Discovery of biased orientation of human DNA motif sequences affecting enhancer-promoter interactions and transcription of genes

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Abstract

Chromatin interactions have important roles for enhancer-promoter interactions (EPI) and regulating the transcription of genes. CTCF and cohesin proteins are located at the anchors of chromatin interactions, forming their loop structures. CTCF has insulator function limiting the activity of enhancers into the loops. DNA binding sequences of CTCF indicate their orientation bias at chromatin interaction anchors – forward-reverse (FR) orientation is frequently observed. DNA binding sequences of CTCF were found in open chromatin regions at about 40% - 80% of chromatin interaction anchors in Hi-C and in situ Hi-C experimental data. It has been reported that long range of chromatin interactions tends to include less CTCF at their anchors. It is still unclear what proteins are associated with chromatin interactions.

To find DNA binding motif sequences of transcription factors (TF) such as CTCF affecting the interaction between enhancers and promoters of transcriptional target genes and their expression, here, I predicted human transcriptional target genes of TF bound in open chromatin regions in enhancers and promoters in monocytes and other cell types using experimental data in public database. Transcriptional target genes were predicted based on enhancer-promoter association (EPA). EPA was shortened at the genomic locations of FR orientation of DNA binding motifs of TF, which were supposed to be at chromatin interaction anchors.

The expression level of the target genes predicted based on EPA was compared with target genes predicted from only promoters. Total 287 biased orientation of DNA motifs [159 forward-reverse (FR) and 153 reverse-forward (RF) orientation, the reverse

complement sequences of some DNA motifs are also registered in databases, so the total number is smaller than the number of FR and RF] affected the expression level of putative transcriptional target genes significantly in $CD14^+$ monocytes of four people in common. The same analysis was conducted in $CD4^+$ T cells of four people. DNA motif sequences of CTCF, cohesin and other transcription factors involved in chromatin interactions were found to be biased orientation. Transposon sequences, which are known to be involved in insulators and enhancers, showed biased orientation. Moreover, EPI predicted using FR or RF orientation of DNA motif sequences were overlapped with chromatin interaction data (Hi-C and HiChIP) more than other EPA.

Keywords: transcriptional target genes, gene expression, transcription factors, enhancer, enhancer-promoter interactions, chromatin interactions, CTCF, cohesin, open chromatin regions

Background

Chromatin interactions have important roles for enhancer-promoter interactions (EPI) and regulating the transcription of genes. CTCF and cohesin proteins are located at the anchors of chromatin interactions, forming their loop structures. CTCF has insulator function limiting the activity of enhancers into the loops (Fig. 1A). DNA binding sequences of CTCF indicate their orientation bias at chromatin interaction anchors – forward-reverse (FR) orientation is frequently observed (de Wit et al. 2015; Guo et al. 2015). About 40% - 80% of chromatin interaction anchors of Hi-C and in situ

55 Hi-C experiments include DNA binding motif sequences of CTCF. However, it has been
56 reported that long range of chromatin interactions tends to include less CTCF at their
57 anchors (Jeong et al. 2017). Other DNA binding proteins such as ZNF143, YY1, and
58 SMARCA4 (BRG1) are found to be associated with chromatin interactions and EPI
59 (Bailey et al. 2015; Barutcu et al. 2016; Weintraub et al. 2017). CTCF, cohesin, ZNF143,
60 YY1 and SMARCA4 have other biological functions as well as chromatin interactions
61 and enhancer-promoter interactions. The DNA binding motif sequences of the
62 transcription factors are found in open chromatin regions near transcriptional start sites
63 (TSS) as well as chromatin interaction anchors.

64 DNA binding motif sequence of ZNF143 was enriched at both chromatin
65 interaction anchors. ZNF143's correlation with the CTCF-cohesin cluster relies on its
66 weakest binding sites, found primarily at distal regulatory elements defined by the
67 'CTCF-rich' chromatin state. The strongest ZNF143-binding sites map to promoters
68 bound by RNA polymerase II (POL2) and other promoter-associated factors, such as the
69 TATA-binding protein (TBP) and the TBP-associated protein, together forming a
70 'promoter' cluster (Bailey et al. 2015).

71 DNA binding motif sequence of YY1 does not seem to be enriched at both
72 chromatin interaction anchors (Z-score < 2), whereas DNA binding motif sequence of
73 ZNF143 is significantly enriched (Z-score > 7; Bailey et al. 2015 Figure 2a). In the
74 analysis of YY1, to identify a protein factor that might contribute to EPI, (Ji et al. 2015)
75 performed chromatin immune precipitation with mass spectrometry (ChIP-MS), using
76 antibodies directed toward histones with modifications characteristic of enhancer and

77 promoter chromatin (H3K27ac and H3K4me3, respectively). Of 26 transcription factors
78 that occupy both enhancers and promoters, four are essential based on a CRISPR
79 cell-essentiality screen and two (CTCF, YY1) are expressed in >90% of tissues
80 examined (Weintraub et al. 2017). These analyses started from the analysis of histone
81 modifications of an enhancer mark rather than chromatin interactions. Other protein
82 factors associated with chromatin interactions may be found from other researches.

83 As computational approaches, machine-learning analyses to predict chromatin
84 interactions have been proposed (Schreiber et al. 2017; Zhang et al. 2017). However,
85 they were not intended to find DNA motif sequences of transcription factors affecting
86 chromatin interactions, EPI, and the expression level of transcriptional target genes,
87 which were examined in this study.

88 DNA binding proteins involved in chromatin interactions are supposed to affect
89 the transcription of genes in the loop formed by chromatin interactions. In my previous
90 analyses, the expression level of human putative transcriptional target genes was
91 affected, according to the criteria of enhancer-promoter association (EPA) (Fig. 1B;
92 (Osato 2018)). EPI predicted based on EPA shortened at the genomic locations of
93 forward-reverse orientation of CTCF binding sites affected the expression level of
94 putative transcriptional target genes the most, compared with the expression level of
95 transcriptional target genes predicted from only promoters (Fig. 1B). The expression
96 level tended to be increased in monocytes and $CD4^+$ T cells, implying that enhancers
97 activated the transcription of genes, and decreased in ES and iPS cells, implying that
98 enhancers repressed the transcription of genes. These analyses suggested that enhancers

99 affected the transcription of genes significantly, when EPI were predicted properly.
100 Other DNA binding proteins involved in chromatin interactions, as well as CTCF,
101 would locate at chromatin interaction anchors with a pair of biased orientation of DNA
102 binding motif sequences, affecting the expression level of putative transcriptional target
103 genes in the loop formed by the chromatin interactions. As experimental issues of the
104 analyses of chromatin interactions, chromatin interaction data are changed, according to
105 experimental techniques, depth of DNA sequencing, and even replication sets of the
106 same cell type. Chromatin interaction data may not be saturated enough to cover all
107 chromatin interactions. Supposing these characters of DNA binding proteins associated
108 with chromatin interactions and avoiding experimental issues of the analyses of
109 chromatin interactions, here I searched for DNA motif sequences of transcription factors
110 and repeat DNA sequences, affecting EPI and the expression level of putative
111 transcriptional target genes in $CD14^+$ monocytes and $CD4^+$ T cells of four people and
112 other cell types without using chromatin interaction data. Then, putative EPI were
113 compared with chromatin interaction data.
114

115 ## Results

116 **Search for biased orientation of DNA motif sequences**

117 Transcription factor binding sites (TFBS) were predicted using open chromatin
118 regions and DNA motif sequences of transcription factors collected from various
119 databases and journal papers (see Methods). Transcriptional target genes were predicted
120 using TFBS in promoters and enhancer-promoter association (EPA) shortened at the

121 genomic locations of DNA binding motif sequences of transcription factors such as
122 CTCF and cohesin (RAD21 and SMC3). To find DNA motif sequences of transcription
123 factors, other than CTCF and cohesin, and repeat DNA sequences affecting the
124 expression level of putative transcriptional target genes, transcriptional target genes of
125 each transcription factor were predicted based on EPA shortened at the genomic
126 locations of DNA motif sequences, and the expression level of the putative
127 transcriptional target genes was compared with those predicted from promoters using
128 Mann Whiteney U test (p-value < 0.05) (Fig. 2). The number of transcription factors
129 showing a significant difference of expression level of putative transcriptional target
130 genes was counted. To examine whether the orientation of DNA motif sequences
131 shortening EPA affected the number of transcription factors showing a significant
132 difference of expression level of putative transcriptional target genes, the number of the
133 transcription factors was compared among forward-reverse, reverse-forward, and any
134 orientation of DNA motif sequences shortening EPA, using chi-square test (p-value <
135 0.05). To avoid missing DNA motif sequences showing a relatively weak statistical
136 significance by multiple testing collection, the above analyses were conducted using
137 monocytes of four people independently, and DNA motif sequences found in monocytes
138 of four people in common were selected. Total 287 of biased (159 forward-reverse and
139 153 reverse-forward) orientation of DNA binding motif sequences of transcription
140 factors have been found in monocytes of four people in common, whereas only one any
141 orientation (i.e. without considering orientation) of DNA binding motif sequences have
142 been found in monocytes of four people in common (Fig. 3; Table 1; Supplemental

143 Table S1). Forward-reverse orientation of DNA motif sequences included CTCF,
144 cohesin (RAD21 and SMC3), ZNF143 and YY1, which are associated with chromatin
145 interactions and enhancer-promoter interactions (EPI). SMARCA4 (BRG1) is
146 associated with topologically associated domain (TAD), which is higher-order
147 chromatin organization. The DNA binding motif sequences of SMARCA4 was not
148 registered in the databases used in this study. Forward-reverse orientation of DNA motif
149 sequences included SMARCC2, a member of the same SWI/SNF family of proteins as
150 SMARCA4.

