Figure 1. The immediate early transcriptional response to TPO signaling. (A) Volcano plot of gene transcription changes induced by 30 min stimulation of HPC-7 cells with TPO relative to serum-starved (SS) control cells, based on chromatin-associated RNA expression. Red and blue shaded regions enclose transcriptionally up- and down-regulated genes (Q-value $<10^{-3}$), respectively. The total number of genes within each category is indicated. Representative hits are labeled. The asterisk denotes the most strongly induced gene (Cish, $Q = 1.8 \times 10^{-241}$), which was repositioned within the plot area. (B, left) Chromatin-associated (Chro), nucleoplasmic (Nup), and cytoplasmic (Cyto) RNA expression heatmap for the top 25 induced and top 25 repressed genes ranked by Q-value. Regularized loge33t for toze ro.7(l5(w-me4(of32(e)]sub)- 0 T)-2)234 TctivBo

RNA levels within 30 min of TPO, indicating that transcripts encoding these key regulators are subjected to rapid turnover. To validate our results in primary cells, we confirmed the rapid TPO-induced down-regulation of *Hlf*, *Sox4* and *Cxcr4*, as well as up-regulation of *Myc* by RT-qPCR in $CD41^+Lin^-Sca1^+c\text{-}Kit^+$ ($CD41^+$LSK) bone marrow cells (Fig. 1C; Nishikii et al. 2015), indicating that TPO elicits a common transcriptional program in megakaryocytic-biased hematopoietic progenitors.

To gain further insights into the nature of the transcriptional programs regulated by TPO, we subjected differentially transcribed genes to a Molecular Signatures Database (MSigDB) and Gene Ontology (GO) enrichment analyses. Transcriptionally up-regulated events were strongly enriched for housekeeping genes involved in RNA and protein metabolism, whose expression is largely driven by a MYC transcriptional program, and for genes that respond to cytokine signaling in the immune system (Fig. 1D,E). However, megakaryocytic-affiliated genes were not induced within 30 min of TPO (Supplemental Fig. S2), consistent with a slower induction kinetic (Park et al. 2015). In contrast, genes in-

or decreased (Loss,

At finer scales, physical interactions between promoter-distal sites appear to be widespread (Fullwood et al. 2009; Phillips-Cremins et al. 2013; Ghavi-Helm et al. 2014; Sahlén et al. 2015) and might function to provide specificity and robustness to enhancer–promoter interactions within cis-regulatory units (Markenscoff-Papadimitriou et al. 2014; Ing-Simmons et al. 2015). Previous work suggests that enhancer elements tend to cluster in the nuclear space in a cohesin-dependent manner (Ing-Simmons et al. 2015), but how enhancer–enhancer interactions are modulated by extracellular signaling remains largely unknown.

To investigate this aspect, we took advantage of the extensive and spatially compartmentalized epigenome remodeling induced by transient TPO signaling and analyzed enhancer–enhancer interactions within and between differentially acetylated TADs using a structured interaction matrix analysis (SIMA) (Lin et al. 2012). This method pools Hi-C interactions across a predefined set of genomic reg63os44866.8(-273l3ri)ent

chromatin fiber, demonstrating that our analysis is well calibrated (Fig. 3G). We then examined the consequences of TPO signaling. Enhancer–enhancer interactions were only moderately perturbed within 30 min of TPO (Fig. 3G). Indeed, homotypic enhancers remained significantly clustered within differentially acetylated TADs, suggesting that TPO selectively modulates enhancer–enhancer interactions rather than altering them at a global scale.

Although by definition intra-TAD interactions occur more frequently than interactions spanning TAD boundaries, topological domains represent a modest twofold enrichment in interaction frequency (Dixon et al. 2012; Nora et al. 2012; Sexton et al. 2012). Therefore, we tested whether enhancers located within neighboring TPO-regulated TADs show evidence of spatial clustering (Methods). We found that, similarly to intra-TAD interactions, inter-TAD enhancer–enhancer interactions were significantly enriched over random expectation and only moderately perturbed by transient TPO signaling (Fig. 3H). This enrichment was further confirmed by an analysis of enhancer–enhancer interactions between CTCF loops (Supplemental Fig. S3F).

