents the rank of the target TR divided by the total number of TRs in the BART library. Center line in the box represents median.

(**b-d**) Comparison of the relative ranks of target TRs in the BART results generated from distance-based normalization against other methods: (**b**) HiChIP profile, (**c**) unnormalized and (**d**) ICE normalization. Each dot represents a dataset from the 84 HiChIP samples. More dots located below the diagonal line indicates that distance-based normalization (y-axis) yields to higher rank in the BART result.

Figure 3

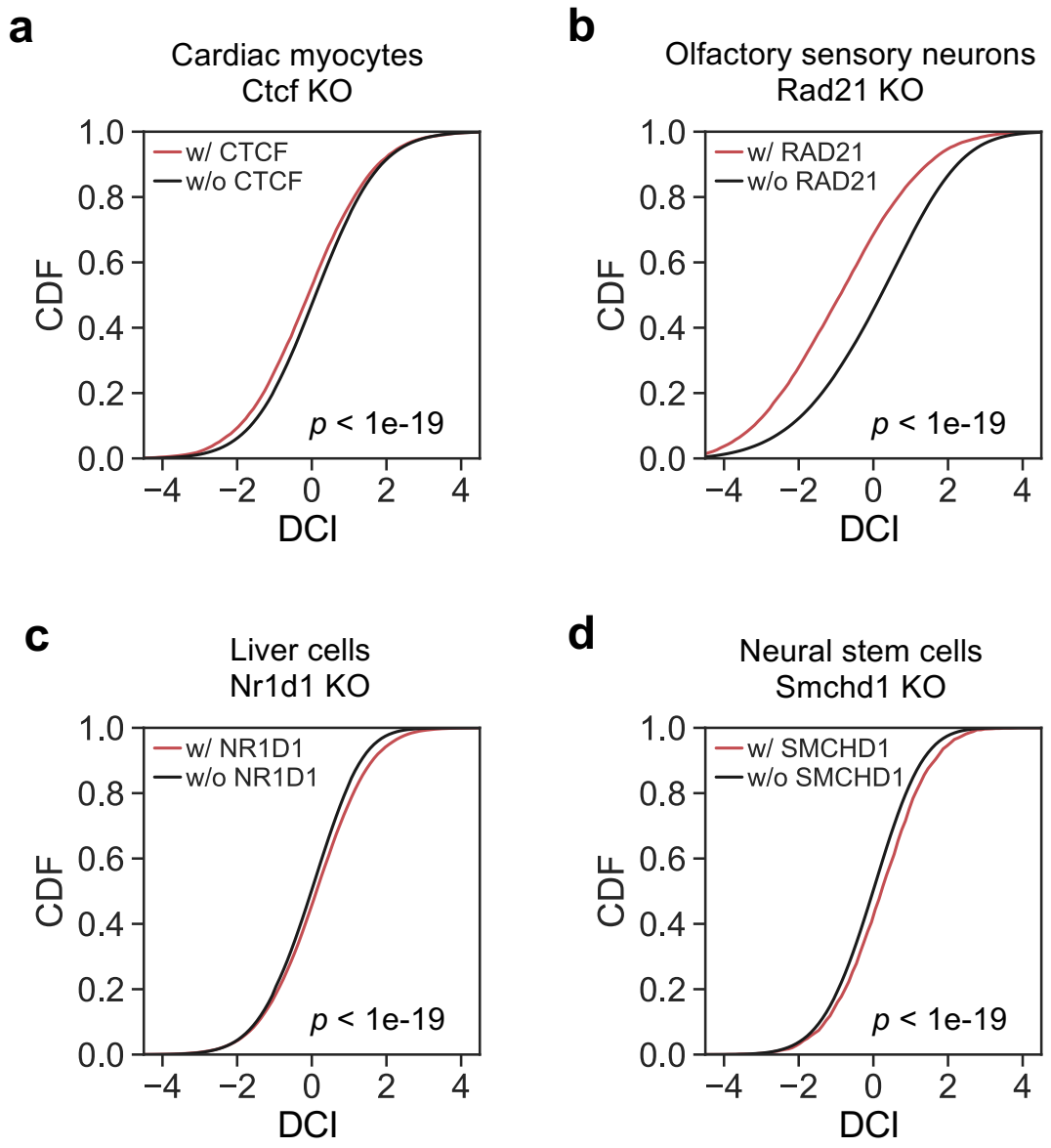


Figure 3. DCI scores at binding sites of KO TRs show significant deviations from the genomic background.

