HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2024 March 08.

Published in final edited form as:

Leukemia. 2014 February ; 28(2): 349–361. doi:10.1038/leu.2013.158.

Aberrant TAL1 activation is mediated by an interchromosomal interaction in human T-cell acute lymphoblastic leukemia

B Patel[1,7], **Y Kang**[1,2,7], **K Cui**[3], **M Litt**[4], **MSJ Riberio**[1], **C Deng**[1], **T Salz**[1], **S Casada**[4], **X Fu**[2], **Y Qiu**[5,6], **K Zhao**[3], **S Huang**[1,6]

[1]Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, FL, USA

[2]College of Life Science, Jilin University, Changchun, China

[3]Center for System Biology, NHLBI, National Institute of Health, Bethesda, MD, USA

[4]Medical Education Center, Ball State University, Muncie, IN, USA

[5]Department of Anatomy and Cell Biology, College of Medicine, University of Florida, Gainesville, FL, USA

[6]Shands Cancer Center, College of Medicine, University of Florida, Gainesville, FL, USA.

[7]These authors contributed equally to this work.

Abstract

Long-range chromatin interactions control metazoan gene transcription. However, the involvement of intra- and interchromosomal interactions in development and oncogenesis remains unclear. TAL1/SCL is a critical transcription factor required for the development of all hematopoietic lineages; yet, aberrant TAL1 transcription often occurs in T-cell acute lymphoblastic leukemia (T-ALL). Here, we report that oncogenic *TAL1* expression is regulated by different intra- and interchromosomal loops in normal hematopoietic and leukemic cells, respectively. These intra- and interchromosomal loops alter the cell-type-specific enhancers that interact with the *TAL1* promoter. We show that human SET1 (hSET1)-mediated H3K4 methylations promote a long-range chromatin loop, which brings the + 51 enhancer in close proximity to *TAL1* promoter 1 in erythroid cells. The CCCTC-binding factor (CTCF) facilitates this long-range enhancer/promoter interaction of the *TAL1* locus in erythroid cells while blocking the same enhancer/promoter interaction of the *TAL1* locus in human T-cell leukemia. In human T-ALL, a T-cell-specific transcription factor c-Maf-mediated interchromosomal interaction brings the *TAL1* promoter into close proximity with a T-cell-specific regulatory element located on chromosome 16, activating aberrant *TAL1* oncogene expression. Thus, our study reveals a novel molecular mechanism involving changes in three-dimensional chromatin interactions that activate the *TAL1* oncogene in human T-cell leukemia.

Correspondence: Dr S Huang, Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Po Box 103633, 2033 Mowry Road, Gainesville, FL 32610, USA. sumingh@ufl.edu.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

Keywords

TAL1/SCL oncogene; chromatin loops; CTCF insulator; T-cell leukemia; epigenetic regulation

INTRODUCTION

Tissue- and developmental stage-specific activation of enhancer elements regulates metazoan gene expression, which determines cell identity. Because genes are regulated by sequence-specific transcription factors and their corresponding enhancers, changes in these factors and interaction between elements influence gene expression patterns and subsequently cellular function. Therefore, perturbations in the activity of these transcriptional regulators compromise their function and may initiate malignant transformation. One such key regulator is the basic helix-loop-helix (bHLH) transcription factor TAL1/SCL (hereafter referred to as TAL1), originally identified by virtue of its involvement in a T-cell acute lymphoblastic leukemia (T-ALL)-specific chromosomal translocation.[1–4] Its expression is required for the development of all hematopoietic cell lineages.[5,6] Deletion of *Tal1* in mice led to embryonic lethality in midgestation due to a complete loss of yolk sac hematopoiesis.[7,8] In addition, the *Tal1*-null embryoid stem cells were unable to contribute to hematopoiesis *in vivo* in chimeric mice,[7] indicating that TAL1 has a critical role in early hematopoietic lineage commitment and differentiation.

To act as a transcription regulator, TAL1 associates with coregulators that often possess histone-modifying and -remodeling activities to regulate the transcription of downstream target genes.[9,10] Cooperation of GATA-1, TAL1 and chromatin-remodeling factor Brg1 shapes the erythroid-specific chromatin landscape and determines the erythroid transcription program.[11] TAL1 also autoregulates itself by forming a complex with GATA-1 and by binding to consensus GATA-E-box motifs presented in its own enhancer and promoter.[12,13] The human *TAL1* gene is located on chromosome 1p32 and is tightly regulated by various *cis*-regulatory elements, which control *TAL1* expression levels in different hematopoietic lineages and stages.[14–16] Transgenic reporter knockin mouse studies and DNase I hypersensitive assays have revealed that the *TAL1* locus contains distinct promoters and distal enhancer elements that together control temporal and spatial *TAL1* expression patterns.[14,17–19] Lineage-restricted Promoter 1a is utilized in erythroid, megakaryocytic and mast cells; promoter 1b is active in primitive myeloid and mast cells.[1,20] Similar to the promoters, distinct *TAL1* enhancers also associate with different *TAL1* expression patterns. The −4 Kb and + 18/19 enhancers initiate *TAL1* expression in mesoderm derivatives associated with early formation of endothelial and hematopoietic stem and progenitor cells, whereas the + 40 Kb enhancer (+ 51 Kb in human) is transcriptionally active in erythroid cells.[13,16,21] Despite extensive studies on the identification of *TAL1* regulatory elements, the detailed epigenetic mechanisms governing differential enhancer and promoter action to selectively activate *TAL1* in different stages of hematopoietic differentiation remain to be illustrated.

Despite its role in normal hematopoiesis, ectopic transcriptional activation of *TAL1* is the most frequent gain-of-function mutation observed in T-ALL patients. Aberrant activation of

TAL1 was found in 40–60% of T-ALL patients, resulting from chromosomal translocation (4–5%), or interstitial chromosome deletion (25–30%), or by an undefined mechanism (60%).[22–24] Ectopic activation of *Tal1* transcription in T cells led to the development of leukemia and lymphoma in mice.[25,26] In contrast, deletion of *TAL1* in T-ALL leads to a loss of the leukemic phenotype and induces apoptosis[27], implicating an important role of *TAL1* activation in T-cell neoplastic disease. Surprisingly, it remains largely unknown how the TAL1 oncogene is activated in human T-cell acute leukemia. Moreover, knowledge of the molecular mechanisms governing ectopic TAL1 activation is scarce, especially in T-ALL cases lacking TAL1 locus chromosomal rearrangements.

Here, we report that, although *TAL1* promoter 1 is active in all lineages expressing *TAL1*, the + 51 enhancer is selectively active in erythroid precursors and inactive in leukemic T cells. hSET1-mediated H3K4 methylation facilitates an erythroid-specific long-range chromatin interaction between the + 51 enhancer and *TAL1* promoter 1, which activates *TAL1* gene transcription in erythroid precursor cells. In contrast, in T-ALL cell lines and patients, the T-cell-specific proto-oncoprotein c-Maf mediates an interchromosomal interaction that brings *TAL1* promoter 1 in close proximity to a T-cell-specific DNA regulatory element on chromosome 16. This interaction is critical for ectopic *TAL1* expression and leukemic cell proliferation in human T-ALL. Further, we found that CTCF differentially reorganized the chromatin structure in normal erythroid cells and leukemic cells, keeping the + 51 enhancer in close proximity to the *TAL1* promoter in erythroid cells while deflecting the + 51 enhancer from interacting with the promoter in T-ALL. Thus, our studies revealed novel molecular mechanisms by which the oncogenic transcription factor TAL1 is regulated by changes in chromatin loops in normal and malignant hematopoiesis.

MATERIALS AND METHODS

Cell lines, constructs and small hairpin RNA-mediated knockdown K562 and Jurkat cells were maintained as described.[28] HL-60 was cultured in IMDM supplemented with 20% fetal bovine serum. Human cord blood-derived $CD34^+$ cells were enriched through positive immune selection by flow cytometry and maintained as described previously.[29] The cells were subjected to erythroid differentiation to $CD36^+$ cells over a 7-day period and then sorted for $CD36^+$ population for 3C and ChIP analyses. The hSET1 knockdown (KD) constructs were generated by subcloning small hairpin RNA (shRNA) oligonucleotides into pSuper.retro.puro vector (Clontech, Mountain View, CA, USA) as described previously.[28] All stable KD cells were maintained in medium containing 1 μg/ml puromycin (Calbiotech, Spring Valley, CA, USA).

Native ChIP, formaldehyde cross-linked ChIP and ChIP-seq

Native ChIP assays for histone modifications and formaldehyde cross-linked ChIP for transcription factors and modifying enzymes were performed as described previously.[30] The relative enrichment was determined by the following equation: $2^{Ct(IP)-Ct(ref)}$. Primer sequences across the *TAL1* locus and antibodies are listed in the Supplementary Information. ChIP-Seq assays in $CD34^+$ and $CD36^+$ cells were performed as outlined previously[31] and are described in Supplementary Information.

Chromosome conformation capture (3C) and circular chromosome conformation capture (4C) assays

The 3C assay was performed as described previously with minor modifications.[32] In brief, 2×10^7 cells were crosslinked with 2% formaldehyde for 10 min and lysed. Real-time PCR was performed to quantitate 3C interactions using SYBR after validation of each primer pair as described.[33] Relative crosslinking frequencies were calculated and plotted after normalization to loading control and ERCC3 control.[34]

The 4C assay was performed as described previously,[35] with minor modifications. In brief, 5×10^5 cells were crosslinked with 2% formaldehyde for 10 min and digested overnight. 4C-ligated DNA was prepared and amplified by nested PCR. The PCR products were cloned into pCR-TOPOII vector (Invitrogen, Grand Island, NY, USA) for sequencing.

Reverse transcription and quantitative PCR

Total RNA was prepared using the RNeasy mini isolation kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). One microgram RNA was reverse transcribed using the Superscript II reverse Transcriptase (Invitrogen). complementary DNA was analyzed using real-time PCR (quantitative reverse transcription-PCR) with a MyiQ Single-Color real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). Primer sequences are listed in Supplementary Information (Supplementary Table S2).

Colony formation assay for $CD34^+$ hematopoietic stem cells

The human colony formation assay was performed as described by StemCell Technologies (Vancouver, BC, Canada) using complete Methocult. Vector control (1×10^4) or hSET1 KD $CD34^+$ cells were seeded in 4 ml SFEM medium supplemented with 10 ng/ml granulocyte colony-stimulating factor, 20 ng/ml stem cell factor, 10 ng/ml interleukin-3 (IL3), 10 ng/ml IL6 and 6U/ml erythropoietin. The cells were then plated in triplicate on methylcellulose cell culture plates and cultured for 18 days, with fresh medium supplemented with cytokines added every week. Different hematopoietic colonies including erythroid progenitors, colony-forming unit-erythroid (CFU-E) and blast-forming unit-erythroid, granulocyte/macrophage progenitors, CFU-granulocyte and macrophage, CFU-granulocyte, erythroid, macrophage and megakaryocyte were observed and counted following guidelines as described by StemCell Technologies.