151 The same analysis was conducted using DNase-seq data of $CD4^+$ T cells of four
152 people. Total 265 of biased (158 forward-reverse and 139 reverse-forward) orientation
153 of DNA binding motif sequences of transcription factors have been found in T cells of
154 four people in common, whereas only four any orientation (i.e. without considering
155 orientation) of DNA binding motif sequences have been found in T cells of four people
156 in common (Supplemental Fig. S1 and Supplemental Table S2). Biased orientation of
157 DNA motif sequences in T cells included CTCF, cohesin (RAD21 and SMC3), ZNF143,
158 YY1, and SMARC. Biased orientation of DNA motif sequences in T cells and
159 monocytes (when using H3K27ac histone modification marks, which is described in the
160 next paragraph) included JUNDM2 (JDP2), which is involved in histone-chaperone
161 activity, promoting nucleosome, and inhibition of histone acetylation (Jin et al. 2006).
162 JDP2 forms a homodimer or heterodimer with various transcription factors
163 (https://en.wikipedia.org/wiki/Jun_dimerization_protein). Among 287, 61 of biased
164 orientation of DNA binding motif sequences of transcription factors were found in both

165 monocytes and T cells in common (Supplemental Table S5). For each orientation, 31
166 forward-reverse and 34 reverse-forward orientation of DNA binding motif sequences of
167 transcription factors were found in both monocytes and T cells in common. Without
168 considering the difference of orientation of DNA binding motif sequences, 84 of biased
169 orientation of DNA binding motif sequences of transcription factors were found in both
170 monocytes and T cells. As a reason for the increase of the number from 61, a
171 transcription factor or an isoform of the same transcription factor may bind to a
172 different DNA binding motif sequence according to cell types and/or in the same cell
173 type. The same transcription factor has several DNA binding motif sequences and in
174 some cases one of the motif sequence is almost the same as the reverse complement
175 sequence of another motif sequence of the same transcription factor. For example,
176 RAD21 showed both forward-reverse and reverse-forward orientation of the DNA motif
177 sequences, but the number of forward-reverse orientation was relatively small in the
178 genome, and reverse-forward orientation of the DNA motif sequence was frequently
179 observed and co-localized with CTCF. I previously found that a complex of
180 transcription factors would bind to a slightly different DNA binding motif sequence
181 from the combination of DNA binding motif sequences of transcription factors
182 composing the complex in *C. elegans* (Tabuchi et al. 2011). From another viewpoint of
183 this study, the expression level of putative transcription target genes of some
184 transcription factors would be different, depending on the genomic locations (enhancers
185 or promoters) of DNA binding motif sequences of the transcription factors in monocytes
186 and T cells of four people.

187 Moreover, using open chromatin regions overlapped with H3K27ac histone
188 modification marks known as enhancer and promoter marks, the same analyses were
189 performed in monocytes and T cells. H3K27ac histone modification marks were used in
190 the analysis of EPI, but were not used in the analysis of TF as insulator like CTCF and
191 cohesin, since in this condition, new biased orientation of DNA motif sequences was
192 found. Total 241 of biased (141 forward-reverse and 120 reverse-forward) orientation of
193 DNA binding motif sequences of transcription factors have been found in monocytes of
194 four people in common, whereas only one any orientation (i.e. without considering
195 orientation) of DNA binding motif sequences have been found (Supplemental Table S3).
196 Though the number of biased orientation of DNA motif sequences was reduced, JDP2
197 was found in monocytes as well as T cells. CTCF, RAD21, SMC3, ZNF143, YY1, and
198 SMARCC2 were also found. For T cells using H3K27ac histone modification marks,
199 total 195 of biased (107 forward-reverse and 110 reverse-forward) orientation of DNA
200 binding motif sequences of transcription factors have been found in T cells of four
201 people in common, whereas only six any orientation of DNA binding motif sequences
202 has been found (Supplemental Table S4). Though the number of biased orientation of
203 DNA motif sequences was reduced, CTCF, RAD21, SMC3, YY1, and SMARCC2 were
204 found. Scores of CTCF, RAD21, and SMC3 were increased compared with the result of
205 T cells without using H3K27ac histone modification marks, and they were ranked in the
206 top six. As summary of the results with and without H3K27ac histone modification
207 marks, total 386 of biased (223 forward-reverse and 214 reverse-forward) orientation of
208 DNA motif sequences has been found in monocytes of four people in common. Total

209 348 of biased (202 forward-reverse and 198 reverse-forward) orientation of DNA motif
210 sequences has been found in T cells of four people in common. Total of these results of
211 monocytes and T cells was 590 biased (368 forward-reverse and 359 reverse-forward)
212 orientation of DNA motif sequences. Biased orientation of DNA motif sequences found
213 in both monocytes and T cells was listed in Supplemental Table S5.

214 To examine whether the biased orientation of DNA binding motif sequences of
215 transcription factors was observed in other cell types, the same analyses were conducted
216 in other cell types. However, for other cell types, experimental data of one sample were
217 available in ENCODE database, so the analyses of DNA motif sequences were
218 performed by comparing with the result in monocytes of four people. Among the biased
219 orientation of DNA binding motif sequences found in monocytes, 69, 82, 56, and 53
220 DNA binding motif sequences were also observed in H1-hESC, iPS, Huvec and MCF-7
221 respectively, including CTCF and cohesin (RAD21 and SMC3) (Table 2; Supplemental
222 Table S6). The scores of DNA binding motif sequences were the highest in monocytes,
223 and the other cell types showed lower scores. The results of the analysis of DNA motif
224 sequences in $CD20^{+}$ B cells and macrophages did not include CTCF and cohesin,
225 because these analyses can be utilized in cells where the expression level of putative
226 transcriptional target genes of each transcription factor show a significant difference
227 between promoters and EPA shortened at the genomic locations of DNA motif
228 sequences. Some experimental data of a cell did not show a significant difference
229 between promoters and EPA (Osato 2018).

230 Instead of DNA binding motif sequences of transcription factors, repeat DNA

231 sequences were also examined. The expression level of transcriptional target genes
232 predicted based on EPA shortened at the genomic locations of repeat DNA sequences
233 was compared with the expression level of transcriptional target genes predicted from
234 promoters. Three reverse-forward orientation of repeat DNA sequences showed a
235 significant difference of expression level of putative transcriptional target genes in
236 monocytes of four people in common (Table 3). Among them, LTR16C repeat DNA
237 sequence was observed in iPS and H1-hESC with enough statistical significance
238 considering multiple tests (p-value $< 10^{-7}$). The same as $CD14^{+}$ monocytes, biased
239 orientation of repeat DNA sequences was examined in $CD4^{+}$ T cells. Three
240 forward-reverse and two reverse-forward orientation of repeat DNA sequences showed
241 a significant difference of expression level of putative transcriptional target genes in T
242 cells of four people in common (Supplemental Table S7). MIRb and MIR3 were also
243 found in the analysis using open chromatin regions overlapped with H3K27ac histone
244 modification marks, which are enhancer and promoter marks. MIR and other transposon
245 sequences are known to be involved in insulators and enhancers (Bejerano et al. 2006;
246 Rebollo et al. 2012; de Souza et al. 2013; Jjingo et al. 2014; Wang et al. 2015).
247

248 **Co-location of biased orientation of DNA motif sequences**

249 To examine the association of 287 biased (forward-reverse and reverse-forward)
250 orientation of DNA binding motif sequences of transcription factors, co-location of the
251 DNA binding motif sequences in open chromatin regions in monocytes was analyzed.
252 The number of open chromatin regions with the same pairs of DNA binding motif

253 sequences was counted, and when the pairs of DNA binding motif sequences were
254 enriched with statistical significance (chi-square test, *p*-value < 1.0×10^{-10}), they were
255 listed (Table 4; Supplemental Table S8). Open chromatin regions overlapped with
256 histone modification of an enhancer mark (H3K27ac) (total 26,095 regions) showed a
257 larger number of enriched pairs of DNA motifs than all open chromatin regions (Table
258 4; Supplemental Table S8). H3K27ac is known to be enriched at chromatin interaction
259 anchors (Phanstiel et al. 2017). As already known, CTCF was found with cohesin such
260 as RAD21 and SMC3 (Table 4). Top 30 pairs of FR and RF orientations of DNA motifs
261 co-occupied in the same open chromatin regions were shown (Table 4). Total number of
262 pairs of DNA motifs was 492, consisting of 115 unique DNA motifs, when the pair of
263 DNA motifs were observed in more than 80% of the number of open chromatin regions
264 with the DNA motifs.

265 The same as $CD14^+$ monocytes, to examine the association of 265 biased
266 orientation of DNA binding motif sequences of transcription factors, co-location of the
267 DNA binding motif sequences in open chromatin regions in $CD4^+$ T cells was analyzed.
268 Top 30 pairs of FR and RF orientations of DNA motifs co-occupied in the same open
269 chromatin regions were shown (Supplemental Table S9). Total number of pairs of DNA
270 motifs was 332, consisting of 118 unique DNA motifs, when the pair of DNA motifs
271 were observed in more than 60% of the number of open chromatin regions with the
272 DNA motifs (chi-square test, *p*-value < 1.0×10^{-10}). Among them, 12 pairs of DNA
273 motif sequences including a pair of CTCF, SMC3, and RAD21 in T cells were found in
274 monocytes in common (Supplemental Table S9).