(**a-d**) Cumulative distributions of DCI scores for genomic regions with (red) and without (black) binding sites of KO TRs, including Ctcf KO in cardiac myocytes (**a**), Rad21 KO in olfactory sensory neurons (**b**), Nr1d1 KO in liver cells (**c**), and Smchd1 KO in neural stem cells (**d**), in mouse cell samples. DCI scores were calculated for each 5kb bin by comparing the normalized contact frequencies with its +/-500kb flanking regions. P-values were calculated by Wilcoxon rank-sum test.

Figure 4

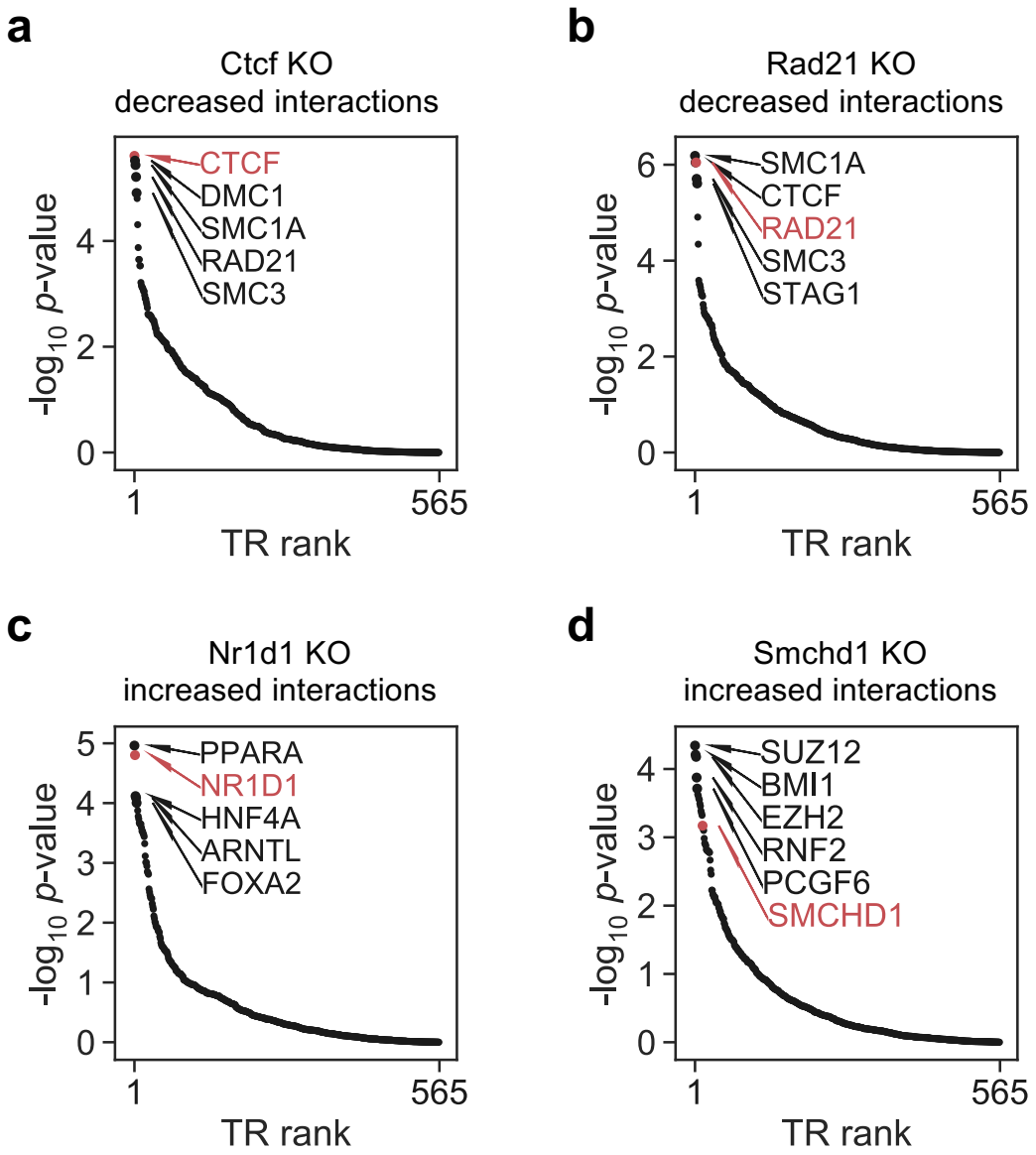


Figure 4. BART3D infers TRs that induce differential chromatin interactions from TR knockout Hi-C data.