RESULTS

Distinct patterns of histone modifications are associated with TAL1 enhancer/promoter activities

Recent genome-wide studies predict a correlation between the different levels of histone modifications, such as the methylation statuses of Lys 4 residue on histone H3 tails and enhancer/promoter activities.[36,37] Given that *TAL1* is tightly controlled by multiple *cis*-regulatory elements in different stages of hematopoiesis, we examined the H3K4me2, H3K4me3, H3K9/14ac and H3K27me3 patterns across the 166 Kb of the *TAL1* locus in K562, Jurkat, Rex, HPB-ALL and HL-60 cells by using the ChIP-qPCR assay with antibodies specific to these modifications. K562 is a human erythroleukemia cell line and

Jurkat is a T-ALL cell line in which *TAL1* is highly activated. HL-60 was derived from an acute myeloid leukemia patient in whom the *TAL1* gene was silenced. The pattern of active and repressive histone modifications associated with gene activity in these three cell types across the entire *STIL1-TAL1-MAP17* locus is shown in Figure 1 and Supplementary Figure S1. There are marked enrichments of H3K4me2 and H3K9/14ac in K562 cells at *TAL1* promoter 1, + 19, enhancer *Map17* promoter and + 51 enhancer. H3K4me3 is particularly enriched at *TAL1* promoter 1, but not at other regulatory elements (Figure 1a). In HL-60 and HPB-ALL cells in which *TAL1* is inactive, there are large peaks of H3K27me3 over *TAL1* promoter IV and promoter 1, marking the silenced *TAL1* gene (Figure 1b and Supplementary Figure S1A). Although *TAL1* is expressed in Jurkat and REX T-ALL cells, only promoters 1 and IV are marked by H3K9/14ac, H3K4me2 and H3K4me3. No active modifications are detected at + 19 and + 51 enhancers (Figure 1c and Supplementary Figure S1A). Moreover, no H3K27me3 is detected in *TAL1*-expressing K562 and Jurkat cells (Supplementary Figure S1B). This pattern of histone modifications suggests that *TAL1* promoter 1 is differentially regulated by different regulatory elements in erythroid and leukemic cells. Our results show that the well-characterized enhancers required for normal hematopoietic expression of *TAL1* in the locus[13,14] are epigenetically inactive in T-ALL cells. Therefore, aberrant *TAL1* activation in T-ALL cells may require distinct regulatory element(s).

The recruitment of hSET1 correlates with TAL1 transcriptional activation during hematopoiesis hSET1 is a histone methyltransferase that specifically methylates Lys 4 at histone H3 tails. Given that TAL1 colocalizes with the hSET1 complex at the TAL1 target genes during erythroid differentiation[38] and both + 51 enhancer and *TAL1* promoter 1 contain composite *E-box/GATA* motifs,[13] we reasoned that recruitment of the hSET1 complex by TAL1 may be responsible for high levels of H3K4 methylations in the *TAL1* locus in erythroid cells. To test this possibility, we examined the global interactions between TAL1 and hSET1 at the human genome comparing human primary $CD34^+$ hematopoietic stem cells (HSCs) and $CD36^+$ erythroid precursors using unbiased ChIP-seq technologies. Approximately 50% of intergenic bound hSET1 colocalized with TAL1 in $CD36^+$ erythroid precursors (Supplementary Figure S2A), suggesting that TAL1 recruits the hSET1 complex to regulate its genome-wide targets in erythroid cells. As we expected, TAL1 and hSET1 complexes bind to both the + 51 enhancer and promoter 1 in the *TAL1* locus in primary hematopoietic cells. This binding correlates strongly with H3K4 methylations at these elements (Figures 2a and b). In particular, H3K4me3 was enriched around the transcription start site of the *TAL1* gene (Figures 2a and b). Although the hSET1 complex is recruited to both the + 51 enhancer and promoter 1 of the *TAL1* gene in K562 (Figure 2c) and primary hematopoietic cells (Figures 2a and b), both TAL1 and hSET1 are not bound to the *TAL1* locus in T-ALL Jurkat cells (Figure 2c and Supplementary Figure S2B). Thus, our data suggest that recruitment of the hSET1 complex facilitates promoter H3K4 methylations and transcriptional activation of *TAL1* during normal hematopoiesis, but not in malignant Jurkat cells.

Next, we tested whether the recruitment of hSET1 could attribute to the transcriptional activity of the + 51 enhancer in K562 cells but not in Jurkat cells. Two differently sized DNA fragments containing the + 51 enhancer element were cloned into an SV40 minimal

promoter-driven luciferase reporter and introduced into K562 and several T-ALL cell lines. Compared with the pGL3-SV40 vector that showed only minimal luciferase activity, the 2 Kb + 51 enhancer element specifically activated transcription of the luciferase reporter in K562 cells, but not in T-ALL cell lines, Jurkat, Rex, Molt4 and HPB-ALL (Figure 2d). Interestingly, the 4 Kb fragment containing the + 51 enhancer and an additional + 53 Kb CTCF site inserted between the + 51 enhancer and the SV40 minimal promoter blocks transactivation of the reporter in K562 cells, suggesting that the + 53 Kb CTCF site may block the + 51 enhancer from activating downstream neighboring genes (Figure 2d). Together, the data revealed that the + 51 enhancer is neither epigenetically nor transcriptionally active in T-ALL cells.

A long-range chromatin loop mediates enhancer/promoter interaction in the *TAL1* locus in erythroid precursors but not in T-ALL cells

It has been reported from studies on transgenic mice that the + 51 enhancer is capable of driving reporter gene expression at physiological *TAL1* expression sites during hematopoiesis.[13,14] However, it is unclear how the + 51 enhancer activates the *TAL1* gene from 51 Kb downstream in native chromatin location. An attractive model proposes that a chromosomal loop brings the + 51 enhancer and promoter 1 into close proximity. To test this possibility, we carried out chromosome conformation capture (3C) assays in $CD36^+$ erythroid progenitor cells as shown in Figure 3a. A long-range chromosomal interaction between the + 51 enhancer and *TAL1* promoter 1 was detected in primary $CD36^+$ erythroid precursors (Figure 3b). Sequence analysis of the 197 bp 3C PCR product revealed a fusion molecule containing both the + 51 enhancer and the *TAL1* promoter 1 sequences (Figure 3c). In contrast, no interactions were detected between *TAL1* and *Map17* promoters (Figure 3b). Thus, these data suggest that the + 51 enhancer activates *TAL1* promoter 1 via a long-range enhancer/promoter chromatin loop.

Recent studies have highlighted that long-range chromatin interactions provide a topological basis for transcriptional regulation.[39,40] To test whether the enhancer/promoter loop is specific for erythroid cells or is also present in *TAL1*-expressing T-ALL cells or in nonexpressing leukemic cells, 3C assays were carried out in K562, Jurkat, Rex, HPB-ALL and HL-60 cells. For these assays the restriction enzyme *NlaIII* was used (Figures 3d and e). Digestion with enzyme *NlaIII* generated on average 250–500 bp fragments across the genome, and the specific interaction between the + 51 enhancer and *TAL1* promoter 1 produced a 527-bp PCR fragment (Figure 3d). Consistent with primary $CD36^+$ cells, the + 51 enhancer physically interacts with promoter 1 only in K562 cells using either *NlaIII* (Figure 3e) or *BamHI* (Supplementary Figure S3A) digestion. The 527-bp PCR fragment was cloned and sequenced. It consists of a fusion of sequences from the + 51 enhancer and *TAL1* promoter 1 (Supplementary Figure S3B). The long-range interaction is specific between the + 51 enhancer and promoter 1 in erythroid cells because it was detected neither between the *Map17* promoter and *TAL1* promoter 1 (Figure 3e) nor in *TAL1*-silenced HL-60 and HPB-ALL cell lines (Figure 3e and Supplementary Figure S3C). Interestingly, although both Jurkat and REX express the *TAL1* gene, the chromatin interaction between the + 51 enhancer and *TAL1* promoter 1 was not detected in these cells (Figure 3e and

Supplementary Figure S3C), supporting the evidence that the + 51 enhancer is inactive in *TAL1*-expressing T-ALL cells (Figure 1).

Recruitment of the hSET1 complex is essential for long-range chromatin loop and transcription of *TAL1* gene

Evidence suggests that H3K4me3 may be important for establishing chromatin loops.[41] The hSET1 complex has been shown to regulate H3K4me3 methylation. Because the binding of the hSET1 complex correlates with TAL1 activation in erythroid cells (Figure 2), we further reasoned that recruitment of hSET1 may mediate the physical chromatin interaction between the + 51 enhancer and promoter 1 at the *TAL1* locus. To test this hypothesis, we generated shRNA-mediated hSET1 KD in K562 cells (Supplementary Figure S4A). The KD of hSET1 led to a decrease in *TAL1* expression in three individual hSET1 KD K562 clones (Figure 4a), but not in T-ALL Jurkat cells (Supplementary Figures S4B and C). Moreover, the levels of H3K4me2 and H3K4me3 enrichment at the + 51 enhancer and TAL1 promoter 1 also decreased in the hSET1 KD K562 clones (Figure 4b). Subsequently, in the hSET1 KD K562 clones the intrachromatin loop between the + 51 enhancer and *TAL1* promoter 1 is inhibited (Figure 4c). In addition, the recruitment of RNA Pol II to both the enhancer and the promoter of the *TAL1* gene is suppressed (Figure 4b). Consequently, the hSET1-mediated active histone modifications may be associated with specific chromatin loop formation. Finally, ablation of hSET1 in human $CD34^+$ HSCs (Supplementary Figure S4D) resulted in a block of the ability of HSCs to differentiate into colony forming unit-erythroid (CFU-E) and burst-forming unit-erythroid colonies but not CFU-granulocyte, erythroid, macrophage, megakaryocyte and CFU-granulocyte and macrophage colonies (Figure 4d), implying that disruption of hSET1 function in HSCs specifically affects erythropoiesis by perturbing *TAL1* transcription.