275

276 **Pairs of biased orientation of DNA motif sequences surrounding genes**

277 CTCF and cohesin are found in chromatin interaction anchors and are considered
278 to form homodimers. Transcription factors are also known to form heterodimers with
279 another transcription factor. In this study, if the DNA binding motif sequence of only the
280 mate to a pair of transcription factors was found in EPA, EPA was shortened at one side,
281 which is the genomic location of the DNA binding motif sequence of the mate to the
282 pair, and transcriptional target genes were predicted using the EPA shortened at the side.
283 In this analysis, the mate to both heterodimer and homodimer of transcription factors
284 can be used to examine the effect on the expression level of transcriptional target genes
285 predicted based on the EPA shortened at one side. To examine whether biased
286 orientation of DNA motif sequences tend to be found as a pair of the same transcription
287 factor or different transcription factors in upstream and downstream of genes, the
288 enrichment of pairs of biased orientation of DNA motif sequences was investigated. The
289 number of genes having a pair of forward-reverse or reverse-forward orientation of
290 DNA motif sequences was counted using all pairs of the DNA motif sequences, and
291 statistical tests (chi-square test) were conducted, which is almost the same as the
292 analysis of co-location of biased orientation of DNA motif sequences using the number
293 of genes instead of open chromatin regions in this analysis. Fig. 4 and Supplemental Fig.
294 S2 showed that not only a pair of the same transcription factor, but also a pair of
295 different transcription factors were found in upstream and downstream of genes. DNA
296 motif sequences of CTCF and cohesin (RAD21 and SMC3) were enriched near the

297 same genes, but cohesin tended to be found with another transcription factor near the
298 same genes as well. The result of the analysis seems to be different between monocytes
299 and T cells. Some of these transcription factors would form homodimers or
300 heterodimers directory or indirectly composing a complex structure, contributing to
301 make chromatin interactions.
302
303 **Comparison with chromatin interaction data**
304 To examine whether the biased orientation of DNA motif sequences is associated
305 with chromatin interactions, enhancer-promoter interactions (EPI) predicted based on
306 enhancer-promoter associations (EPA) were compared with chromatin interaction data
307 (Hi-C). Due to the resolution of Hi-C experimental data used in this study (50kb), EPI
308 were adjusted to 50kb resolution. EPI were predicted based on three types of EPA: (i)
309 EPA without being shortened by the genomic locations of DNA motif sequences, (ii)
310 EPA shortened at the genomic locations of DNA motif sequences of transcription factors
311 such as CTCF and cohesin (RAD21, SMC3) without considering their orientation, and
312 (iii) EPA shortened at the genomic locations of forward-reverse or reverse-forward
313 orientation of DNA motif sequences of transcription factors. EPA (iii) showed the
314 highest ratio of EPI overlapped with chromatin interactions (Hi-C) using DNA binding
315 motif sequences of CTCF and cohesin (RAD21 and SMC3), compared with the other
316 two types of EPA (Supplemental Fig. S3). Total 59 biased orientation (40 FR and 23
317 RF) of DNA motif sequences including CTCF, cohesin, and YY1 showed a higher ratio
318 of EPI overlapped with Hi-C chromatin interactions than the other types of EPA in

319 monocytes (Supplemental Table S10). Chromatin interaction data were obtained using
320 different samples from DNase-seq, open chromatin regions, so individual differences
321 seemed to be large from the results of this analysis. Since, for some DNA motif
322 sequences of transcription factors, the number of EPI overlapped with chromatin
323 interactions was small, if higher resolution of chromatin interaction data (such as
324 HiChIP, in situ DNase Hi-C, and in situ Hi-C data, or a tool to improve the resolution
325 such as HiCPlus) is available, the number of EPI overlapped with chromatin
326 interactions would be increased and the difference of the numbers among three types of
327 EPA might be larger and more significant than the current results (Rao et al. 2014;
328 Ramani et al. 2016; Mumbach et al. 2017; Zhang et al. 2018).

329 After the analysis of $CD14^+$ monocytes, to utilize HiChIP chromatin interaction
330 data in $CD4^+$ T cells, the same analysis for $CD14^+$ monocytes was performed using
331 DNase-seq data of four donors in $CD4^+$ T cells (Mumbach et al. 2017). EPI predicted
332 based on EPA were compared with HiChIP chromatin interaction data in $CD4^+$ T cells.
333 The resolutions of HiChIP chromatin interaction data and EPI were adjusted to 5kb. EPI
334 were predicted based on the three types of EPA in the same way as $CD14^+$ monocytes
335 using top 60% of all the transcript. Conditions of the analysis was determined to include
336 known DNA motif sequences involved in chromatin interactions such as CTCF and
337 cohesin in the result and the result was consistent with the result using Hi-C chromatin
338 interaction data6. EPA (iii) showed the highest ratio of EPI overlapped with chromatin
339 interactions (HiChIP) using DNA binding motif sequences of CTCF and cohesin
340 (RAD21 and SMC3), compared with the other two types of EPA (Fig. 5). Total 121

341 biased orientation (73 FR and 58 RF) of DNA motif sequences including CTCF, cohesin,
342 ZNF143, and SMARC showed a higher ratio of EPI overlapped with HiChIP chromatin
343 interactions than the other types of EPA in T cells (Table 5). As expected, the number of
344 EPI overlapped with chromatin interactions (HiChIP) was increased, compared with
345 Hi-C chromatin interactions. However, the same as $CD14^+$ monocytes, chromatin
346 interaction data were obtained using different samples from DNase-seq, open chromatin
347 regions in $CD4^+$ T cells, so individual differences seemed to be large from the results of
348 this analysis, and (Mumbach et al. 2017) suggested that individual differences of
349 chromatin interactions were larger than those of open chromatin regions. ATAC-seq data,
350 open chromatin regions were available in $CD4^+$ T cells in the paper, however, when
351 using ATAC-seq data, the result of the analysis of biased orientation of DNA motif
352 sequences was different from DNase-seq data, and not included a part of CTCF and
353 cohesin. Thus, DNase-seq data collected from ENCODE and Blueprint projects were
354 employed in this study.
355

356 **Discussion**

357 To find DNA motif sequences of transcription factors and repeat DNA sequences
358 affecting human putative transcriptional target genes, DNA motif sequences were
359 searched from open chromatin regions of monocytes of four people. Total 287 biased
360 (159 forward-reverse and 153 reverse-forward) orientation of DNA motif sequences
361 were found in monocytes of four people in common, whereas only one any orientation
362 (i.e. without considering orientation) of DNA motif sequence was found to affect

363 putative transcriptional target genes, suggesting that EPA shortened at the genomic
364 locations of forward-reverse or reverse-forward orientation of DNA motif sequences of
365 transcription factors is an important character for EPI and transcription of genes.

366 When DNA motif sequences were searched from monocytes of one person, a
367 larger number of biased orientation of DNA motif sequences affecting human putative
368 transcriptional target genes has been found. When the number of people, from which
369 experimental data were obtained, was increased, the number of DNA motif sequences
370 found in all people in common decreased and in some cases, known transcription
371 factors involved in chromatin interactions such as CTCF and cohesin (RAD21 and
372 SMC3) were not identified by statistical tests. This would be caused by individual
373 difference of the same cell type, low quality of experimental data, and experimental
374 errors. Moreover, though forward-reverse orientation of DNA binding motif sequences
375 of CTCF and cohesin is frequently observed at chromatin interaction anchors, the
376 percentage of forward-reverse orientation is not 100, and other orientations of the DNA
377 binding motif sequences are also observed. Though DNA binding motif sequences of
378 CTCF and cohesin are found in various open chromatin regions, DNA binding motif
379 sequences of some transcription factors would be observed less frequently in open
380 chromatin regions. The analyses of cells of a number of people would avoid missing
381 relatively weak statistical significance of DNA motif sequences in cells of each person
382 by multiple testing correction of thousands of statistical tests. A DNA motif sequence
383 was found with p-value < 0.05 in cells of one person and the DNA motif sequence
384 found in the same cell type of four people in common would have p-value $< (0.05)^4 =$

385 0.00000625. Actually, DNA motif sequences with *p*-value slightly less than 0.05 in
386 monocytes of one person were observed in monocytes of four people in common.

387 EPI were compared with chromatin interactions (Hi-C) in monocytes. EPA
388 shortened at the genomic locations of DNA binding motif sequences of CTCF and
389 cohesin (RAD21 and SMC3) showed a significant difference of the ratios of EPI
390 overlapped with chromatin interactions, according to three types of EPA (FR, any, and
391 others). Using open chromatin regions overlapped with ChIP-seq experimental data of
392 histone modification of an enhancer mark (H3K27ac), the ratio of EPI not overlapped
393 with Hi-C was reduced. (Phanstiel et al. 2017) also reported that there was an especially
394 strong enrichment for loops with H3K27 acetylation peaks at both ends (Fisher's Exact
395 Test, $p = 1.4 \times 10^{-27}$). However, the total number of EPI overlapped with chromatin
396 interactions was also reduced using H3K27ac peaks, so more chromatin interaction data
397 would be needed to obtain reliable results in this study. As an issue of experimental data,
398 chromatin interaction data were obtained using different samples from DNase-seq, open
399 chromatin regions, so individual differences were not reflected to chromatin interaction
400 data. Moreover, the resolution of chromatin interaction data used in monocytes is about
401 50kb, thus the number of chromatin interactions was relatively small (72,284 at 50kb
402 resolution with a cutoff score of CHiCAGO tool > 1). EPA shortened at the genomic
403 locations of DNA binding motif sequences of transcription factors found in various
404 open chromatin regions such as CTCF and cohesin (RAD21 and SMC3) tend to be
405 overlapped with a larger number of chromatin interactions than DNA binding motif
406 sequences of transcription factors less frequently observed in open chromatin regions.

