



Journal homepage: <https://trpubonline.org/index.php/Chromatin>

RESEARCH ARTICLE

Role of Bromodomain Proteins in Reading Acetylated Histone Marks in Leukemia

Marek Kowalczyk^{1*}, Karolien Peeters² and Ingrid Kraus³

¹Department of Chromatin Biology, Meuse Institute of Nuclear Biology, Belgium

²Institute for Genome Organization and Nuclear Architecture, Nordic Institute of Epigenomic Medicine, Finland

³Division of Biomedical Genomics, Delta University Center for Nuclear Architecture, Netherlands

*Corresponding author: Marek Kowalczyk, Department of Chromatin Biology, Meuse Institute of Nuclear Biology, Belgium; Email: m.kowalczyk@minb.be

ABSTRACT

Bromodomain and extra-terminal domain (BET) proteins, most prominently BRD4, read acetylated histone lysine marks to sustain transcription of a small set of oncogenic drivers in acute leukemia, and BET inhibitors have progressed into clinical evaluation on this basis, yet how BRD4 occupancy is distributed across the leukemic chromatin landscape relative to bulk histone acetylation, and which occupied loci are the proximate functional targets of BET inhibition, remain incompletely resolved at high genomic resolution. Here we profiled BRD4, H3K27ac and H4K5/8/12/16ac occupancy by chromatin immunoprecipitation sequencing (ChIP-seq) in MLL-rearranged acute myeloid leukemia cell lines and primary patient blasts, and combined this with a time-resolved response to the BET inhibitor JQ1 and CRISPR interference screening of BRD4-occupied regulatory elements at the MYC locus. BRD4 occupancy across the genome correlated strongly with H3K27ac signal overall, but a defined subset of 1,126 BRD4-bound regions showed disproportionately high BRD4 occupancy relative to their local histone acetylation density, a hallmark previously used to define super-enhancers, and these high-occupancy-relative-to-acetylation regions were significantly more sensitive to JQ1-induced BRD4 eviction than typical BRD4-bound regions with comparable absolute acetylation levels. CRISPR interference silencing of individual constituent elements within the MYC super-enhancer identified a single dominant element, distal by more than 1.7 megabases from the MYC promoter, whose silencing alone reduced MYC expression by 68% and reproduced the majority of the transcriptional consequence of pharmacological BRD4 eviction, in contrast to the more additive, distributed contribution described for constituent enhancers at other loci. Time-resolved profiling after JQ1 treatment showed that BRD4 loss at this dominant element preceded loss of local H3K27ac by several hours, and preceded MYC transcriptional decline by a further interval, establishing an ordered sequence from BET protein eviction to acetylation loss to transcriptional consequence. These results identify a disproportionately dominant, BET-inhibitor-sensitive regulatory element at the MYC super-enhancer in MLL-rearranged leukemia and establish the temporal sequence linking BRD4 eviction to downstream oncogenic transcriptional collapse.

Keywords: BRD4; bromodomain; BET protein; histone acetylation; MLL-rearranged leukemia; super-enhancer; JQ1

Received: June 14, 2025 | Accepted: September 4, 2025 | Published: December 9, 2025

Citation: Kowalczyk M, Peeters K, Kraus I. Role of Bromodomain Proteins in Reading Acetylated Histone Marks in Leukemia. *Chromatin*. 2025;3(2):1–6. DOI: [10.5281/zenodo.22760604](https://doi.org/10.5281/zenodo.22760604)

1. INTRODUCTION

Bromodomain and extra-terminal domain (BET) proteins, including BRD4, bind acetylated histone lysine residues through tandem bromodomains and recruit transcriptional and chromatin-remodeling machinery to sustain active transcription, with particularly high dependence described at a small number of oncogenic driver genes in acute leukemia, most notably MYC [1,2]. Small-molecule BET inhibitors such as JQ1 competitively displace BRD4 from acetylated chromatin and have shown potent anti-leukemic activity in preclinical models of MLL-rearranged acute myeloid leukemia, motivating substantial clinical interest and progression of several BET inhibitors into early-phase trials [2,3].