(**a-d**) BART3D results focusing on decreased (**a,b**) or increased (**c,d**) chromatin interactions from differential Hi-C datasets in TR KO mouse samples: Ctcf KO in cardiac myocytes (**a**), Rad21 KO in olfactory sensory neurons (**b**), Nr1d1 KO in liver cells (**c**), and Smchd1 KO in neural stem cells (**d**). P-value scores were calculated from rank sum using the null hypothesis under the Irwin-Hall distribution. Top ranked TRs were labeled, and the KO TRs were marked in red.

Figure 5

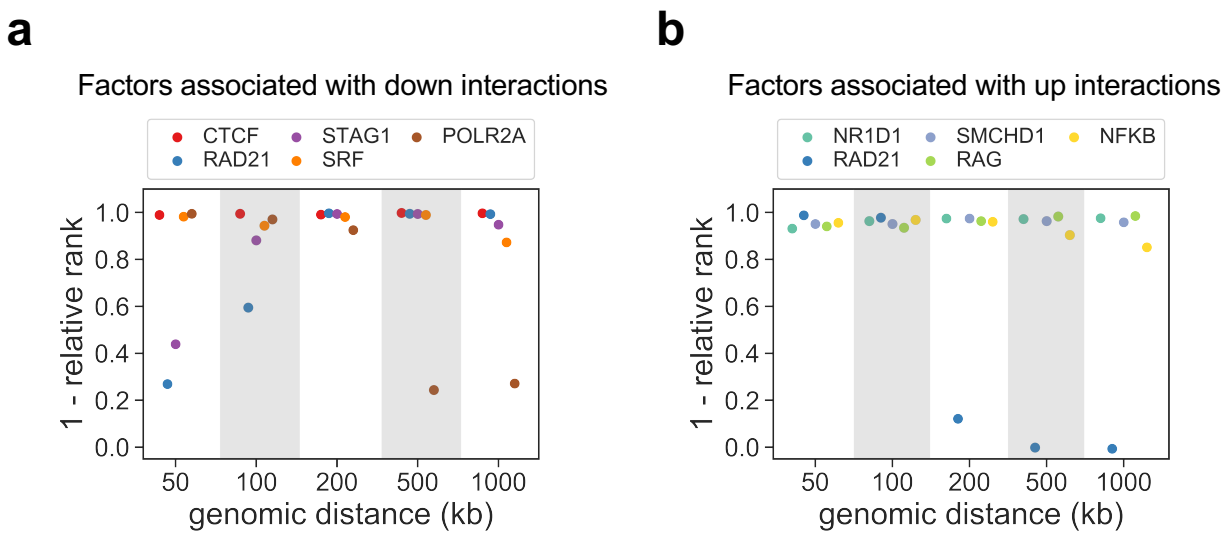
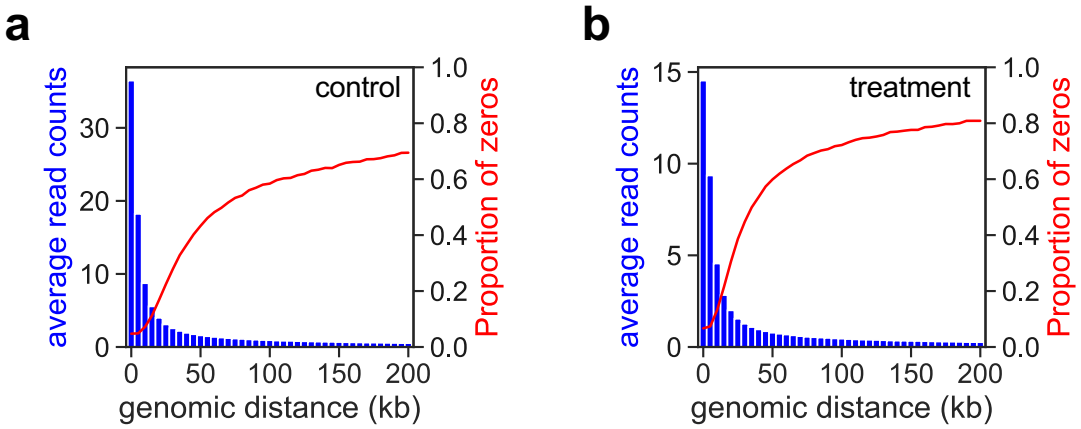