407 Therefore, to examine the difference of the numbers of EPI overlapped with chromatin
408 interactions, according to three types of EPA (FR or RF, any, and others), the number of
409 chromatin interactions should be large enough.

410 As HiChIP chromatin interaction data were available in $CD4^+$ T cells, biased
411 orientations of DNA motif sequences of transcription factors were examined in T cells
412 using DNase-seq data of four people. The resolutions of chromatin interactions and EPI
413 were adjusted to 5kb by fragmentation of genome sequences. In monocytes, the
414 resolution of Hi-C chromatin interaction data was converted by extending anchor
415 regions of chromatin interactions to 50kb length and merging chromatin interactions
416 overlapped with each other. Fragmentation of genome sequences may affect the
417 classification of chromatin interactions of which anchors are located near the border of a
418 fragment, but the number of chromatin interactions would not be decreased, compared
419 with merging chromatin interactions. The number of HiChIP chromatin interactions was
420 19,926,360 at 5kb resolution and 666,149 at 5kb resolution with more than 1,000 counts
421 of chromatin interactions. As expected, the number of EPI overlapped with chromatin
422 interactions was increased, and about half of biased orientation of DNA motif sequences
423 of transcription factors showed a statistical significance in EPI predicted based on EPA
424 shortened at the genomic locations of the DNA motif sequences, compared with the
425 other types of EPA. False positive predictions of EPI would be decreased by using
426 H3K27ac marks and other features. The ratio of EPI overlapped with Hi-C chromatin
427 interactions was increased by using H3K27ac marks in both monocytes and T cells. The
428 ratio of EPI overlapped with HiChIP chromatin interactions was also increased by using

429 H3K27ac marks. However, the number of biased orientation of DNA motif sequences
430 showing higher ratio of EPI overlapped with HiChIP chromatin interactions than the
431 other types of EPA was decreased using H3K27ac marks (Supplemental material 2).

432 Though the number of known DNA binding proteins and transcription factors
433 involved in chromatin interactions would be less than 20 or so, about 300 DNA binding
434 proteins showed enrichment of biased orientation of their DNA binding sequences, and
435 known DNA binding proteins involved in chromatin interactions were found in the
436 biased orientation of DNA binding sequences. JUNDM2 (JDP2) found in T cells and
437 monocytes is not known DNA binding proteins involved in chromatin interactions.
438 JDP2 is involved in histone and nucleosome-related functions such as
439 histone-chaperone activity, promoting nucleosome, and inhibition of histone acetylation
440 (Jin et al. 2006). JDP2 forms a homodimer or heterodimer with various transcription
441 factors (https://en.wikipedia.org/wiki/Jun_dimerization_protein). Thus, the main
442 function of JDP2 may not be associated with chromatin interactions, but it forms a
443 homodimer or heterodimer with transcription factors, potentially forming a chromatin
444 interaction through the binding of JDP2 and another transcription factor, like chromatin
445 interactions formed by YY1 transcription factor (Weintraub et al. 2017). In the analysis
446 of HiChIP chromatin interaction data, JDP2 was shown in the list of biased orientation
447 of DNA motif sequences showing higher ratio of EPI overlapped with HiChIP
448 chromatin interactions than the other types of EPA, using chromatin interactions with
449 more than 6,000 counts (Supplemental material 2). When forming a homodimer or
450 heterodimer with another transcription factor, transcription factors may bind to genome

451 DNA with a specific orientation of their DNA binding sequences. From the analysis of
452 biased orientation of DNA motif sequences of transcription factors, such transcription
453 factors would also be found. If the DNA binding motif sequence of only the mate to a
454 pair of transcription factors was found in EPA, EPA was shortened at one side, which is
455 the genomic location of the DNA binding motif sequence of the mate to the pair, and
456 transcriptional target genes were predicted using the EPA shortened at the side. In this
457 analysis, the mate to both heterodimer and homodimer of transcription factors can be
458 used to examine the effect on the expression level of transcriptional target genes
459 predicted based on the EPA shortened at one side. For heterodimer, biased orientation of
460 DNA motif sequences may also be found in forward-forward or reverse-reverse
461 orientation.

462 I reported that the ratio of the number of functional enrichment of putative
463 transcriptional target genes in the total number of putative transcriptional target genes
464 changed according to the types of EPA, in the same way as gene expression level (Osato
465 2018). JDP2 showed a higher ratio of functional enrichment of putative transcriptional
466 target genes using EPA shortened at genomic locations of forward-reverse orientation of
467 the DNA binding motif sequence, compared with the other types of EPA
468 (reverse-forward or any orientation) in monocytes and T cells, among monocytes, T
469 cells and $CD20^+$ B cells. While the genome-wide analysis of functional enrichments of
470 putative transcriptional target genes takes computational time, the genome-wide
471 analysis of gene expression level is finished with less computational time. The analysis
472 of gene expression level can be used for a cell type and experimental data where the

473 expression levels of transcriptional target genes predicted based on EPA are
474 significantly different from those predicted from only promoter.

475 It has been reported that CTCF and cohesin-binding sites are frequently mutated
476 in cancer (Katainen et al. 2015). Some biased orientation of DNA motif sequences
477 would be associated with chromatin interactions and might be associated with diseases
478 including cancer.

479 The analyses in this study revealed novel characters of DNA binding motif
480 sequences of transcription factors and repeat DNA sequences, and would be useful to
481 analyze transcription factors involved in chromatin interactions and/or forming a
482 homodimer, heterodimer or complex with other transcription factors, affecting the
483 expression and transcriptional regulation of genes.

484

485 Methods

486 Search for biased orientation of DNA motif sequences

487 To examine transcriptional regulatory target genes, bed files of hg38 of Blueprint
488 DNase-seq data for $CD14^+$ monocytes of four donors (EGAD00001002286; Donor ID:
489 C0010K, C0011I, C001UY, C005PS) were obtained from Blueprint project web site
490 (http://dcc.blueprint-epigenome.eu/#/home), and the bed files of hg38 were converted
491 into those of hg19 using Batch Coordinate Conversion (liftOver) web site
492 (https://genome.ucsc.edu/util.html). Bed files of hg19 of ENCODE H1-hESC
493 (GSM816632; UCSC Accession: wgEncodeEH000556), iPSC (GSM816642; UCSC
494 Accession: wgEncodeEH001110), HUVEC (GSM1014528; UCSC Accession:

495 wgEncodeEH002460), and MCF-7 (GSM816627; UCSC Accession:
496 wgEncodeEH000579) were obtained from the ENCODE websites
497 (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeUwDgf/;
498 http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeUwDnase/).
499 As high resolution of chromatin interaction data using HiChIP became available
500 in $CD4^+$ T cells, to promote the same analysis of $CD14^+$ monocytes in $CD4^+$ T cells,
501 DNase-seq data of four donors were obtained from ENCODE and Blueprint projects
502 web sites. DNase-seq data of only one donor was available in Blueprint project for
503 $CD4^+$ T cells (Donor ID: S008H1), and three other DNase-seq data were obtained from
504 NCBI Gene Expression Omnibus (GEO) database. Though the peak calling of
505 DNase-seq data available in GEO database was different from other DNase-seq data in
506 ENCODE and Blueprint projects where 150bp length of peaks were usually predicted
507 using HotSpot (John et al. 2011), FASTQ files of DNase-seq data were downloaded
508 from NCBI Sequence Read Archive (SRA) database (SRR097566, SRR097618, and
509 SRR171574). Read sequences of FASTQ files were aligned to the hg19 version of the
510 human genome reference using BWA (Li and Durbin 2009), and the BAM files
511 generated by BWA were converted into SAM files, sorted, and indexed using Samtools
512 (Li et al. 2009). Peaks of the DNase-seq data were predicted using HotSpot-4.1.1.
513 To identify transcription factor binding sites (TFBS) from the DNase-seq data,
514 TRANSFAC (2013.2), JASPAR (2010), UniPROBE, BEEML-PBM, high-throughput
515 SELEX, Human Protein-DNA Interactome, and transcription factor binding sequences
516 of ENCODE ChIP-seq data were used (Wingender et al. 1996; Newburger and Bulyk

517 2009; Portales-Casamar et al. 2010; Xie et al. 2010; Zhao and Stormo 2011; Jolma et al.
518 2013; Kheradpour and Kellis 2014). Position weight matrices of transcription factor
519 binding sequences were transformed into TRANSFAC matrices and then into MEME
520 matrices using in-house scripts and transfac2meme in MEME suite (Bailey et al. 2009).
521 Transcription factor binding sequences of transcription factors derived from vertebrates
522 were used for further analyses. Searches were conducted for transcription factor binding
523 sequences from each narrow peak using FIMO with *p*-value threshold of 10^{-5} (Grant et
524 al. 2011). Transcription factors corresponding to transcription factor binding sequences
525 were searched computationally by comparing their names and gene symbols of HGNC
526 (HUGO Gene Nomenclature Committee) -approved gene nomenclature and 31,848
527 UCSC known canonical transcripts
528 (http://hgdownload.cse.ucsc.edu/goldenpath/hg19/database/knownCanonical.txt.gz), as
529 transcription factor binding sequences were not linked to transcript IDs such as UCSC,
530 RefSeq, and Ensembl transcripts.