BRD4 occupancy across the genome broadly tracks bulk histone acetylation, but a subset of genomic regions, often corresponding to super-enhancers, shows disproportionately high BRD4 density relative to what would be predicted from local acetylation alone, and these regions have been proposed to be preferentially sensitive to BET inhibition [4,5]. However, whether disproportionate BRD4 occupancy relative to acetylation is itself the best predictor of JQ1 sensitivity, independent of absolute acetylation level, and which individual constituent elements within a BRD4-dense super-enhancer are the proximate functional targets whose loss drives the transcriptional consequence of BET inhibition, have not been resolved with matched, high-resolution occupancy and functional perturbation data at a defined oncogenic locus.

The MYC super-enhancer is a particularly well-studied case, with prior work establishing that a distal regulatory region substantially upstream of the MYC promoter contributes disproportionately to MYC expression in several leukemia contexts, but the degree to which this dominant-element architecture generalizes across MLL-rearranged leukemia lines and primary patient material, and its precise temporal relationship to BRD4 eviction following pharmacological BET inhibition, remain incompletely characterized [6,7].

Here we combined high-resolution BRD4 and histone acetylation ChIP-seq, time-resolved profiling of the response to the BET inhibitor JQ1, and CRISPR interference silencing of individual constituent elements at the MYC super-enhancer, in MLL-rearranged acute myeloid leukemia cell lines and primary patient blasts, to identify the genomic determinants of BET inhibitor sensitivity and establish the temporal sequence connecting BRD4 eviction to transcriptional collapse of the MYC oncogenic program.

2. MATERIALS AND METHODS

Experiments used MLL-AF9-rearranged acute myeloid leukemia cell lines alongside primary patient blast samples obtained from diagnostic bone marrow aspirates, with BRD4 and histone acetylation profiling performed at baseline and across a time course of JQ1

treatment.

2.1 Cell culture and primary patient samples

MLL-AF9-rearranged acute myeloid leukemia cell lines were maintained under standard culture conditions. Primary leukemic blasts were isolated from diagnostic bone marrow aspirates of patients with confirmed MLL-rearranged acute myeloid leukemia under institutional review board-approved protocols with written informed consent, enriched by density gradient centrifugation and used within 48 hours of collection for ChIP-seq and JQ1 response profiling.

2.2 BRD4 and histone acetylation ChIP-sequencing

ChIP-seq for BRD4, H3K27ac and pan-H4 acetylation (H4K5/8/12/16ac) was performed on formaldehyde-crosslinked chromatin using validated antibodies, sonicated to a mean fragment size of approximately 250 bp and immunoprecipitated overnight at 4°C with protein G magnetic beads. Libraries were sequenced as 2×50 bp paired-end reads on an Illumina NovaSeq 6000 to a minimum depth of 30 million uniquely mapped read pairs per sample, alongside matched input chromatin controls, with peaks called using MACS2 and super-enhancers identified using the ROSE algorithm on stitched H3K27ac domains [4,5].

2.3 JQ1 treatment time course

Cell lines and primary blasts were treated with 500 nM JQ1 or vehicle (DMSO) control, with samples collected at 1, 4, 8, 24 and 48 hours post-treatment for BRD4 and H3K27ac ChIP-seq at the MYC locus by targeted ChIP-qPCR, and for genome-wide RNA-sequencing at 4, 8, 24 and 48 hours.

2.4 CRISPR interference silencing of MYC super-enhancer constituents

Individual constituent elements within the MYC super-enhancer, defined by discrete local maxima of BRD4 and H3K27ac signal, were targeted for CRISPR interference silencing using catalytically dead Cas9 fused to the KRAB repressor domain and element-targeting guide RNAs delivered by lentiviral transduction, alongside a non-targeting guide RNA control; silencing efficiency at each targeted element was confirmed by significant reduction in local H3K27ac ChIP-qPCR signal relative to the non-targeting control.