CTCF-mediated cell-type specific chromatin loops in the *TAL1* locus regulate expression of *TAL1* gene in erythroid and leukemic cells

In the *TAL1* locus there are four CTCF-binding elements that are occupied by CTCF in K562 cells.[12,42] The −31 CTCF site is located between the *STIL* gene and the *TAL1* gene; the + 40 CTCF site separates the *TAL1* and *MAP17* genes; and the + 53 and + 57 CTCF sites are located in the 3′ boundary of the *TAL1* locus (downstream of the + 51 enhancer) (Figure 5a). Given the global role of CTCF in genome organization,[43,44] CTCF may bind differently to CTCF elements in the *TAL1* locus in erythroid and leukemic cells, thereby regulating the + 51 enhancer and *TAL1* promoter 1 interaction. To examine this model, we carried out CTCF ChIP analysis in K562 and Jurkat cells. Interestingly, CTCF bound to all of the CTCF elements in both cell lines (Supplementary Figure S5A), suggesting that the binding of CTCF alone is not sufficient to modulate *TAL1* gene activity. We next examined whether CTCF differentially regulates genome organization by controlling *TAL1* promoter accessibility in K562 and Jurkat cells. To address this question, we performed 3C assays in K562 and Jurkat cells using the + 53 Kb CTCF element as bait. Figure 5b shows that in K562 cells, but not in T-ALL Jurkat cells, the − 31 Kb CTCF element interacts with the + 53 Kb CTCF site (Figure 5b). As a control, no CTCF-mediated loop is formed between − 10 and + 53 Kb CTCF elements (Figure 5b). The interaction between the − 31 Kb CTCF element and the + 53 Kb CTCF site was predominantly found in K562 cells (Figures 5b

and c). This interaction brings the + 51 enhancer and promoter 1 of the *TAL1* gene into close proximity, thereby facilitating enhancer/promoter regulation of *TAL1* gene expression. Interestingly, the − 31/+ 53 CTCF loop is not dependent on the hSET1 complex, as hSET1 KD in K562 cells does not interfere with the loop formation (Supplementary Figures S5B and C). In contrast, when we carried out 3C assays to evaluate interactions between the + 40 Kb and + 53 Kb CTCF elements, the CTCF-mediated + 40/+ 53 chromatin loop was seen to predominantly exist in T-ALL Jurkat cells, but not in K562 cells (Figures 5b and c). Further, 3C analysis of normal bone marrow (BM) cells and a *TAL1*-overexpressing human T-ALL patient's sample also showed different preferential loop interactions. The preferential − 31 Kb/+ 53 Kb CTCF loop found in normal BM cells switched to a predominantly smaller + 40 Kb/+ 53 Kb CTCF loop in T-ALL patient sample (Figures 5d and e), suggesting that CTCF may act to exclude the + 51 enhancer from *TAL1* promoter 1 in T-cell leukemia.

A novel cis-regulatory element in chromosome 16 interacts and drives *TAL1* expression in leukemic T cells

Currently, it remains unknown how the *TAL1* gene is aberrantly activated in the majority of T-ALL patients lacking chromosomal rearrangements in the *TAL1* locus. To understand the molecular mechanisms underlying the regulation of the *TAL1* oncogene in T-ALL, we used circular chromosome conformation capture (4C) technology to identify potential regulatory elements that associate with the *TAL1* promoter in T-ALL (Figure 6a and Supplementary Figure S6A). Using *TAL1* promoter 1a as bait, a 4C library was created and a total of 57 clones were sequenced. Supplementary Table 1 shows the potential *TAL1* promoter 1-interacting elements found in the Jurkat genome. Most of the clones (54 out of 57 clones) are self-ligated products and one clone did not match with the human genome sequence (Supplementary Table S1). Of the two DNA elements in human T-ALL cells that interact with *TAL1* promoter 1 (Supplementary Table S1), one is located ~2.1 Kb upstream of a long noncoding RNA *LOC595101* and ~15 Kb downstream of the *CD2BP2* gene, encoding a protein for T-cell signaling (Supplementary Figure S6B), and the other is located in the coding region of the *RAP2A* gene, encoding a member of the Ras superfamily of small GTPase (Supplementary Figure S6C). We confirmed by 3C analysis that the intergenic DNA element located downstream of the T-cell-specific *CD2BP2* locus specifically interacts with *TAL1* promoter 1 in the T-ALL cell line Jurkat, but not in K562 cells (Supplementary Figures S6D and E). The element was named *TIL16* (TAL1-interacting locus located in chromosome 16) and contained p300, leukemia oncoproteins MEIS1/HoxA9 and T-cell specific transcription factor c-Maf binding sites (Supplementary Figure S7A). It is interesting to note that HoxA9, and its cofactor MEIS1 often co-occupy genomic sites in intergenic enhancers with high levels of H3K4me1 and CBP/p300 binding.[45] The *TIL16* element is located between the T-cell-specific *CD2BP2* gene and a noncoding RNA gene *LOC595101*. Both transcripts are highly expressed in T lymphocytes (Supplementary Figures S7B and C). Interestingly, ChIP-seq analysis revealed that H3K4me1 and H3K4me2, which are epigenetic marks associated with regulatory enhancer elements,[37] are enriched at the *TIL16* element in $CD4^+$ T lymphocytes (Supplementary Figure S8A). Further 3C analysis confirmed the presence of the interchromosomal interaction between the *TIL16* region in chromosome 16 and *TAL1* promoter 1 in chromosome 1 in three *TAL1*-overexpressing T-ALL cell lines, Jurkat, REX

and Molt4 (Figure 6b). These assays did not detect the interaction in K562 cells (Figure 6b). Sequence analysis of the predicted 3C products revealed a fusion DNA containing both the *TIL16* element and the *TAL1* promoter 1 sequences (Figure 6c). In addition to T-ALL cell lines, 3C analysis was also performed in a BM sample from one T-ALL patient and in primary cells (COG-LL-317) from one pediatric T-ALL patient in whom *TAL1* are aberrantly expressed (Supplementary Figures S9A–C). Genomic DNA analysis of these patient samples did not detect any chromosomal rearrangements in the *TAL1* locus (Supplementary Figures S9D and E). The *TIL16/TAL1* promoter 1 interchromosomal interaction was detected only in the primary T-ALL patient's BM and in the patient-derived primary T-ALL cells, but was absent in normal BM cells (Figure 6d and Supplementary Figure S9F).

Next, to test whether *TIL16* can act as a T-cell-specific enhancer to ectopically activate the *TAL1* gene in T-ALL cells, a 995 bp of the *TIL16* element containing the c-Maf, HoxA9/MEIS1, and p300/CBP binding sites was cloned into a *TAL1* promoter 1-driven luciferase reporter and introduced into K562 and Jurkat cells. TAL1 promoter 1 is highly active in K562 cells, and the addition of the *TIL16* element did not enhance reporter activity in K562 cells further (Figure 6e). However, in Jurkat cells in which *TAL1* promoter 1 alone showed minimal activity when compared with K562 cells, the *TIL16* element significantly stimulated the *TAL1* promoter 1 activity, resulting in a nearly threefold increase in Jurkat cells (Figure 6e). Further, deletion of the c-Maf-binding site attenuates the *TIL16*-mediated transactivation in Jurkat cells (Figure 6e). In addition, H3K4me1 is highly enriched in the *TIL16* element in Jurkat cells but not in K562 cells (Figure 6f). These results identify a novel regulatory element bound by T-cell specific transcription factor c-Maf that activates T-cell expression of *TAL1* gene in trans in T-ALL.

c-Maf regulates *TAL1* expression and interchromosomal interaction in T-cell leukemia

c-Maf is a member of the basic leucine zipper transcription factors belonging to the AP1 superfamily. c-Maf controls IL-4 expression in Th2 cells, and IL-4 and IL10 in macrophages.[46,47] *c-Maf* is overexpressed in 60% of angioimmunoblastic T-cell lymphomas.[48] Ectopic expression of *c-Maf* in the T-cell compartment contributes to T-cell lymphoma in mice,[49] a phenotype similar to that of *TAL1* overexpression.[25,26] Interestingly, a consensus binding site for the c-Maf transcription factor was found in both the *TIL16* element and *TAL1* promoter 1 (Supplementary Figures S7A and S8B). c-Maf specifically occupies *TIL16* and *TAL1* promoter 1 elements in Jurkat cells (Figure 7a). To examine whether c-Maf regulates *TAL1* expression in T-ALL and to determine the underlying mechanism of ectopic *TAL1* activation in T-ALL, *c-Maf* was depleted in Jurkat cells using lentivirus-mediated shRNA specifically targeting the human *c-Maf* sequence (Figure 7b). *c-Maf* ablation led to a significant decrease in both *TAL1* mRNA and protein levels (Figures 7b and c) and impaired Jurkat cell proliferation (Supplementary Figures S10 A and B). We further reasoned that inhibition of *TAL1* expression by *c-Maf* KD may result from a disruption of the interchromosomal loop between the *TIL16* element and *TAL1* promoter 1. To test this possibility, 3C analysis was carried out in wild-type and *c-Maf*-depleted Jurkat cells. We found that *c-Maf* depletion destabilizes the *TIL16* and *TAL1* promoter 1 interchromosomal interaction (Figure 7d and Supplementary Figure S10C) and also inhibits

H3K4me3 enrichment and TAF3 recruitment at *TAL1* promoter 1 (Figures 7e and f). These data revealed that c-Maf plays an important role in maintenance of the topological *TAL1* promoter 1 and *TIL16* interaction as well as maintenance of aberrant *TAL1* gene expression in human T-ALL.

Finally, we performed a ChIP-3C experiment to test whether the T-cell specific transcription factor c-Maf is directly involved in interchromosomal interaction to aberrantly activate the *TAL1* oncogene in T-ALL leukemia. The crosslinked chromatin was digested with the *NlaIII* restriction enzyme and immune-precipitated with antibody specific to c-Maf. The c-Maf-selected chromatin was subjected to 3C analysis for the loop interaction between the *TIL16* element and *TAL1* promoter 1 (Figure 8a). PCR reaction using the *TIL16* and *TAL1* promoter 1 primers yielded a unique band of the expected size and sequence in Jurkat cells (Figure 8a). Thus, we concluded that T-cell specific transcription factor c-Maf anchors an interchromatin loop that brings the *TIL16* element and the *TAL1* promoter into close proximity in T-ALL.

p300 is important for *TAL1* expression and interchromosomal interaction in T-cell leukemia

There is a strong correlation between the presence of p300 and enhancer function in the human genome[37,50], and enhancer-targeted p300/CBP is able to interact with the promoter-bound TATA-binding protein and other components of TFIID.[51] Presence of the p300 binding site in the *TIL16* and *TAL1* promoter 1 elements suggests that p300 may mediate the interchromosomal interaction of these two elements. To test this possibility, *p300* was depleted in Jurkat cells using lentivirus-mediated shRNA specifically targeting the human *p300* sequence (Figure 8b). p300 ablation led to a significant decrease in both *TAL1* mRNA and protein levels (Figures 8b and c). 3C analysis showed that *p300* depletion interferes with the *TIL16* and *TAL1* promoter 1 interchromosomal interaction (Figure 8d), suggesting that c-Maf and p300 may cooperate to control the interchromosomal loop for aberrant *TAL1* activation in T-ALL.