531 Target genes of a transcription factor were assigned when its TFBS was found in
532 DNase-seq narrow peaks in promoter or extended regions for enhancer-promoter
533 association of genes (EPA). Promoter and extended regions were defined as follows:
534 promoter regions were those that were within distances of ±5 kb from transcriptional
535 start sites (TSS). Promoter and extended regions were defined as per the following
536 association rule, which is the same as that defined in Figure 3A of a previous study
537 (McLean et al. 2010): the single nearest gene association rule, which extends the
538 regulatory domain to the midpoint between the TSS of the gene and that of the nearest

539 gene upstream and downstream without the limitation of extension length. Extended
540 regions for EPA were shortened at the genomic locations of DNA binding sites of
541 transcription factors that were the closest to a transcriptional start site, and
542 transcriptional target genes were predicted from the shortened enhancer regions using
543 TFBS. Furthermore, promoter and extended regions for EPA were shortened at the
544 genomic locations of forward–reverse orientation of DNA binding sites of transcription
545 factors. When forward or reverse orientation of DNA binding sites were continuously
546 located in genome sequences several times, the most external forward–reverse
547 orientation of DNA binding sites were selected. The genomic positions of genes were
548 identified using 'knownGene.txt.gz' file in UCSC bioinformatics sites (Karolchik et al.
549 2014). The file 'knownCanonical.txt.gz' was also utilized for choosing representative
550 transcripts among various alternate forms for assigning promoter and extended regions
551 for EPA. From the list of transcription factor binding sequences and transcriptional
552 target genes, redundant transcription factor binding sequences were removed by
553 comparing the target genes of a transcription factor binding sequence and its
554 corresponding transcription factor; if identical, one of the transcription factor binding
555 sequences was used. When the number of transcriptional target genes predicted from a
556 transcription factor binding sequence was less than five, the transcription factor binding
557 sequence was omitted.

558 Repeat DNA sequences were searched from the hg19 version of the human
559 reference genome using RepeatMasker (Smit, AFA & Green, P RepeatMasker at
560 http://www.repeatmasker.org) and RepBase RepeatMasker Edition

561 (http://www.girinst.org).

562 For gene expression data, RNA-seq reads mapped onto human hg19 genome
563 sequences were obtained, including ENCODE long RNA-seq reads with poly-A of
564 H1-hESC, iPSC, HUVEC, and MCF-7 (GSM26284, GSM958733, GSM2344099,
565 GSM2344100, GSM958734, and GSM765388), and UCSF-UBC human reference
566 epigenome mapping project RNA-seq reads with poly-A of naive $CD4^+$ T cells
567 (GSM669617). Two replicates were present for H1-hESC, iPSC, HUVEC, and MCF-7,
568 and a single one for $CD4^+$ T cells. RPKMs of the RNA-seq data were calculated using
569 RSeQC (Wang et al. 2012). For monocytes, Blueprint RNA-seq RPKM data
570 (GSE58310, GSE58310_GeneExpression.csv.gz, Monocytes_Day0_RPMI) was used
571 (Saeed et al. 2014). Based on RPKM, UCSC transcripts with expression levels among
572 top 30% of all the transcripts were selected in each cell type.

573 The expression level of transcriptional target genes predicted based on EPA
574 shortened at the genomic locations of DNA motif sequences of transcription factors or
575 repeat DNA sequences was compared with the expression level of transcriptional target
576 genes predicted from promoter. For each DNA motif sequence shortening EPA,
577 transcriptional target genes were predicted using about 3,000 – 5,000 DNA binding
578 motif sequences of transcription factors, and the expression level of putative
579 transcriptional target genes of each transcription factor was compared between EPA and
580 only promoter using Mann-Whitney test (p-value < 0.05). The number of transcription
581 factors showing a significant difference of expression level of putative transcriptional
582 target genes between EPA and promoter was compared among forward-reverse,

583 reverse-forward, and any orientation of DNA motif sequences shortening EPA using
584 chi-square test (p-value < 0.05). When DNA motif sequences of transcription factors or
585 repeat DNA sequences shortening EPA showed a significant difference of expression
586 level of putative transcriptional target genes in forward-reverse, reverse-forward, or any
587 orientation in monocytes of four people in common, the DNA motif sequences were
588 listed.

589 Though forward-reverse orientation of DNA binding motif sequences of CTCF
590 and cohesin is frequently observed at chromatin interaction anchors, the percentage of
591 forward-reverse orientation is not 100, and other orientations of the DNA binding motif
592 sequences are also observed. Though DNA binding motif sequences of CTCF and
593 cohesin are found in various open chromatin regions, DNA binding motif sequences of
594 some transcription factors would be observed less frequently in open chromatin regions.
595 The analyses of cells of a number of people would avoid missing relatively weak
596 statistical significance of DNA motif sequences in cells of each person by multiple
597 testing correction of thousands of statistical tests. A DNA motif sequence was found
598 with p-value < 0.05 in cells of one person and the DNA motif sequence found in the
599 same cell type of four people in common would have p-value < $0.05^4 = 0.00000625$.

600

601 **Co-location of biased orientation of DNA motif sequences**

602 Co-location of biased orientation of DNA binding motif sequences of
603 transcription factors was examined. The number of open chromatin regions with the
604 same pairs of DNA binding motif sequences was counted, and when the pairs of DNA

605 binding motif sequences were enriched with statistical significance (chi-square test,
606 p-value < 1.0×10^{-10}), they were listed.
607 For histone modification of an enhancer mark (H3K27ac), bed files of hg38 of
608 Blueprint ChIP-seq data for $CD14^+$ monocytes (EGAD00001001179) and $CD4^+$ T cells
609 (Donor ID: S000RD) was obtained from Blueprint web site
610 (http://dcc.blueprint-epigenome.eu/#/home), and the bed files of hg38 were converted
611 into those of hg19 using Batch Coordinate Conversion (liftOver) web site
612 (https://genome.ucsc.edu/util.html).
613
614 **Comparison with chromatin interaction data**
615 For comparison of EPA in monocytes with chromatin interactions,
616 'PCHiC_peak_matrix_cutoff0.txt.gz' file was downloaded from 'Promoter Capture
617 Hi-C in 17 human primary blood cell types' web site (https://osf.io/u8tzp/files/), and
618 chromatin interactions for Monocytes with scores of CHiCAGO tool > 1 were extracted
619 from the file (Javierre et al. 2016).
620 EPI were predicted using three types of EPA in monocytes: (i) EPA shortened at
621 the genomic locations of forward-reverse or reverse-forward orientation of DNA motif
622 sequences of transcription factors, (ii) EPA shortened at the genomic locations of any
623 orientation (i.e. without considering orientation) of DNA motif sequences of
624 transcription factors, and (iii) EPA without being shortened by DNA motif sequences.
625 EPI predicted using the three types of EPA in common were removed. First, EPI
626 predicted based on EPA (i) were compared with chromatin interactions (Hi-C). The

627 resolution of chromatin interaction data used in this study was 50kb, so EPI were
628 adjusted to 50kb before their comparison. The number and ratio of EPI overlapped with
629 chromatin interactions were counted. Second, EPI were predicted based on EPA (ii), and
630 EPI predicted based on EPA (i) were removed from the EPI. The number and ratio of
631 EPI overlapped with chromatin interactions were counted. Third, EPI were predicted
632 based on EPA (iii), and EPI predicted based on EPA (i) and (ii) were removed from the
633 EPI. The number and ratio of EPI overlapped with chromatin interactions were counted.
634 The number and ratio of the EPI compared two times between EPA (i) and (iii), and
635 EPA (i) and (ii) (binomial distribution, p-value < 0.025 for each test).

636 For comparison of EPA with chromatin interactions (HiChIP) in $CD4^+$ T cells,
637 'GSM2705049_Naive_HiChIP_H3K27ac_B2T1_allValidPairs.txt' file was downloaded
638 from Gene Expression Omnibus (GEO) database (GSM2705049). The resolutions of
639 chromatin interaction data and EPI were adjusted to 5kb before their comparison.
640 Chromatin interactions with more than 6,000 and 1,000 counts were used in this study.