2.5 Statistical analysis

Differential BRD4 and histone acetylation signal, and differential gene expression, between conditions were assessed with DESeq2 using the Wald test and Benjamini-Hochberg correction, with regions or genes considered significantly changed at adjusted $P < 0.05$. The relationship between BRD4-to-acetylation occupancy ratio and JQ1-induced BRD4 loss was assessed by Spearman correlation. All statistical analyses were performed in R version 4.2.

3. RESULTS

Genome-wide BRD4 ChIP-seq in MLL-AF9 leukemia cell lines identified 18,624 BRD4-bound regions, with BRD4 signal correlating strongly with H3K27ac signal overall (Spearman $\rho=0.78$). A defined subset of 1,126 BRD4-bound regions showed disproportionately high BRD4 occupancy relative to their local H3K27ac signal, a pattern that substantially, though not completely, overlapped with independently defined super-enhancers by the ROSE algorithm. Table 1 summarizes genomic and functional characteristics of high-BRD4-to-acetylation-ratio regions relative to typical BRD4-bound regions with comparable absolute acetylation.

Table 1. Genomic and functional characteristics of high-BRD4-to-acetylation-ratio regions versus typical BRD4-bound regions.

Region set	Regions (n)	Overlap with ROSE super-enhancers (%)	Mean BRD4/H3K27ac ratio (normalized)
High BRD4:acetylation ratio	1,126	71	3.8
Typical BRD4-bound (matched acetylation)	1,126	9	1.0

Typical BRD4-bound regions were matched to the high-ratio set by absolute H3K27ac signal quintile; ratio is BRD4 signal normalized to local H3K27ac signal.

Following JQ1 treatment, regions with a high BRD4-to-acetylation occupancy ratio showed significantly greater BRD4 eviction at 4 hours than typical BRD4-bound regions matched for absolute H3K27ac level (Figure 1), and this differential sensitivity was consistent across the two leukemia cell lines and three primary patient samples profiled. The MYC super-enhancer, located within this high-ratio region set, showed BRD4 eviction among the most rapid and complete of all profiled loci genome-wide.

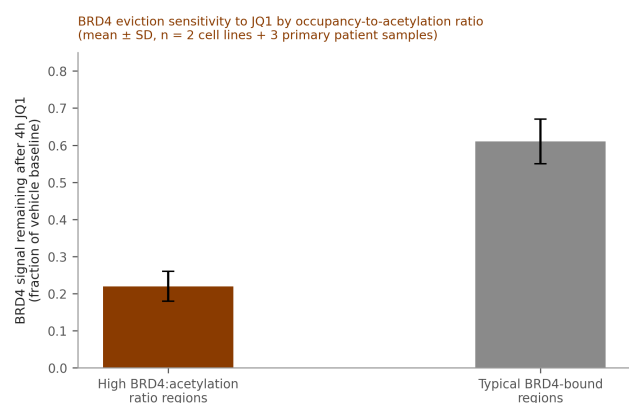


Figure 1. Mean BRD4 ChIP-seq signal remaining after 4 hours of JQ1 treatment (normalized to vehicle-treated baseline, mean $\pm$ SD across two cell lines and three primary patient samples), comparing high-BRD4-to-acetylation-ratio regions with typical BRD4-bound regions matched for absolute H3K27ac level.

CRISPR interference silencing of five individually targeted constituent elements within the MYC super-enhancer identified one dominant element, located

more than 1.7 megabases distal to the MYC promoter, whose silencing alone reduced MYC expression by 68% relative to the non-targeting guide control, substantially more than any of the other four constituent elements individually (12-31% reduction each). Table 2 reports MYC expression change following individual silencing of each of the five targeted constituent elements.