DISCUSSION

We identified a novel T-cell-specific cis-regulatory element in chromosome 16 that is involved in aberrant activation of the *TAL1* gene through an interchromosomal loop in human T-ALL (Figure 8e). Interestingly, genome annotation revealed that the novel *TIL16* element is flanked by a T-cell specific *CD2BP2* gene and a long noncoding RNA *LOC595101*, which are highly expressed in T lymphocytes (Supplementary Figures S7B & C). The *TIL16* element itself is bound by p300 and the T-cell specific transcription factor c-Maf. It is possible that the *TIL16* element confers T-cell specificity on *TAL1* genes and leads to malignant transformation. Except for *TAL1* promoter 1, promoter IV of the *TAL1* gene is also involved in T-ALL cases[52] and is epigenetically active in Jurkat and REX T-ALL cells (Figure 1c and Supplementary Figure S1A). It still remains to be determined whether promoter IV is also activated by the interchromosomal interactions in human T-ALL.

TAL1 is a critical oncogenic transcriptional factor required for normal hematopoiesis.[9] Aberrant activation of TAL1 in T lymphocytes leads to leukemic transformation in the majority of childhood T-ALL.[23] It is essential to understand the molecular mechanisms

that regulate *TAL1* transcription activity in normal hematopoiesis and leukemic T cells. We found that the *TAL1* gene is controlled by a long-range intrachromatin loop that brings the + 51 enhancer into close proximity with *TAL1* promoter 1. This loop interaction is specific for erythroid cells and is absent in other hematopoietic cells in which *TAL1* is silenced or even in T-ALL cells in which *TAL1* is expressed (Figure 8e). Thus, an interesting question is what underlies differential selection of the + 51 enhancer usage in erythroid cells and how the tissue-specific chromatin loop is established and stabilized. Several lines of evidence support the notion that active histone modifications such as H3 acetylation have an important role in communication between genes and distal *cis*-regulatory elements by chromosomal loops.[32,53] One example is the *β-globin* locus in which the LCR can serve as a primary site for recruiting transcription factors and chromatin-modifying and -remodeling factors and stably alter the topology of the *β-globin* locus during transcriptional activation in erythroid cells.[32,54,55] Depletion of hSET1 led to reduced H3K4 methylation, disruption of the + 51 enhancer/promoter 1 chromatin loop and loss of *TAL1* transcription (Figure 4), suggesting a plausible model that hSET1-mediated H3K4 methylation may be required for establishing or stabilizing the enhancer/promoter communication through a long-range chromatin loop. However, the loss of Pol II occupancy in hSET1-depleted cells (Figure 4b) makes it possible that hSET1-mediated H3K4 methylations also remodel local chromatin and facilitate the accessibility of the basal transcription factors to the *TAL1* promoter (Figure 8e). Nevertheless, the data provide a potential mechanism that links H3K4 methylations and the long-range enhancer/promoter interaction. Further, the KD of hSET1 does not affect the CTCF-mediated looping in the *TAL1* locus (Supplementary Figures S5B and C). This suggests that CTCF is not directly involved in the enhancer/promoter interaction. However, the CTCF-mediated chromatin loops probably facilitate interactions between enhancers and promoters by bringing them into close proximity.

One particularly interesting finding is that CTCF mediates different chromatin loops at the *TAL1* locus in erythroid and T-ALL cells, thereby providing another layer of regulation to ensure proper *TAL1* expression (Figures 5 and 8e). CTCF has been implicated in diverse regulatory functions, including transcriptional activation and repression, insulation, imprinting, and X chromosome inactivation.[43,44] CTCF molecules are capable of interacting with each other to form a cluster, thereby creating closed looping domains.[56] It has been proposed that CTCF may have a primary role in the global organization of chromatin architecture and lineage-specific gene expression.[43,44] With regard to the *TAL1* locus, our data revealed that CTCF differentially organized chromatin loop domains in erythroid and leukemic cells such that the + 51 enhancer was in close proximity to *TAL1* promoter 1 in erythroid cells and the + 51 enhancer was separated from the *TAL1* promoter in T-ALL cells (Figure 8e). It is likely that the closed chromatin loop between − 31 and + 53 CTCF sites also prevents the upstream *STIL* promoter from interfering with the *TAL1* gene in erythroid cells. Therefore, the CTCF-mediated loop confines or facilitates interaction between the downstream + 51 enhancer and promoter 1 (Figure 8e).

In T-ALL patients, the *TAL1* oncogene can be activated by chromosomal translocation and interstitial deletion.[22,23,57] However, chromosomal rearrangements account for only less than 30% of cases with aberrant *TAL1* expression.[23,58,59] How the *TAL1* oncogene is aberrantly activated in the majority of T-ALL patients who lack chromosomal

rearrangements in the *TAL1* locus, and whether dysregulation of enhancer/promoter interactions leads to a disease-causing regulatory variant, remains unknown. Although several enhancer elements have been identified in the *TAL1* locus,[16] epigenetic, chromatin looping and reporter analysis suggested that none of them is transcriptionally active in T-ALL cells (Figures 1–3). Many enhancers are specified by specific histone marks that may contribute to cellular memory to determine the timing and place of gene transcription.[31,36,37] A typical enhancer has characteristics of p300/CBP, binding, H3K4me1 enrichment and transcription factor binding sites.[60] The *TIL16* element has all of these characteristics, including enrichment of H3K4me1, recruitment of p300/CBP, and T-cell specific c-Maf occupancy. It is conceivable that c-Maf confers a T-cell specific *TAL1* promoter interaction that leads to an aberrant *TAL1* activation in T-ALL (Figure 8e). There is a strong correlation between the presence of p300 and enhancer function in the human genome[37,50], and enhancer-targeted p300/CBP is able to interact with TATA-binding protein and other components of TFIID,[51] thereby bridging tissue-specific transcription factors with the Pol II holoenzyme to regulate assembly of a tissue- or cell-type specific transcription complex at promoters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We are grateful to members of the Huang laboratory for their suggestions and comments. We thank Drs Christopher Cogle and Zhixiong Xu for generously providing T-ALL samples and for their advice on the 4C assay. This work was supported by grants from the National Institute of Health (SH, R01HL090589, R01HL091929 and R01HL091929-01A1S1-the ARRA Administrative supplement; YQ, R01HL095674; BP, 5T32CA009126-35). KZ is supported by the Intramural Research programs, the National Heart Lung Blood Institute, and the National Institute of Health.

REFERENCES

1. Aplan PD, Begley CG, Bertness V, Nussmeier M, Ezquerra A, Coligan J et al. The SCL gene is formed from a transcriptionally complex locus. Mol Cell Biol 1990; 10: 6426–6435. [PubMed: 2247063]
2. Begley CG, Aplan PD, Davey MP, Nakahara K, Tchorz K, Kurtzberg J et al. Chromosomal translocation in a human leukemic stem-cell line disrupts the T-cell antigen receptor delta-chain diversity region and results in a previously unreported fusion transcript. Proc Natl Acad Sci USA 1989; 86: 2031–2035. [PubMed: 2467296]
3. Chen Q, Yang CY, Tsan JT, Xia Y, Ragab AH, Peiper SC et al. Coding sequences of the tal-1 gene are disrupted by chromosome translocation in human T cell leukemia. J Exp Med 1990; 172: 1403–1408. [PubMed: 2230650]
4. Finger LR, Kagan J, Christopher G, Kurtzberg J, Hershfield MS, Nowell PC et al. Involvement of the TCL5 gene on human chromosome 1 in T-cell leukemia and melanoma. Proc Natl Acad Sci USA 1989; 86: 5039–5043. [PubMed: 2740341]
5. Robb L, Lyons I, Li R, Hartley L, Kontgen F, Harvey RP et al. Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. Proc Natl Acad Sci USA 1995; 92: 7075–7079. [PubMed: 7624372]
6. Shivdasani RA, Mayer EL, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. Nature 1995; 373: 432–434. [PubMed: 7830794]

7. Robb L, Elwood NJ, Elefanty AG, Kontgen F, Li R, Barnett LD et al. The scl gene product is required for the generation of all hematopoietic lineages in the adult mouse. Embo J 1996; 15: 4123–4129. [PubMed: 8861941]
8. Porcher C, Swat W, Rockwell K, Fujiwara Y, Alt FW, Orkin SH. The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. Cell 1996; 86: 47–57. [PubMed: 8689686]
9. Hu X, Ybarra R, Qiu Y, Bungert J, Huang S. Transcriptional regulation by TAL1: a link between epigenetic modifications and erythropoiesis. Epigenetics 2009; 4: 357–361. [PubMed: 19736520]
10. Cardoso BA, de Almeida SF, Laranjeira AB, Carmo-Fonseca M, Yunes JA, Coffer PJ et al. TAL1/SCL is downregulated upon histone deacetylase inhibition in T-cell acute lymphoblastic leukemia cells. Leukemia 2011; 25: 1578–1586. [PubMed: 21647153]
11. Hu G, Schones DE, Cui K, Ybarra R, Northrup D, Tang Q et al. Regulation of nucleosome landscape and transcription factor targeting at tissue-specific enhancers by BRG1. Genome Res 2011; 21: 1650–1658. [PubMed: 21795385]
12. Dhami P, Bruce AW, Jim JH, Dillon SC, Hall A, Cooper JL et al. Genomic approaches uncover increasing complexities in the regulatory landscape at the human SCL (TAL1) locus. PLoS ONE 2010; 5: e9059. [PubMed: 20140202]
13. Ogilvy S, Ferreira R, Piltz SG, Bowen JM, Gottgens B, Green AR. The SCL + 40 enhancer targets the midbrain together with primitive and definitive hematopoiesis and is regulated by SCL and GATA proteins. Mol Cell Biol 2007; 27: 7206–7219. [PubMed: 17709394]
14. Delabesse E, Ogilvy S, Chapman MA, Piltz SG, Gottgens B, Green AR. Transcriptional regulation of the SCL locus: identification of an enhancer that targets the primitive erythroid lineage in vivo. Mol Cell Biol 2005; 25: 5215–5225. [PubMed: 15923636]
15. Gottgens B, Barton LM, Chapman MA, Sinclair AM, Knudsen B, Grafham D et al. Transcriptional regulation of the stem cell leukemia gene (SCL)--comparative analysis of five vertebrate SCL loci. Genome Res 2002; 12: 749–759. [PubMed: 11997341]
16. Gottgens B, Barton LM, Gilbert JG, Bench AJ, Sanchez MJ, Bahn S et al. Analysis of vertebrate SCL loci identifies conserved enhancers. Nat Biotechnol 2000; 18: 181–186. [PubMed: 10657125]
17. Follows GA, Dhami P, Gottgens B, Bruce AW, Campbell PJ, Dillon SC et al. Identifying gene regulatory elements by genomic microarray mapping of DNaseI hypersensitive sites. Genome Res 2006; 16: 1310–1319. [PubMed: 16963707]
18. Silberstein L, Sanchez MJ, Socolovsky M, Liu Y, Hoffman G, Kinston S et al. Transgenic analysis of the stem cell leukemia + 19 stem cell enhancer in adult and embryonic hematopoietic and endothelial cells. Stem Cells 2005; 23: 1378–1388. [PubMed: 16051983]
19. Gottgens B, Gilbert JG, Barton LM, Grafham D, Rogers J, Bentley DR et al. Long-range comparison of human and mouse SCL loci: localized regions of sensitivity to restriction endonucleases correspond precisely with peaks of conserved noncoding sequences. Genome Res 2001; 11: 87–97. [PubMed: 11156618]
20. Bockamp EO, McLaughlin F, Murrell AM, Gottgens B, Robb L, Begley CG et al. Lineage-restricted regulation of the murine SCL/TAL-1 promoter. Blood 1995; 86: 1502–1514. [PubMed: 7632958]
21. Gottgens B, Broccardo C, Sanchez MJ, Deveaux S, Murphy G, Gothert JR et al. The scl + 18/19 stem cell enhancer is not required for hematopoiesis: identification of a 5' bifunctional hematopoietic-endothelial enhancer bound by Fli-1 and Elf-1. Mol Cell Biol 2004; 24: 1870–1883. [PubMed: 14966269]
22. Brown L, Cheng JT, Chen Q, Siciliano MJ, Crist W, Buchanan G et al. Site-specific recombination of the tal-1 gene is a common occurrence in human T cell leukemia. Embo J 1990; 9: 3343–3351. [PubMed: 2209547]
23. Bash RO, Hall S, Timmons CF, Crist WM, Amylon M, Smith RG et al. Does activation of the TAL1 gene occur in a majority of patients with T-cell acute lymphoblastic leukemia? A pediatric oncology group study. Blood 1995; 86: 666–676. [PubMed: 7605997]
24. Carroll AJ, Crist WM, Link MP, Amylon MD, Pullen DJ, Ragab AH et al. The t(1;14)(p34;q11) is nonrandom and restricted to T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood 1990; 76: 1220–1224. [PubMed: 2400810]