641

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Figures

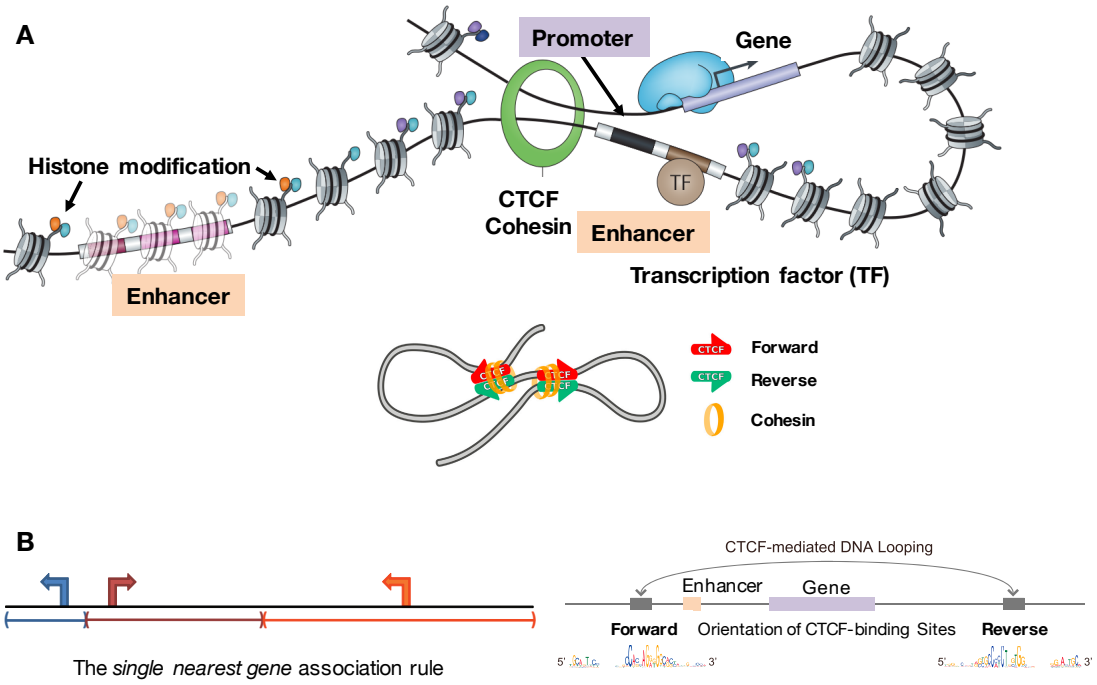
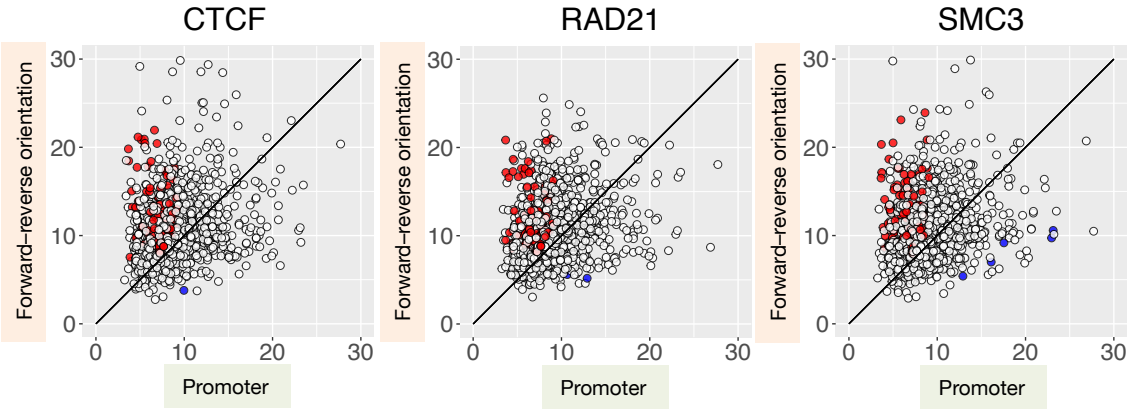


Figure 1. Chromatin interactions and enhancer-promoter association. (A) Forward–reverse orientation of CTCF-binding sites are frequently found in chromatin interaction anchors. CTCF can block the interaction between enhancers and promoters limiting the activity of enhancers to certain functional domains (de Wit et al. 2015; Guo et al. 2015). (B) Computationally-defined regulatory domains for enhancer-promoter association (McLean et al. 2010). The *single nearest gene* association rule extends the regulatory

663 domain to the midpoint between this gene's TSS and the nearest gene's TSS both
664 upstream and downstream. Enhancer-promoter association was shortened at the
665 genomic locations of forward-reverse orientation of DNA binding motif sequences of
666 transcription factors (e.g. CTCF in the figure).
667
668



669
670 **Figure 2.** Comparison of median expression level of putative transcriptional target
671 genes. The median expression levels of target genes of the same transcription factor
672 binding sequences were compared between promoters and EPA shortened at the
673 genomic locations of forward-reverse orientation of DNA motif sequences of CTCF and
674 cohesin (RAD21 and SMC3) in monocytes. Red and blue dots show statistically
675 significant difference (Mann-Whitney test) of the distribution of expression levels of
676 target genes between promoters and EPA. Red dots show the median expression level of
677 target genes was higher based on EPA than promoters, and blue dots show the median
678 expression level of target genes was lower based on EPA than promoters. The
679 expression level of target genes predicted based on the EPA tended to be higher than
680 promoters, implying that transcription factors acted as enhancers of target genes.
681
682

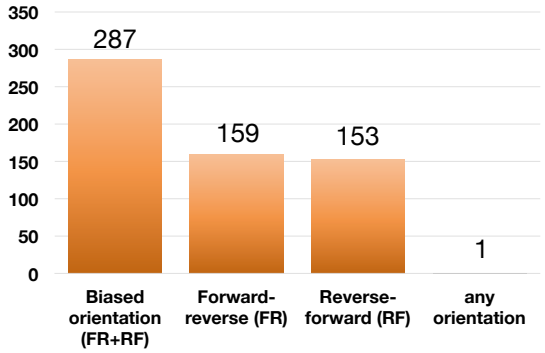


Figure 3. Biased orientation of DNA motif sequences of transcription factors in monocytes. Total 287 of biased orientation of DNA binding motif sequences of transcription factors have been found to affect the expression level of putative transcriptional target genes in monocytes of four people in common, whereas only one any orientation (i.e. without considering orientation) of DNA binding motif sequences have been found to affect the expression level.

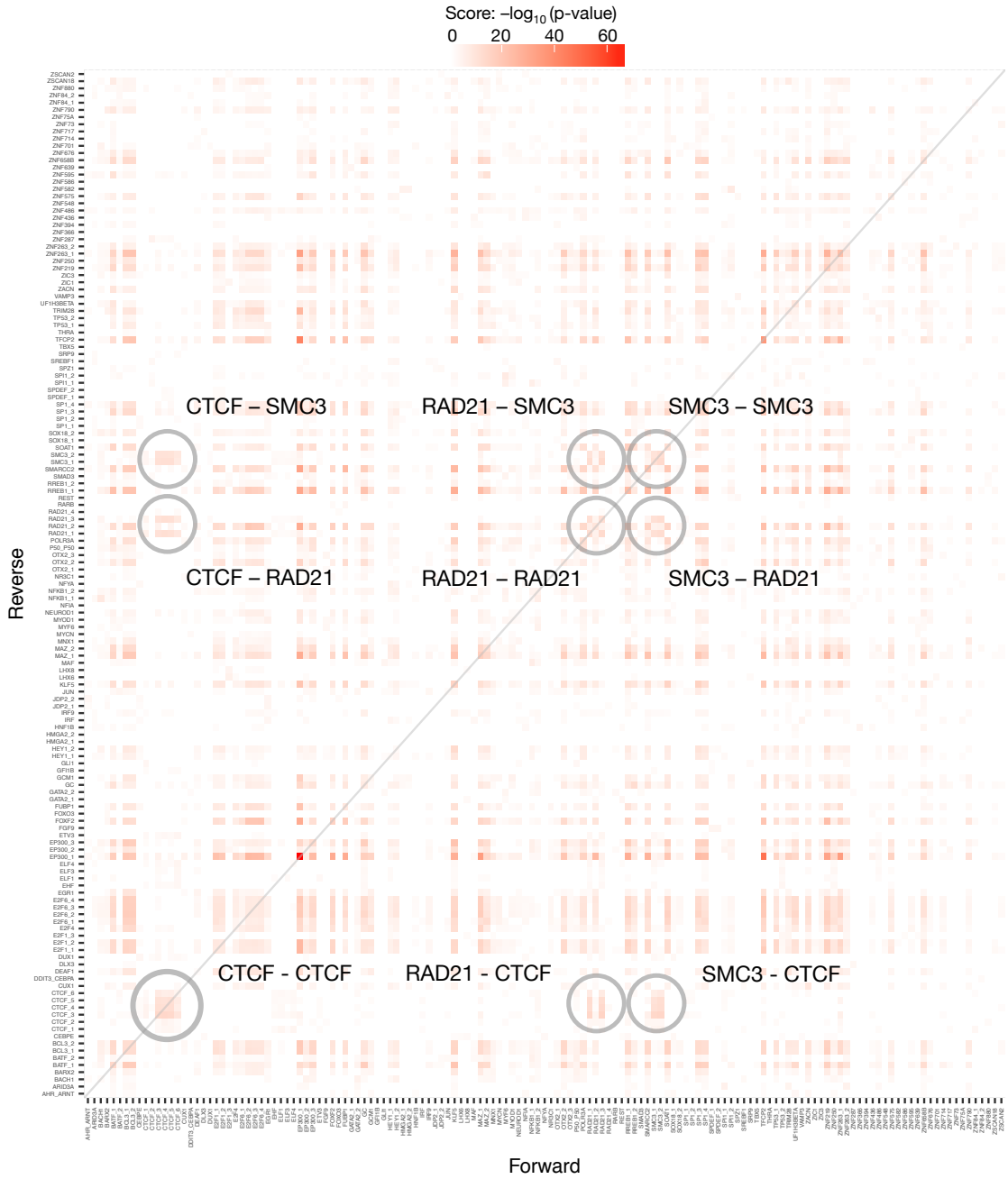


Figure 4. Pairs of biased orientation of DNA binding motif sequences of transcription factors enriched in upstream and downstream of some genes. The number of genes having a pair of forward-reverse orientation of DNA motif sequences was counted using all pairs of the DNA motif sequences, and statistical tests (chi-square test) were conducted in T cells. Gray circles show pairs of DNA motif sequences of CTCF and cohesin (RAD21 and SMC3). CTCF and cohesin are known to be involved in chromatin interactions and have biased orientation of DNA binding motif sequences to form

chromatin loops. Other pairs of forward-reverse orientation of DNA motif sequences were also enriched near genes.