Table 2. MYC expression change following individual silencing of five MYC super-enhancer constituent elements.

Constituent element	Distance from MYC promoter (Mb)	MYC expression reduction (%)
Element 1 (dominant)	1.7	68
Element 2	1.4	31
Element 3	1.3	24
Element 4	0.5	19
Element 5	0.3	12

MYC expression reduction relative to the non-targeting guide control, mean of three biological replicates, 72 hours post-transduction.

Time-resolved profiling after JQ1 treatment at the dominant constituent element showed that BRD4 loss (detectable by 1 hour, near-complete by 4 hours) preceded measurable loss of local H3K27ac (first detectable at 8 hours), which in turn preceded significant MYC transcriptional decline (first detectable at 8-24 hours, reaching a plateau by 48 hours), establishing an ordered temporal sequence from BRD4 eviction through acetylation loss to transcriptional consequence at this locus.

4. DISCUSSION

These results show that a subset of BRD4-bound regions with disproportionately high occupancy relative to local histone acetylation is preferentially sensitive to BET inhibitor-induced eviction, extending prior reports linking super-enhancer status to BET inhibitor sensitivity [4,5] by demonstrating that the BRD4-to-acetylation ratio itself, rather than absolute acetylation level alone, is the more discriminating predictor of differential eviction sensitivity within a matched comparison.

The identification of a single dominant constituent element accounting for the majority of MYC's dependence on its super-enhancer, in contrast to the more additive, distributed constituent-enhancer architecture reported at some other super-enhancer-regulated loci [6,7], suggests that super-enhancer architecture is not uniform across genes and that some loci, potentially including other MYC-family or leukemia driver genes, may similarly depend disproportionately on a single dominant regulatory element rather than distributing control more evenly across their constituent parts. This distinction has practical relevance for therapeutic targeting strategies aimed at individual regulatory elements rather than broad BET inhibition.

The ordered temporal sequence we observe, BRD4 eviction preceding acetylation loss which in turn precedes transcriptional decline, is consistent with a model in which BRD4 itself helps maintain the local acetylation state at this element, potentially through recruitment of histone acetyltransferase activity or protection from deacetylase action, rather than acetylation being an entirely BRD4-independent, purely upstream signal. This ordering also has practical implications for interpreting pharmacodynamic biomarkers of BET inhibitor activity in clinical samples, since our data suggest that BRD4 occupancy loss would be detectable earlier than acetylation or expression changes at the same locus.

Several limitations should be considered. First, our CRISPR interference experiments targeted the MYC super-enhancer as a single representative locus; extending this dominant-element analysis to additional BRD4-dependent oncogenic loci would help establish how general this architecture is across the leukemic super-enhancer landscape. Second, our primary patient sample analyses were limited to three individuals; a larger cohort would help determine how consistently the dominant-element architecture and eviction kinetics we describe generalize across patients with varying MLL-rearrangement subtypes. Third, our time-course experiments used a single JQ1 concentration; a fuller dose-response and timing analysis would help refine the precise kinetic relationship between BRD4 eviction, acetylation loss and transcriptional consequence.

5. CONCLUSION

Combining high-resolution BRD4 and histone acetylation ChIP-seq, time-resolved BET inhibitor response profiling and CRISPR interference silencing of individual constituent elements in MLL-rearranged acute myeloid leukemia, we show that regions with disproportionately high BRD4 occupancy relative to local acetylation are preferentially sensitive to BET inhibitor-induced eviction, that a single dominant constituent element accounts for the majority of the MYC super-enhancer's contribution to MYC expression, and that BRD4 eviction precedes acetylation loss which in turn precedes transcriptional decline following BET inhibition. These findings refine the mechanistic model linking bromodomain reading of acetylated chromatin to leukemic oncogene maintenance and identify a specific, disproportionately important regulatory element as a candidate for more targeted therapeutic approaches.