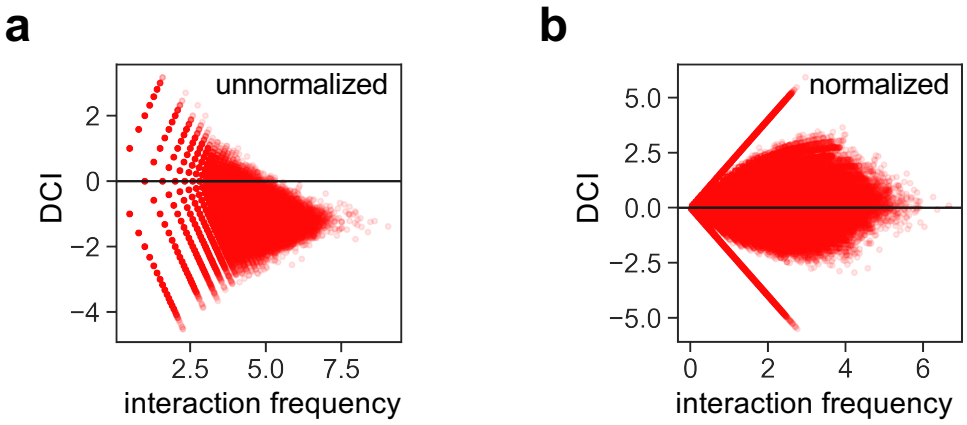
Figure 5. BART3D results on dynamic Hi-C datasets with TR perturbation under different genomic distance settings. The data were separated for TRs associated with decreased (**a**) and increased (**b**) chromatin interactions. The 1 - relative rank of the perturbed TR in BART3D results were shown for each dataset. Higher scores correspond to higher ranked TRs.

Supplementary Figure S1



Supplementary Figure S1. Hi-C read count negatively correlates with genomic distance between bin pairs. Average read counts and percentage of zeros in all bin pairs at the same genomic distance in two Hi-C datasets. (a) control: GSM2790405; (b) treatment: GSM2790406.

Supplementary Figure S2



Supplementary Figure S2. Effect of normalization. MA plots of averaged interaction frequency (x-axis) and differential chromatin interaction (DCI, y-axis) with unnormalized (a) and normalized (b) Hi-C contact matrices between treatment (GSM2790406) and control (GSM2790405).