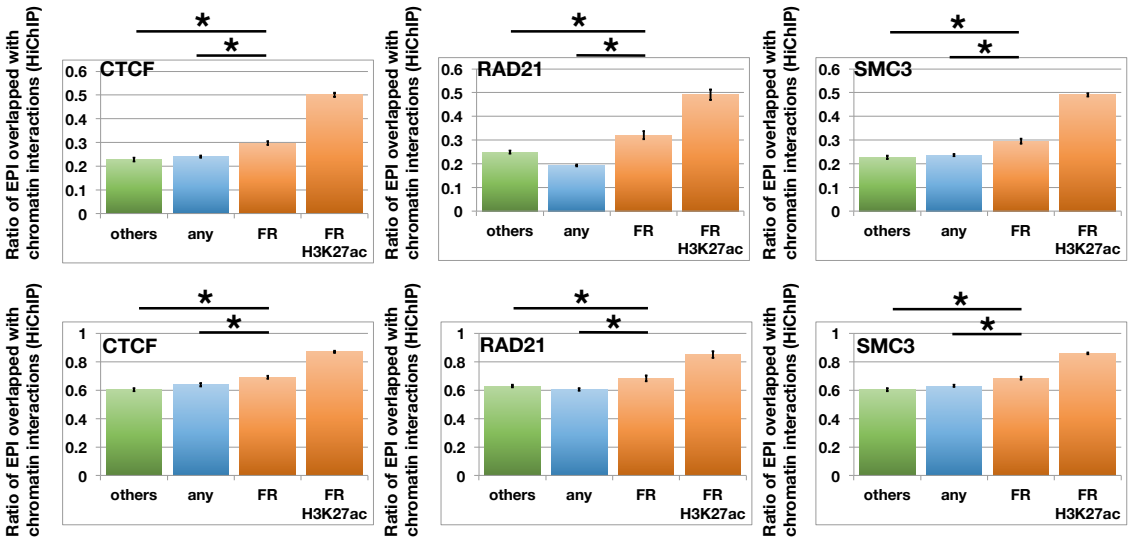


Figure 5. Comparison of DNA binding motif sequences of transcription factors with chromatin interactions in T cells. monocytes. Total 121 biased orientation (73 FR and 58 RF) of DNA motif sequences including CTCF and cohesin showed a higher ratio of EPI overlapped with HiChIP than the other types of EPA in monocytes. Upper part is a comparison of EPI with HiChIP chromatin interactions with more than 6,000 counts, and lower part is a comparison of EPI with HiChIP chromatin interactions with more than 1,000 counts. FR: forward-reverse orientation of DNA motifs. any: any orientation of DNA motifs. others: enhancer-promoter association not shortened at the genomic locations of DNA motifs. FR H3K27ac: forward-reverse orientation of DNA motifs found from open chromatin regions overlapped with H3K27ac histone modification marks. * p-value < 10^{-4}.

Tables

Table 1. Top 90 biased orientation of DNA binding motif sequences of transcription factors in monocytes. TF: DNA binding motif sequences of transcription factors. Score: $-\log_{10}$ (p-value).

Forward-reverse orientation

DNA motif of TF	Score	TF	Score	TF	Score
SRF	75.45	STAT5A	31.24	SMARCC2	21.86
IRF7	71.05	E2F8	30.62	ZNF695	21.78
MINI20	61.68	TP53	30.56	ZNF28	21.77
CTCF	61.62	STAT5B	29.94	ZNF316	21.66
E2F	59.32	STAT3	29.85	TBP	21.60
STAT1	58.94	FOS	29.75	NFIB	21.45
ZNF317	55.97	CEBPZ	29.42	PHOX2B	20.82
CEBPG	55.46	RAD21	28.67	DEC	20.62
ZNF623	54.71	SMAD2	28.60	ZNF709	20.58
ZNF93	54.23	GATA2	28.52	YY1	20.57
HMBOX1	52.99	KLF6	28.02	EIF5A2	20.55
SMAD2_SMAD3_SMAD4	50.66	HOXB1	27.62	ZNF148	20.45
FOXN2	49.88	GLI1	27.09	PAX5	20.05
SPEF1	49.52	GCM1	26.73	POU6F1	19.93
ZNF646	43.95	ZNF676	26.66	FOXO4	19.72
TFE3	42.46	TCF3	26.32	EBF	19.26
MYB	41.98	EHF	26.32	ZNF648	19.12
STAT4	40.54	TCF12	26.29	HENMT1	19.02
ZNF716	40.39	MEF2A	26.22	PARG	18.78
KLF14	39.64	HIC1	25.34	ZNF460	18.64
FOXA1	38.13	EGR2	24.92	SLC25A20	17.83
USF	37.74	NR2F2	24.86	KLF5	17.77
EN1	37.01	PLAGL1	24.65	XRCC4	17.53
ZNF219	35.37	REST	24.53	WT1	17.34
IKZF1	33.79	ZNF143	23.67	SULT1A2	17.34
ZIC1	33.60	YBX1	22.99	FUBP1	17.33
SMC3	33.37	PRDM15	22.63	DNAAF2	17.26
ESR2	32.07	ZNF92	22.22	ZNF521	17.25
TP63	31.98	ZNF214	22.11	XBP1	16.85
ZNF836	31.66	PTF1A	22.09	SP1	16.84

Reverse-forward orientation

TF	Score	TF	Score	TF	Score
RFX5	102.30	TFAP2B	34.55	ZNF449	27.96
TCF7	75.34	TBP	34.47	ZNF233	27.66
SRF	65.52	ZNF670	34.33	SOX2	26.46
ZNF28	63.89	ZNF350	34.30	RXRA	26.07
HNF1B	62.46	ZNF687	33.82	MYF6	25.85
ZNF225	62.18	NFKB2	33.05	MAFK	25.81
CEBPB	59.69	RFX3	32.74	CEBPZ	25.73
NFY	58.96	NFE2L3	32.69	POU2F2	25.59
ZNF286B	54.73	TRIM28	32.27	ETS2	25.05
CTCF	53.16	SP3	32.22	HSF1	24.76
PAX5	52.57	FLI1	32.15	AHR	23.56
STAT1	52.11	RBAK	31.92	PAX6	23.48
ISL1	49.73	NR2F1	31.80	IRF4	22.47
ETV7	47.08	NKX3-1	31.55	ETV3	21.90
KLF16	46.45	ZIC2	31.40	FOXP2	21.72
SP1	46.03	PARP1	31.19	CDX2	21.45
SMAD3	45.27	ZNF195	31.06	NFATC1	21.24
STAT5B	44.72	BDP1	30.84	SP4	21.14
TEAD1	43.00	SOX9	30.72	USF1	20.92
ZNF579	42.94	SP8	30.49	MSX2	20.84
ZBTB2	42.28	ZNF121	30.04	PPARG	20.70
ZNF343	41.01	ZNF202	30.01	MYEF2	20.23
DOBOX5	39.21	CD59	29.85	MYC_MAX	20.22
NANOG	38.84	RXRB	29.57	ZNF711	19.84
NFE2L2	38.53	STAT5A	29.31	ZBTB18	19.60
OBOX2	38.00	SOX11	29.09	HSF2	19.51
ZNF652	37.84	WT1	29.06	DNAAF2	19.00
ZNF30	37.59	CREM	28.65	IRF3	18.88
MYOG	36.34	ZNF316	28.34	TFAP2E	18.87
GABPB1_GABPB2	35.96	HAND1_TCF3	28.07	BCL11A	18.59

753 **Table 2.** Top 30 forward-reverse orientation of DNA binding motif sequences of

754 transcription factors in MCF-7 and iPS cells. TF: DNA binding motif sequences of

755 transcription factors. Score: $-\log_{10}$ (*p*-value).

756 MCF-7 iPS

TF	Score	TF	Score
RAD21	16.21	ZIC3	19.32
SMC3	15.63	ZNF521	18.93
CTCF	15.12	TFE3	16.63
E2F	10.14	ZNF317	16.43
SLC25A20	9.62	NKX2-5	16.25
HOXA7	8.29	HES1	15.34
STAT5B	7.55	ZBTB24	14.21
REST	7.25	SMC3	13.76
SIRT6	7.05	FOS	13.69
KLF3	6.53	RAD21	13.05
YY1	5.30	ZNF836	11.64
PLAGL1	4.97	TBX5	11.48
ZNF219	4.91	CTCF	11.24
STAT1	4.43	STAT1	11.19
SNTB1	4.42	RUNX2	10.28
ETS	3.16	WT1	10.27
XRCC4	3.15	ZIC1	9.60
ZBTB6	3.12	MAX	8.55
DBP	2.53	KLF3	8.29
SETDB1	2.53	KLF14	8.25
RREB1	2.50	ZNF143	7.81
E2F5	2.38	E2F5	7.66
KLF5	2.33	SIRT6	7.56
SPEF1	2.22	EN1	7.49
ZNF143	2.20	YY1	7.02
ZNF460	2.19	STAT5B	6.73
FOS	2.18	MAZ	6.39
GATA2	1.97	ZNF148	6.20
MAZ	1.95	TCF3	5.73
PARG	1.94	EGR2	5.45

757

758

759

760 **Table 3.** Biased orientation of repeat DNA sequences in monocytes. Score: $-\log_{10}$

761 (*p*-value).