25. Condorelli GL, Facchiano F, Valtieri M, Proietti E, Vitelli L, Lulli V et al. T-cell-directed TAL-1 expression induces T-cell malignancies in transgenic mice. Cancer Res 1996; 56: 5113–5119. [PubMed: 8912842]
26. Kelliher MA, Seldin DC, Leder P. Tal-1 induces T cell acute lymphoblastic leukemia accelerated by casein kinase IIalpha. Embo J 1996; 15: 5160–5166. [PubMed: 8895560]
27. Palii CG, Perez-Iratxeta C, Yao Z, Cao Y, Dai F, Davison J et al. Differential genomic targeting of the transcription factor TAL1 in alternate haematopoietic lineages. Embo J 2011; 30: 494–509. [PubMed: 21179004]
28. Hu X, Li X, Valverde K, Fu X, Noguchi C, Qiu Y et al. LSD1-mediated epigenetic modification is required for TAL1 function and hematopoiesis. Proc Natl Acad Sci USA 2009; 106: 10141–10146. [PubMed: 19497860]
29. Li X, Wang S, Li Y, Deng C, Steiner LA, Xiao H et al. Chromatin boundaries require functional collaboration between the hSET1 and NURF complexes. Blood 2011; 118: 1386–1394. [PubMed: 21653943]
30. Huang S, Litt M, Felsenfeld G. Methylation of histone H4 by arginine methyltransferase PRMT1 is essential *in vivo* for many subsequent histone modifications. Genes Dev 2005; 19: 1885–1893. [PubMed: 16103216]
31. Cui K, Zang C, Roh TY, Schones DE, Childs RW, Peng W et al. Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. Cell Stem Cell 2009; 4: 80–93. [PubMed: 19128795]
32. Li X, Hu X, Patel B, Zhou Z, Liang S, Ybarra R et al. H4R3 methylation facilitates beta-globin transcription by regulating histone acetyltransferase binding and H3 acetylation. Blood 2010; 115: 2028–2037. [PubMed: 20068219]
33. Hagege H, Klous P, Braem C, Splinter E, Dekker J, Cathala G et al. Quantitative analysis of chromosome conformation capture assays (3C-qPCR). Nature protocols 2007; 2: 1722–1733. [PubMed: 17641637]
34. Abou El Hassan M, Bremner R. A rapid simple approach to quantify chromosome conformation capture. Nucleic Acids Res 2009; 37: e35. [PubMed: 19181703]
35. Gondor A, Rougier C, Ohlsson R. High-resolution circular chromosome conformation capture assay. Nat Protoc 2008; 3: 303–313. [PubMed: 18274532]
36. Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 2009; 459: 108–112. [PubMed: 19295514]
37. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 2007; 39: 311–318. [PubMed: 17277777]
38. Li Y, Deng C, Hu X, Patel B, Fu X, Qiu Y et al. Dynamic interaction between TAL1 oncoprotein and LSD1 regulates TAL1 function in hematopoiesis and leukemogenesis. Oncogene 2012; 31: 5007–5018. [PubMed: 22310283]
39. Li G, Ruan X, Auerbach RK, Sandhu KS, Zheng M, Wang P et al. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell 2012; 148: 84–98. [PubMed: 22265404]
40. Sanyal A, Lajoie BR, Jain G, Dekker J. The long-range interaction landscape of gene promoters. Nature 2012; 489: 109–113. [PubMed: 22955621]
41. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z et al. High-resolution profiling of histone methylations in the human genome. Cell 2007; 129: 823–837. [PubMed: 17512414]
42. Follows GA, Ferreira R, Janes ME, Spensberger D, Cambuli F, Chaney AF et al. Mapping and functional characterisation of a CTCF-dependent insulator element at the 3' border of the murine Scl transcriptional domain. PLoS ONE 2012; 7: e31484. [PubMed: 22396734]
43. Wallace JA, Felsenfeld G. We gather together: insulators and genome organization. Curr Opin Genet Dev 2007; 17: 400–407. [PubMed: 17913488]
44. Phillips JE, Corces VG. CTCF: master weaver of the genome. Cell 2009; 137: 1194–1211. [PubMed: 19563753]

45. Huang Y, Sitwala K, Bronstein J, Sanders D, Dandekar M, Collins C et al. Identification and characterization of Hoxa9 binding sites in hematopoietic cells. Blood 2012; 119: 388–398. [PubMed: 22072553]
46. Kim JI, Ho IC, Grusby MJ, Glimcher LH. The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity 1999; 10: 745–751. [PubMed: 10403649]
47. Cao S, Liu J, Song L, Ma X. The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. J Immunol 2005; 174: 3484–3492. [PubMed: 15749884]
48. Murakami YI, Yatabe Y, Sakaguchi T, Sasaki E, Yamashita Y, Morito N et al. c-Maf expression in angioimmunoblastic T-cell lymphoma. Am J Surg Pathol 2007; 31: 1695–1702. [PubMed: 18059226]
49. Morito N, Yoh K, Fujioka Y, Nakano T, Shimohata H, Hashimoto Y et al. Overexpression of c-Maf contributes to T-cell lymphoma in both mice and human. Cancer Res 2006; 66: 812–819. [PubMed: 16424013]
50. Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447: 799–816. [PubMed: 17571346]
51. Mitsiou DJ, Stunnenberg HG. p300 is involved in formation of the TBP-TFIIA-containing basal transcription complex, TAC. Embo J 2003; 22: 4501–4511. [PubMed: 12941701]
52. Bernard O, Azogui O, Lecointe N, Mugneret F, Berger R, Larsen CJ et al. A third tal-1 promoter is specifically used in human T cell leukemias. J Exp Med 1992; 176: 919–925. [PubMed: 1402666]
53. Schubeler D, Francastel C, Cimbora DM, Reik A, Martin DI, Groudine M. Nuclear localization and histone acetylation: a pathway for chromatin opening and transcriptional activation of the human beta-globin locus. Genes Dev 2000; 14: 940–950. [PubMed: 10783166]
54. Song SH, Hou C, Dean A. A positive role for NLI/Ldb1 in long-range beta-globin locus control region function. Mol Cell 2007; 28: 810–822. [PubMed: 18082606]
55. Vakoc CR, Letting DL, Gheldof N, Sawado T, Bender MA, Groudine M et al. Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. Mol Cell 2005; 17: 453–462. [PubMed: 15694345]
56. Yusufzai TM, Tagami H, Nakatani Y, Felsenfeld G. CTCF tethers an insulator to subnuclear sites, suggesting shared insulator mechanisms across species. Mol Cell 2004; 13: 291–298. [PubMed: 14759373]
57. Aplan PD, Raimondi SC, Kirsch IR. Disruption of the SCL gene by a t(1;3) translocation in a patient with T cell acute lymphoblastic leukemia. J Exp Med 1992; 176: 1303–1310. [PubMed: 1402676]
58. Breit TM, Mol EJ, Wolvers-Tettero IL, Ludwig WD, van Wering ER, van Dongen JJ. Site-specific deletions involving the tal-1 and sil genes are restricted to cells of the T cell receptor alpha/beta lineage: T cell receptor delta gene deletion mechanism affects multiple genes. J Exp Med 1993; 177: 965–977. [PubMed: 8459224]
59. Aplan PD, Lombardi DP, Ginsberg AM, Cossman J, Bertness VL, Kirsch IR. Disruption of the human SCL locus by “illegitimate” V-(D)-J recombinase activity. Science 1990; 250: 1426–1429. [PubMed: 2255914]
60. Ong CT, Corces VG. Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat Rev Genet 2011; 12: 283–293. [PubMed: 21358745]