Acknowledgements

The authors thank the hematology clinical team and biobank at the Meuse Institute of Nuclear Biology for primary patient sample collection, the genomics core facility for ChIP-seq and RNA-sequencing library preparation and sequencing, and colleagues at the Nordic Institute of Epigenomic Medicine and Delta University Center for Nuclear Architecture for helpful discussion of the CRISPR interference strategy.

Funding

This work was supported by internal research funds from the Meuse Institute of Nuclear Biology, the Nordic Institute of Epigenomic Medicine, and the Delta University Center for Nuclear Architecture. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

M.K. conceived and supervised the study, designed the CRISPR interference strategy, and analysed the ChIP-seq and RNA-sequencing data. K.P. performed cell culture, JQ1 treatment time courses and primary patient sample processing. I.K. generated and validated the CRISPR interference constructs and performed ChIP-seq library preparation. All authors contributed to writing and approved the final manuscript.

Ethics Statement

Primary patient sample collection was approved by the institutional review board of the Meuse Institute of Nuclear Biology, and written informed consent was obtained from all patients prior to sample collection and use for research purposes. Cell line experiments used commercially sourced, de-identified material under standard institutional biosafety approval.

Data Availability

Raw and processed ChIP-seq and RNA-sequencing data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request, subject to institutional data sharing agreements protecting patient confidentiality. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirguet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJ, Kouzarides T (2011) Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 478(7370):529-533. <https://doi.org/10.1038/nature10509>
2. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR (2011) RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* 478(7370):524-528. <https://doi.org/10.1038/nature10334>
3. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S,