Forward-reverse (FR) orientation (when using H3K27ac histone modification marks)

Repeat DNA seq.	Score
MLT1F1	6.75

762

Reverse-forward (RF) orientation

Repeat DNA seq.	Score
LTR16C	69.39
L1ME4b	23.90
AluSg	23.61

763

764 **Table 4.** Top 30 of co-locations of biased orientation of DNA binding motif sequences
765 of transcription factors in monocytes. Co-locations of DNA motif sequences of CTCF
766 with other biased orientation of DNA motif sequences were shown in a separate table.
767 Motif 1,2: DNA binding motif sequences of transcription factors. # both: the number of
768 open chromatin regions including both Motif 1 and Motif 2. # motif 1: the number of
769 open chromatin regions including Motif 1. # motif 2: the number of open chromatin
770 regions including Motif 2. # others: the number of open chromatin regions not including
771 Motif 1 or Motif 2. Open chromatin regions overlapped with histone modification
772 (H3K27ac) were used (Total 26,095 regions).

773

Motif 1	Motif 2	# both	# motif 1	# motif 2	# others	*p*-value
CTCF	SMAD2	4527	781	4478	16309	0
CTCF	RREB1	4379	929	4757	16030	0
CTCF	ZNF263	4357	951	4875	15912	0
CTCF	SP1	4321	987	4052	16735	0
CTCF	RAD21	3804	1504	225	20562	0
CTCF	KLF6	3695	852	3800	17748	0
CTCF	SMC3	1259	178	1977	22681	0
CTCF	RXRA	1220	259	2559	22057	0

774

Motif 1	Motif 2	# both	# motif 1	# motif 2	# others	*p*-value
SMAD2	ZNF263	7427	1578	1805	15285	0
SMARCC2	ZNF263	7354	1192	1893	15656	0
RREB1	SP1	7208	1957	1165	15765	0
SIRT6	ZNF263	7126	1154	2121	15694	0
MAZ	SP1	7052	909	1321	16813	0
MAZ	ZNF263	7022	999	2225	15849	0
SP1	ZNF263	7009	1364	2223	15499	0
SMAD2	SP1	6925	2080	1448	15642	0
MAZ	RREB1	6920	967	2245	15963	0
SIRT6	SMAD2	6766	1514	2239	15576	0
SIRT6	SMARCC2	6668	1612	1878	15937	0
PLAGL1	SMAD2	6637	1074	2368	16016	0
MAZ	SMAD2	6627	1334	2378	15756	0
PARG	SMAD2	6602	1012	2403	16078	0
PLAGL1	ZNF263	6571	1140	2661	15723	0
EGR2	RREB1	6565	787	2600	16143	0
KLF6	RREB1	6563	932	2573	16027	0
MAZ	SMARCC2	6562	1459	1984	16090	0
RREB1	ZNF263	6551	1607	2681	15256	0
KLF6	SP1	6541	954	1832	16768	0
PLAGL1	RREB1	6496	1215	2640	15744	0
EGR2	SP1	6492	860	1881	16862	0
KLF6	SMAD2	6491	1004	2514	16086	0
PARG	ZNF263	6390	1224	2842	15639	0
PLAGL1	SP1	6318	1393	2055	16329	0
KLF6	ZNF263	6304	1191	2928	15672	0
EGR2	SMAD2	6270	1082	2735	16008	0
EGR2	ZNF263	6253	1099	2979	15764	0
EGR2	MAZ	6219	1133	1742	17001	0
PARG	RREB1	6219	1395	2917	15564	0

775 **Table 5.** Comparison of biased orientation of DNA binding motif sequences of
776 transcription factors with chromatin interaction data in T cells. TF: DNA binding motif
777 sequences of transcription factors. Score: $-\log_{10}$ (p-value). Inf: p-value = 0. Score 1:
778 Comparison of EPA shortened at genomic locations of FR or RF orientation of DNA
779 binding sequences with EPA not shortened. Score 2: Comparison of EPA shortened at
780 genomic locations of FR or RF orientation of DNA binding sequences with EPA
781 shortened at genomic locations of any orientation of DNA binding sequences.

782

783 Forward-reverse (FR)

TF	Score 1	Score 2
APEX1	13.57	3.85
AR	Inf	8.30
CDC5L	65.46	12.23
CTCF	101.79	14.94
DDIT3_CEBPA	249.72	4.78
EBF1	48.10	2.27
EGR1	97.56	6.09
EGR3	130.86	2.73
EGR4	59.94	2.91
ELF5	119.97	2.85
EP300	28.41	15.12
ETV4	270.06	2.16
FOXF2	42.79	5.16
FOXN4	76.81	4.80
FOXO3	323.31	5.49
FOXO6	63.29	1.77
GATA	104.41	2.17
GATA5	89.03	4.38
GFI1B	184.13	11.81
GLIS2	32.14	1.87
HBP1	281.23	3.05
HIC1	101.63	3.27
HOXA13	103.63	3.71
HOXA2	323.31	5.81
INSM2	323.31	2.02
IRF	206.25	2.09
IRF5	39.54	3.78
KLF1	146.64	3.51
LHX8	245.03	2.34
MAF	66.63	3.85
MINI20	102.68	3.29
MYC	39.24	2.64
MZF1	24.10	1.64
NFATC2	37.81	1.92
NFKB1	195.32	11.43
NR0B1	13.24	1.84
NR2C2	137.27	12.28
OTX2	40.38	2.77
P50_P50	131.79	6.49
PITX1	37.22	2.17

TF	Score 1	Score 2
PRDM15	Inf	3.21
RAD21	56.16	4.21
RARB	113.99	2.28
REL	104.66	7.95
RREB	117.64	3.13
RREB1	116.79	3.39
SMC3	90.56	17.59
SOAT1	22.63	1.88
SOX18	113.13	6.71
SRP9	117.49	3.47
TAL1	70.39	1.82
TCF7L2	93.32	8.22
TFCP2	260.99	4.13
THRA	145.85	4.05
TP53	323.31	9.13
TP63	172.00	3.90
YBX1	164.76	5.23
ZBTB7A	66.49	4.74
ZFP14	97.38	5.25
ZNF219	32.59	7.48
ZNF253	323.31	21.30
ZNF585A	185.37	6.09
ZNF616	129.96	8.66
ZNF639	193.43	1.77
ZNF681	323.31	35.04
ZNF701	41.48	1.65
ZNF721	225.60	5.52
ZNF75A	43.99	3.51
ZNF770	66.18	2.95
ZNF786	323.31	15.68
ZNF790	74.59	6.71
ZNF84	149.37	3.38
ZNF846	Inf	10.92

Reverse-forward (RF) orientation

TF	Score 1	Score 2
BCL11B	101.77	5.58
CEBPA	323.31	2.37
CREB3	139.16	13.51
CTCF	119.43	20.80
DBP	25.72	1.84
EGR1	247.74	1.65
EP300	135.93	6.30
ESR1	101.75	9.30
ETS1	250.92	1.76
ETV1	272.94	3.43
ETV3	323.31	2.99
FOS	101.53	9.02
FOXA	323.31	9.16
FOXA1	323.31	8.99
FOXG1	235.13	2.77
FOXN4	61.07	5.39
FOXO3	306.90	5.33
GATA1	314.93	1.91
HLTF	82.82	1.85
HOXA10	29.26	1.81
HOXB6	197.15	2.21
IRF5	Inf	6.92
KLF13	148.08	4.91
KLF8	120.36	2.97
NFE2L2	155.83	3.83
NFKB1	106.02	7.09
NKX2-1	323.31	4.01
NKX2-4	Inf	5.73
NKX2-5	323.31	5.80
NR4A1	116.75	2.99
PPARGC1A	57.39	3.36
RAD21	41.87	3.16
RUNX2	124.18	5.84
SIX6	95.00	3.28
SMARC	323.31	6.02
SRF	323.31	6.78
TAL1	124.35	4.80
TCF12	74.48	2.21
ZBTB18	240.63	9.05
ZBTB4	323.31	2.06

TF	Score 1	Score 2
ZFP2	323.31	6.24
ZNF143	134.24	1.81
ZNF174	159.72	7.65
ZNF257	172.88	3.75
ZNF28	323.31	3.53
ZNF282	100.44	4.02
ZNF300	Inf	3.91
ZNF341	94.91	5.59
ZNF44	165.34	3.45
ZNF484	301.75	12.63
ZNF526	82.28	4.67
ZNF580	55.73	2.98
ZNF625	323.31	2.31
ZNF658	92.95	4.92
ZNF718	165.94	2.09
ZNF721	86.39	7.28
ZNF773	219.31	13.43
ZNF777	137.69	1.96

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