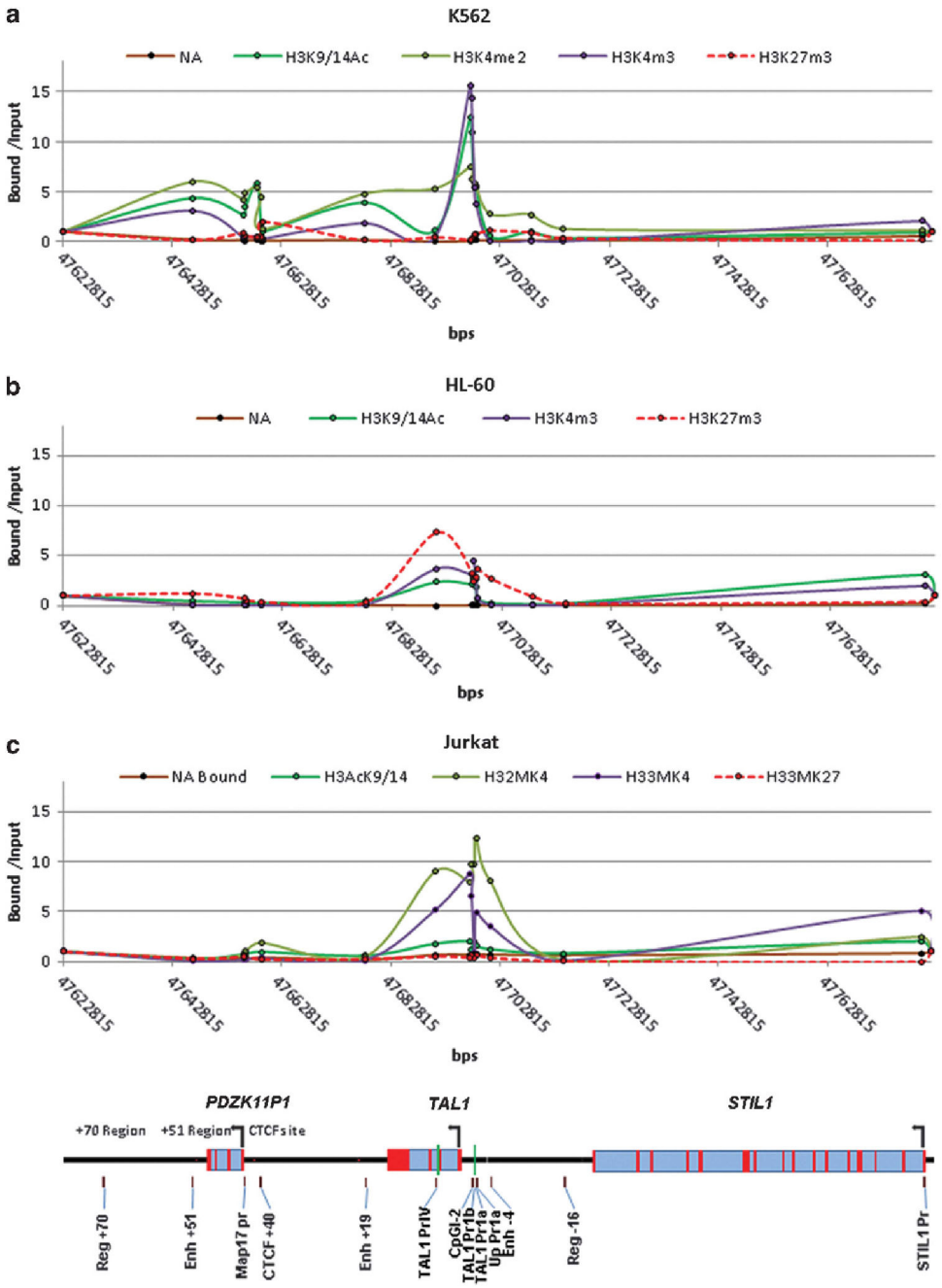

Figure 1.

Distinct histone modification patterns are associated with cell-type-specific enhancer/promoter activities. ChIP analysis of histone modifications across the *STIL-TAL1-MAP17* loci in K562 (**a**), HL-60 (**b**) and Jurkat (**c**) cells.

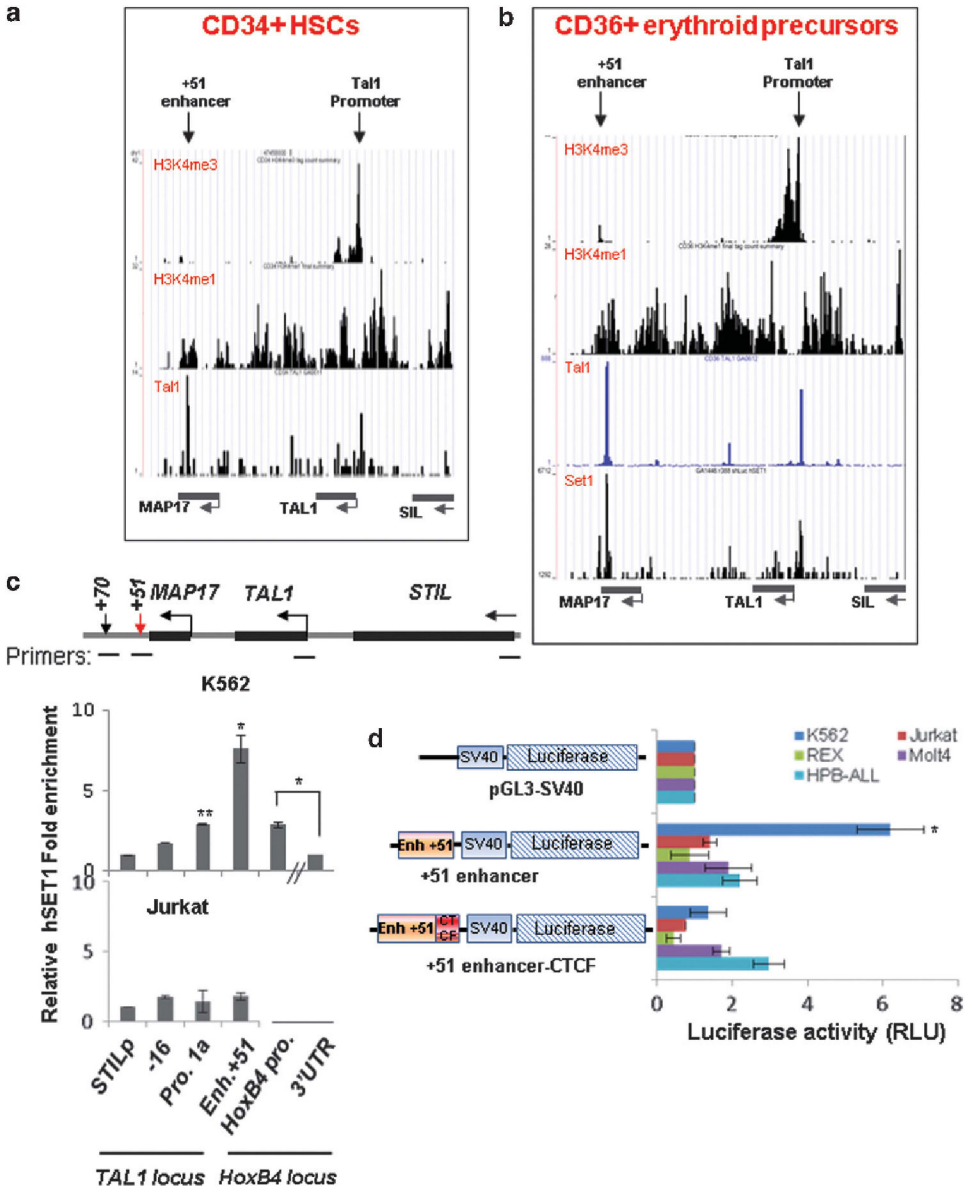

Figure 2.

The + 51 enhancer activates *TAL1* promoter 1 in the erythroid lineage by recruiting the hSET1 HMT complex. (**a**) ChIP-seq analyses of H3K4me1, H3K4me3 and TAL1 binding at the *TAL1* locus in CD34^{+} HSCs. (**b**) ChIP-seq analyses of TAL1 binding, hSET1 recruitment, H3K4me1 and H3K4me3 at the *TAL1* locus in CD36^{+} erythroid precursors. (**c**) ChIP analysis of hSET1 recruitment at the *TAL1* locus in K562 cells (top) and T-ALL Jurkat (bottom) cells. Data are shown as mean ± s.d. **P* < 0.05; ***P* < 0.01. (**d**) K562 and several T-ALL cells were transfected with a control pGL3-SV40 luciferase reporter, a + 51 enhancer-driven pGL3-SV40 luciferase reporter, or a + 51 enhancer-driven pGL3-SV40 luciferase reporter containing a + 53 CTCF site inserted between the + 51 enhancer and the SV40 promoter. A CMV-driven renilla luciferase plasmid was used as a transfection control. Transfected cells were cultured for 48 h and lysed for the measurement of luciferase activity (RLU). Data are shown as mean ± s.d. **P* < 0.05.

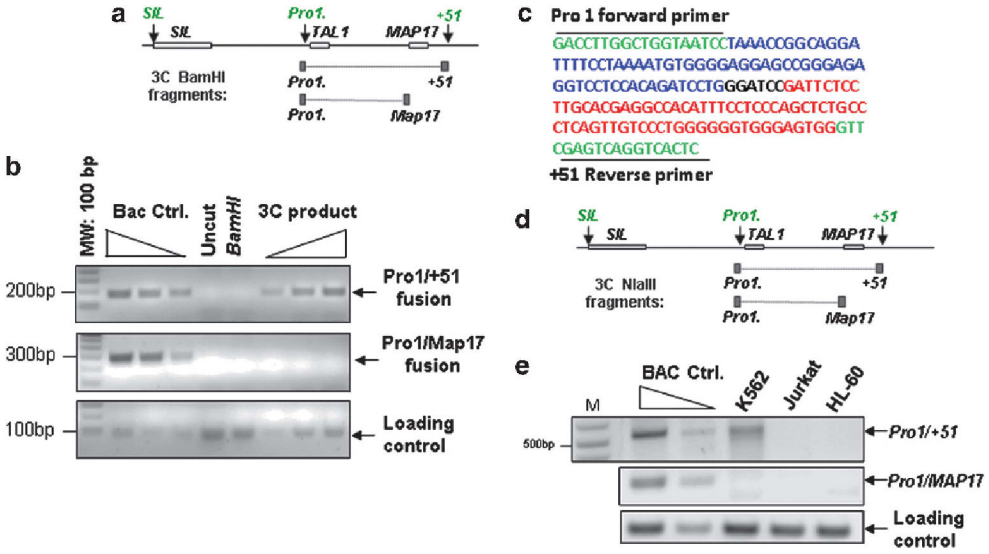

Figure 3.

The *TAL1* enhancer and promoter interact through a long-range chromatin loop in erythroid cells. (**a**) Schematic representation of the *TAL1* locus. The *STIL, TAL1* and *MAP17* genes are indicated by gray boxes, and promoters and enhancers are indicated by arrows. The predicted 3C *BamHI* restriction fragments are shown in black boxes. (**b**) 3C analysis of the interaction between *TAL1* promoter 1 and the + 51 enhancer in $CD36^{+}$ erythroid precursors. (**c**) Sequencing analysis of the + 51/*TAL1* promoter 1 3C fusion product. (**d**) Schematic representation of the *TAL1* locus and the predicted 3C *NlaIII* restriction fragments. (**e**) 3C analysis of the interaction between *TAL1* promoter 1 and the + 51 enhancer in K562, Jurkat and HL-60 cells.