Together, these results indicate that TPO-responsive enhancers engage in preferential long-range intra- and inter-TAD interactions resulting ing6o3(6omo(by)lD)-2404(et)-234u3aiupplr

We found that the vast majority of cis-regulatory units exhibited little or no correlation between changes in cis-regulatory activity and chromatin looping (Fig. 5E).

This result was further supported by a permutation test in which the connectivity of cis-regulatory units was randomly scrambled to derive a null distribution for the correlation coefficients (Methods). Our data revealed that only(that)2j96.(on313.493 0 Our)- 0 Td [mf6e2fe(tin)edeT*(s)tialn313.57onneran8(td)-5ib-220.59ryre

Figure 5. Rapid modulation of cis-regulatory activities within poised chromatin architectures. (A) Normalized Hi-C contact frequencies for intra-TAD and intra-SE interactions (per kilobase of element), and for interactions anchored at DARs spanning more than 20 kb. Spearman's rank correlation coefficients (r) are shown. Box plots (right) summarize normalized interaction fold change distributions. (B) Distribution of the number of promoter-interacting regions (PIRs) for all baited promoters (All) and promoters of transcriptionally up- and down-regulated genes. (C) Distribution of interaction distances for all baited promoters (All) and promoters of transcriptionally up- and down-regulated genes. P-value is from a Wilcoxon rank-sum test. (D) Percentage of significant promoter Capture Hi-C interactions anchored at DARs. (X) any HindIII restriction fragment located outside DARs. (E) Relationship between cis-regulatory activity and chromatin architectures at cis-regulatory units for transcriptionally up- and down-regulated genes (Methods). Genes are ranked based on the Spearman's rank correlation coefficient (r) between normalized H3K27ac fold change (TPO/SS) and normalized Capture Hi-C interaction fold change at target DARs. Only promoters exhibiting significant interactions with at least five distinct DAR-containing HindIII restriction fragments were considered. Genes exhibiting significant correlations are colored, and representative hits are labeled. (F) Representative examples from the analysis in E. Each dot corresponds to an HindIII restriction fragment. (LFC) $\log_2$ fold change. (G) Epigenomic configuration of the Hlf locus. Statistically significant promoter Capture Hi-C interactions within the Hlf TAD are shown. The gray shaded rectangle denotes the position of the baited Hlf promoter (P). (str) strand.

subjected differentially acetylated DHSs to a de novo motif discovery analysis. We found that both activated and repressed DARs were strongly enriched for ETS motifs, which feature a central 5′-GGAA-3′ core, and for motifs recognized by the RUNX and GATA families of TFs (Fig. 6A,B). These motifs exhibited similar en-

Figure 6. TF binding patterns accurately predict rapid cis-regulatory responses to TPO. (A) Top transcription factor motifs (position weight matrices

and hormone receptor binding by 3C or circular chromosome conformation capture (4C) sequencing (Jin et al. 2013; Melo et al. 2013; Eijkelenboom et al. 2014). Our results indicate that poised

instructions. Quantitative RT-PCR was carried out using SYBR Green Brilliant II low Rox and a Stratagene Mx3000P machine (Agilent Technologies). For primer sequences, see Supplemental Table S2.

Subcellular fractionation

Subcellular fractionation and RNA preparation were performed essentially as described (Bhatt et al. 2012) with modifications detailed in Supplemental Methods.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays on serum-starved and TPO-stimulated HPC-7 cells were performed as previously described (Supplemental Methods; Wilson et al. 2010).

Hi-C

Hi-C libraries were generated essentially as described (Schoenfelder et al. 2015) with modifications detailed in Supplemental Methods.

Promoter Capture Hi-C

Promoter Capture Hi-C libraries were generated essentially as described (Schoenfelder et al. 2015) with modifications detailed in Supplemental Methods.

Computational analyses

Subcellular RNA-seq, ChIP-seq, Hi-C, and promoter Capture Hi-C data processing and all computational analyses are detailed in Supplemental Methods.

Data access

RNA-seq, ChIP-seq, Hi-C, and promoter Capture Hi-C raw and processed data from this study have been submitted to the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE100835.

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Thrombopoietin signaling to chromatin elicits rapid and pervasive epigenome remodeling within poised chromatin architectures

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