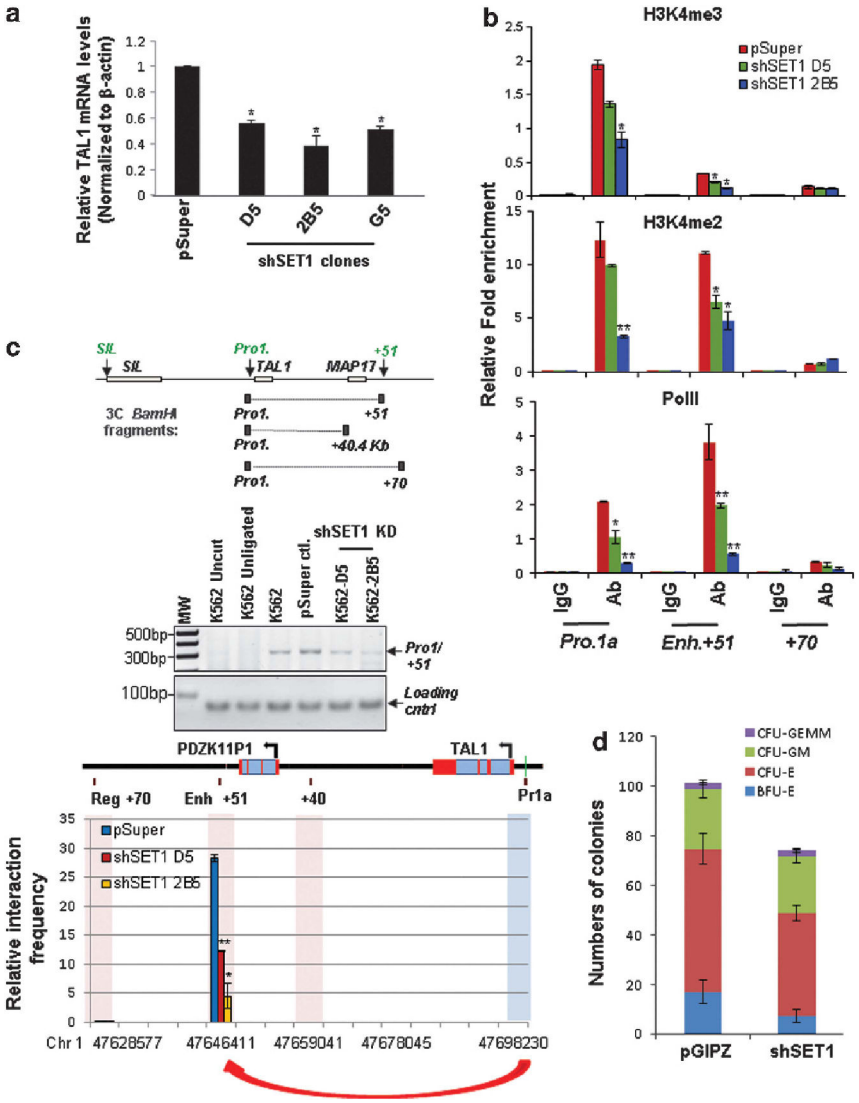

Figure 4.

Loss of hSET1 recruitment disrupts the erythroid-specific enhancer/promoter long-range interaction in the *TAL1* locus. (**a**) Real-time reverse transcription-quantitative PCR (RT-qPCR) analyses of *TAL1* mRNA transcript levels in the vector control and three KD clones harboring shRNA specific for hSET1. Data are shown as mean ± s.d. **P* < 0.05. (**b**) ChIP analyses of H3K4me2 and me3 levels as well as RNA PolII recruitment at the + 51 enhancer and *TAL1* promoter 1 upon hSET1 KD. Data are shown as mean ± s.d. **P* < 0.05; ***P* < 0.01. (**c**) The 3C analysis of the interaction between the + 51 enhancer and *TAL1* promoter 1 in the *TAL1* locus in the vector control and shSET1-transduced clones. (Top) Shown is gel electrophoresis of the 3C PCR products. (bottom) Shown is real-time qPCR quantitation of the 3C products. Data are shown as mean ± s.d. of three independent experiments. **P* < 0.05; ***P* < 0.01. (**d**) CFC assay of $CD34^+$ HSCs transduced with the vector control and shRNA-targeting hSET1.

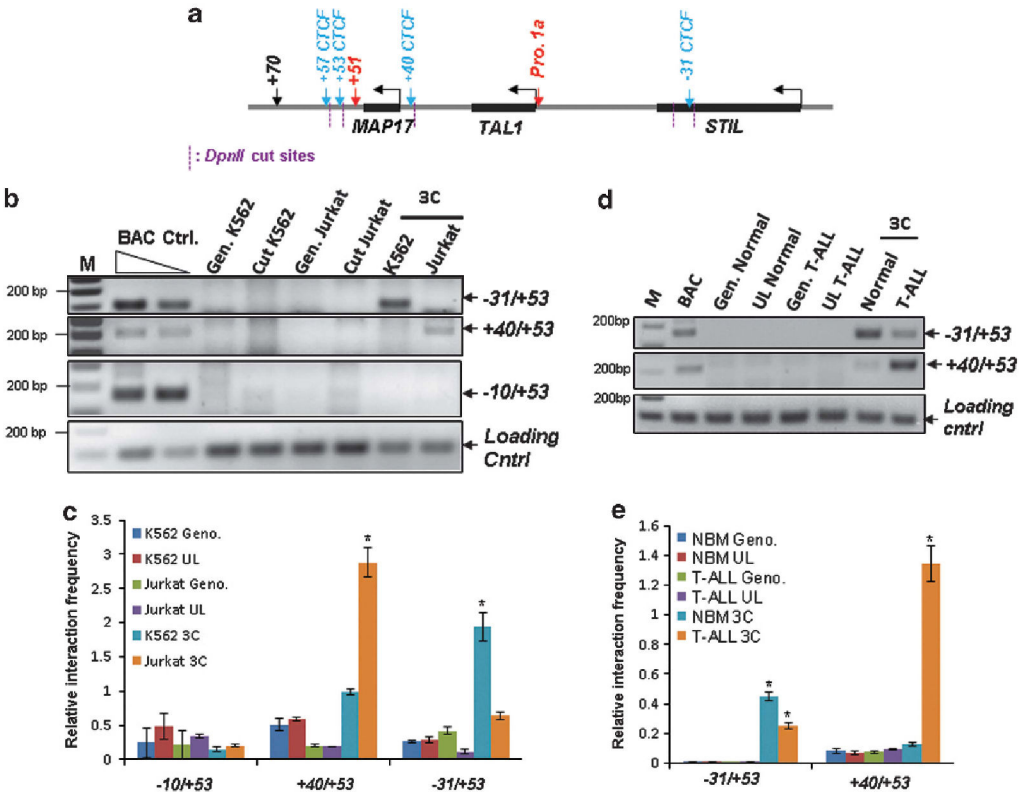

Figure 5.

CTCF-mediated genome organization regulates enhancer and promoter interaction in the *TAL1* locus. (**a**) Schematic representation of the *TAL1* locus and *CTCF* sites is indicated by blue arrows. The predicted *DpnII* restriction fragments are indicated by magenta dashed lines. (**b**) The 3C analysis of long-range genome interaction among *CTCF* sites in the *TAL1* locus comparing K562 and Jurkat cells. (**c**) A total of three independent 3C experiments were quantitated by densitometry. Data are shown as mean ± s.d. of three independent experiments. **P* < 0.05. (**d**) The 3C analysis of long-range genome interaction among *CTCF* sites in the *TAL1* locus comparing normal BM and T-ALL patient BM cells without chromosomal rearrangement detected in the *TAL1* locus. (**e**) A total of three independent 3C experiments were quantitated by densitometry. Data are shown as mean ± s.d. of three independent experiments. **P* < 0.05.

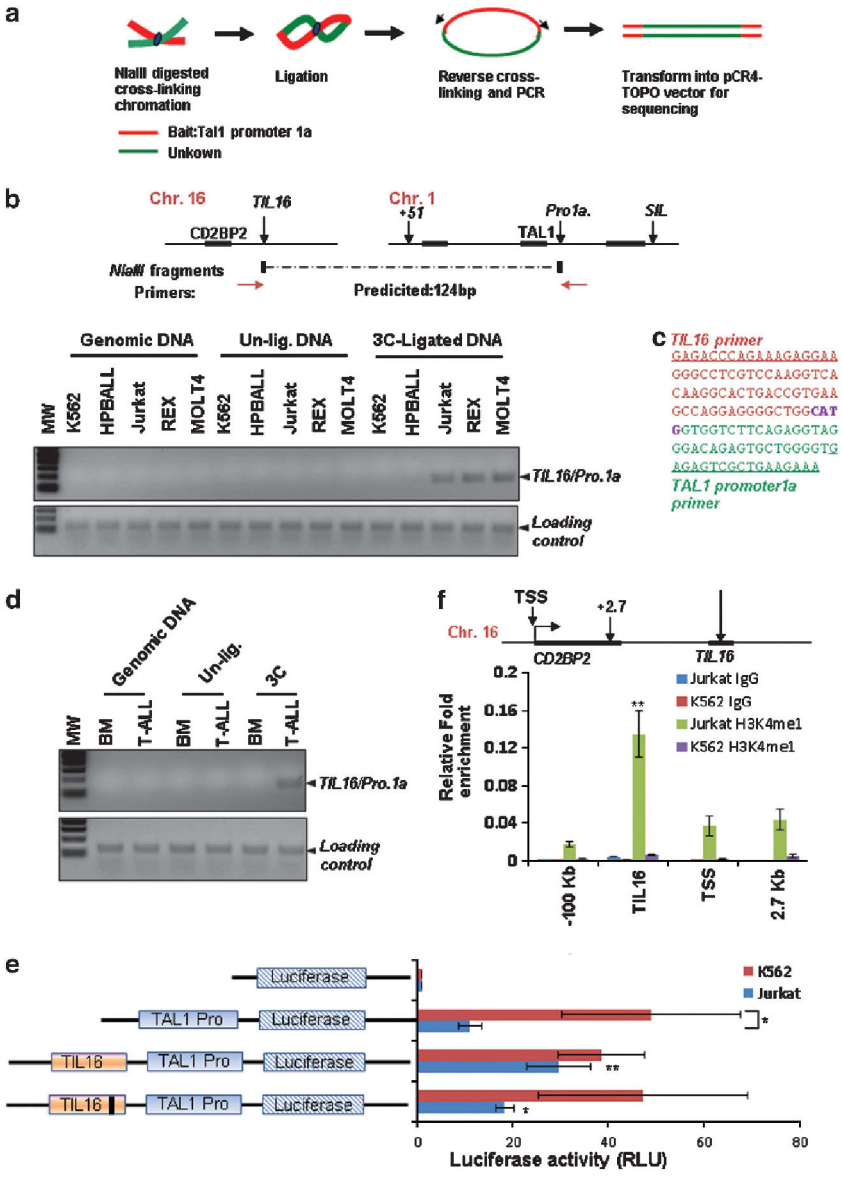

Figure 6.

A T-cell-specific DNA element interacts with and activates the *TAL1* promoter 1 via an interchromosomal interaction in leukemic T cells. (**a**) Schematic representation of the 4C procedure using *TAL1* promoter 1 as bait. (**b**) The 3C analysis confirming the interchromosomal interaction identified by 4C experiments in K562 and a variety of T-ALL cell lines. (**c**) The predicted 3C product was confirmed by DNA sequencing (right). (**d**) 3C analysis of the interchromosomal interaction between *TAL1* promoter 1 on chromosome 1 and the *TIL16* element on chromosome 16 comparing normal BM and T-ALL patient BM cells. (**e**) K562 and Jurkat cells were transfected with the *TAL1* promoter 1a-driven luciferase reporter, a *TIL16* element linked to the *TAL1* promoter 1-luciferase reporter, or a c-Maf site-deleted *TIL16* element linked to the *TAL1* promoter 1-driven luciferase reporter. A CMV-driven renilla luciferase plasmid was used as transfection control. Transfected cells were cultured for 48 h and lysed for the measurement of luciferase activity. Data are shown

as mean ± s.d. *$P < 0.05$; **$P < 0.01$. (**f**) ChIP analysis of the enrichment of characteristic enhancer histone marker H3K4me1 in the *CD2BP2* locus in Jurkat and K562 cells. Data are shown as mean ± s.d. **$P < 0.01$.

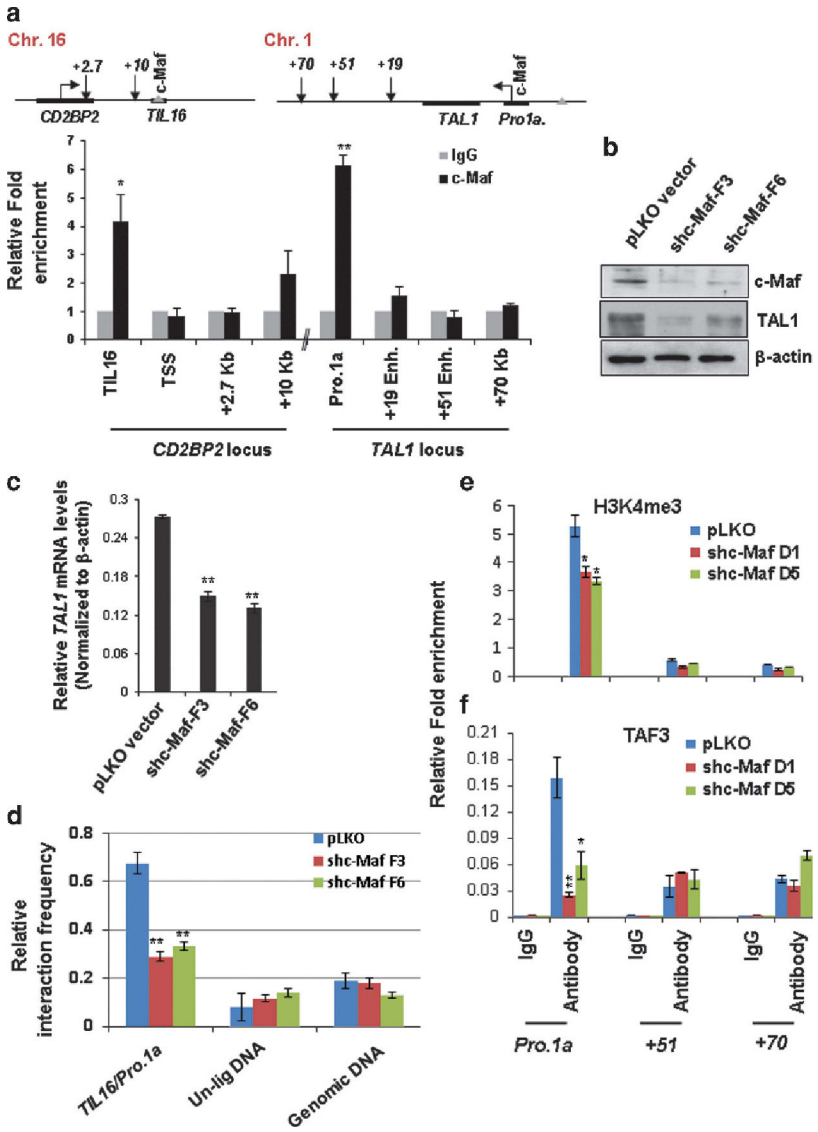

Figure 7.

KD of *c-Maf* leads to disruption of the interchromosomal interaction and decrease in *TAL1* transcription in T-ALL cells. (**a**) ChIP analysis of T-cell-specific transcription factor c-Maf binding in the *CD2BP2* and *TAL1* loci in Jurkat cells. Data are shown as mean ± s.d. of three independent experiments. **P* < 0.05; ***P* < 0.01. (**b**, **c**) Western blot (**b**) and RT-qPCR (**c**) analyses of *TAL1* expression in the vector control and two *c-Maf* KD clones. (**d**) 3C analysis of interchromosomal interaction between *TAL1* promoter 1 and the *TIL16* element comparing the vector control and *c-Maf* KD Jurkat clones. A total of three independent 3C experiments were quantitated by densitometry. (**e**, **f**) ChIP analyses of H3K4me3 levels (**e**) as well as TAF3 recruitment (**f**) at the + 51 enhancer and *TAL1* promoter 1 upon *c-Maf* KD. Data are shown as mean ± s.d. of three independent experiments. **P* < 0.05; ***P* < 0.01.

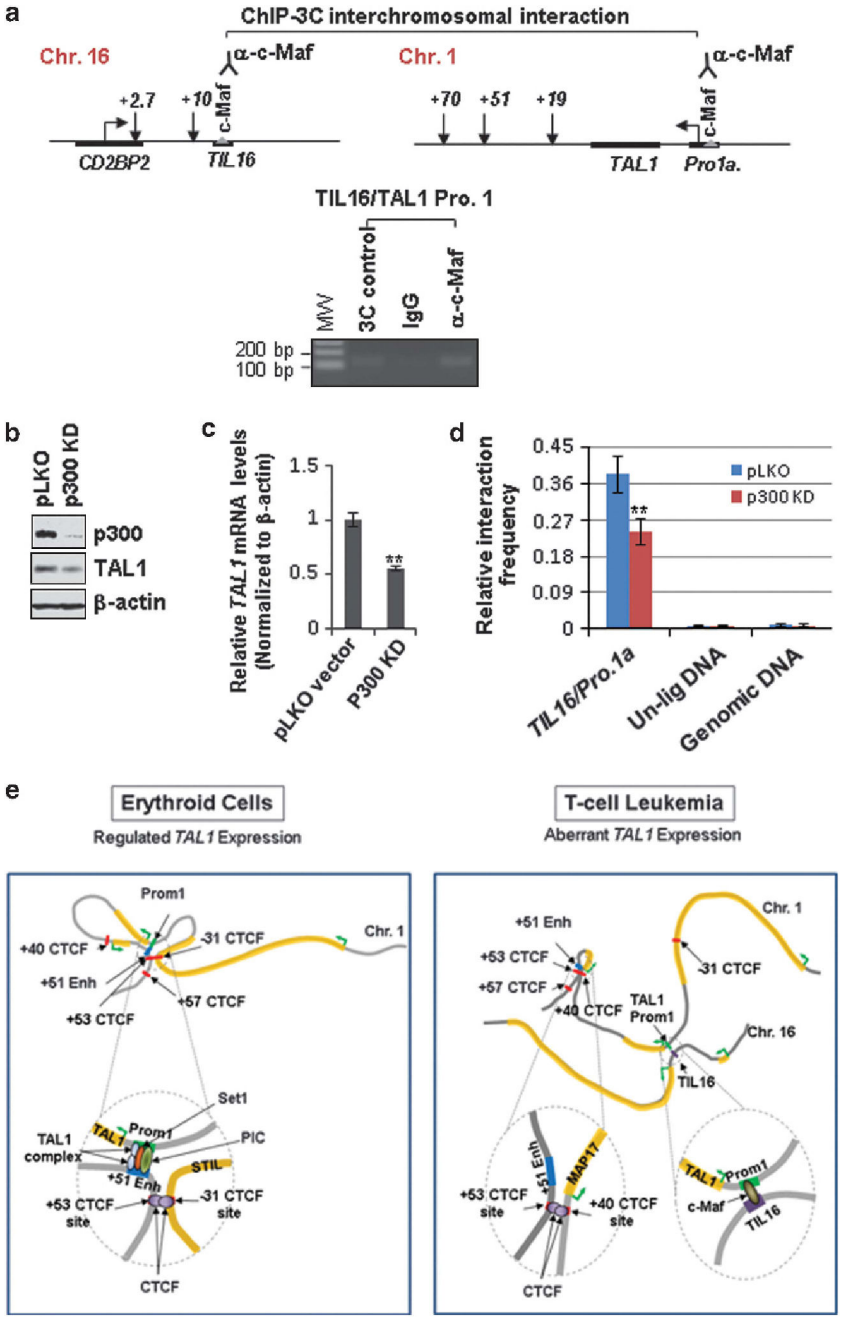

Figure 8.

c-Maf anchors interchromosomal interaction between the *TIL16* element and the *TAL1* promoter in aberrant TAL1-expressed T-ALL cells. (**a**) The ChIP-3C assay was carried out in the *NlaIII*-digested Jurkat chromatin. The chromatin was selected with antibodies specific to c-Maf or control immunoglobulin G (IgG), followed by ligation and PCR amplification using the *TIL16* and *TAL1* promoter primers. (**b**, **c**) Western blot (**b**) and RT-qPCR (**c**) analyses of *TAL1* expression in the vector control and *p300* KD pool. (**d**) 3C analysis of interchromosomal interaction between *TAL1* promoter 1 and the *TIL16* element comparing the vector control and *p300* KD Jurkat cells. A total of three independent 3C experiments

were quantitated by densitometry. Data are shown as mean ± s.d. of three independent experiments. $^{**}P < 0.01$. (**e**) A model depicting that cell-type-specific *TAL1* transcription is regulated by CTCF, transcription factors and cofactor-mediated intra- or interchromosomal loops.