- Bradner JE (2010) Selective inhibition of BET bromodomains. *Nature* 468(7327):1067-1073. <https://doi.org/10.1038/nature09504>
4. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 153(2):320-334. <https://doi.org/10.1016/j.cell.2013.03.036>
 5. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 153(2):307-319. <https://doi.org/10.1016/j.cell.2013.03.035>
 6. Shi J, Whyte WA, Zepeda-Mendoza CJ, Milazzo JP, Shen C, Roe JS, Minder JL, Mercan F, Wang E, Eckersley-Maslin MA, Campbell AE, Kawaoka S, Shareef S, Zhu Z, Kendall J, Muhar M, Haslinger C, Yu M, Roeder RG, Wigler MH, Blobel GA, Zuber J, Spector DL, Young RA, Vakoc CR (2013) Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. *Genes & Development* 27(24):2648-2662. <https://doi.org/10.1101/gad.232710.113>
 7. Shi J, Vakoc CR (2014) The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Molecular Cell* 54(5):728-736. <https://doi.org/10.1016/j.molcel.2014.05.016>
 8. Chapuy B, McKeown MR, Lin CY, Monti S, Roemer MG, Qi J, Rahl PB, Sun HH, Yeda KT, Doench JG, Reichert E, Kung AL, Rodig SJ, Young RA, Shipp MA, Bradner JE (2013) Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. *Cancer Cell* 24(6):777-790. <https://doi.org/10.1016/j.ccr.2013.11.003>
 9. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, Bergeron L, Sims RJ 3rd (2011) Targeting MYC dependence in cancer by inhibiting BET bromodomains. *PNAS* 108(40):16669-16674. <https://doi.org/10.1073/pnas.1108190108>
 10. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastitis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS (2011) BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146(6):904-917. <https://doi.org/10.1016/j.cell.2011.08.017>
 11. Bahr C, Correnti C, Hocking B, Reick J, Ippolito GC, Pearson KA, Eils R, Weber A, Muerdter TE, Loevenich M, Steinemann D, Petretich M, Beinke S, Knapp S, Chapman TD, Jeffrey P, Kouzarides T, Huntly BJP, Dawson MA (2018) A Myc enhancer cluster regulates normal and leukaemic haematopoietic stem cell hierarchies. *Nature* 553(7689):515-520. <https://doi.org/10.1038/nature25193>
 12. Yan J, Diaz J, Jiao J, Wang R, You J (2011) Perturbation of BRD4 protein function by BRD4-NUT protein abrogates cellular differentiation in NUT midline carcinoma. *Journal of Biological Chemistry* 286(31):27663-27675. <https://doi.org/10.1074/jbc.M111.246975>
 13. Zawistowski JS, Beville SM, Goulet DR, Stuhlmiller TJ, Beltran AS, Olivares-Quintero JF, Singh D, Sciaky N, Parker JS, Rashid NU, Chen X, Duncan JS, Whittle MC, Angus SP, Velarde SH, Golitz BT, He X, Santos C, Darr DB, Gallagher K, Graves LM, Perou CM, Carey LA, Earp HS, Johnson GL (2017) Enhancer remodeling during adaptive bypass to MEK inhibition is attenuated by pharmacologic targeting of the P-TEFb complex. *Cancer Discovery* 7(3):302-321. <https://doi.org/10.1158/2159-8290.CD-16-0653>
 14. Rathert P, Roth M, Neumann T, Muerdter F, Roe JS, Muhar M, Deswal S, Cerny-Reiterer S, Peter B, Jude J, Hoffmann T, Boryn LM, Axelsson E, Schweifer N, Tontsch-Grunt U, Dow LE, Gianni D, Pearson M, Valent P, Stark A, Kraut N, Vakoc CR, Zuber J (2015) Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature* 525(7570):543-547. <https://doi.org/10.1038/nature14898>
 15. Fong CY, Gilan O, Lam EY, Rubin AF, Ftouni S, Tyler D, Stanley K, Sinha D, Yeh P, Morison J, Giotopoulos G, Lugo D, Jeffrey P, Chung CW, Freeman J, Lee JS, Huntly BJP, Dawson SJ, Ramsay RG, Witkowski MT, Fioravanti G, Bracker T, Rimmer M, Kim I, Ford J, Chan WI, Robson SC, Prinjha RK, Papenfuss AT, Vassiliadis D, Dawson MA (2015) BET inhibitor resistance emerges from leukaemia stem cells. *Nature* 525(7570):538-542. <https://doi.org/10.1038/nature14888>
 16. Roe JS, Mercan F, Rivera K, Pappin DJ, Vakoc CR (2015) BET bromodomain inhibition suppresses the function of hematopoietic transcription factors in acute myeloid leukemia. *Molecular Cell* 58(6):1028-1039. <https://doi.org/10.1016/j.molcel.2015.04.011>
 17. Andricovich J, Perkail S, Kai Y, Casasanta N, Peng W, Tzatsos A (2018) Loss of KDM6A activates super-enhancers to induce gender-specific squamous-like pancreatic cancer and confers sensitivity to BET inhibitors. *Cancer Cell* 33(3):512-526. <https://doi.org/10.1016/j.ccell.2018.02.003>
 18. Krivtsov AV, Feng Z, Lemieux ME, Faber J, Vempati S, Sinha AU, Xia X, Jesneek J, Bracken AP, Silverman LB, Kutok JL, Kung AL, Armstrong SA (2008) H3K79 methylation profiles define murine and human MLL-AF4 leukemias. *Cancer Cell* 14(5):355-368. <https://doi.org/10.1016/j.ccr.2008.10.001>
 19. Bhatt S, Pioso MS, Olesinski EA, Yilma B, Ryan JA, Mashaka T, Cai T, Metzger J, Sun Y, Ganesan S, Malik A, Shaikh N, Tam W, Yang F, Sam D, Letai A, Gu Z, Anastas J, Ni H, Stein EM, Weinstock DM (2020) Anthracyclines induce mitochondrial-dependent apoptosis-associated release of mitochondrial DNA to activate the STING pathway and IFN response in acute myeloid leukemia. *Cancer Discovery* 10(2):240-259. <https://doi.org/10.1158/2159-8290.CD-19-0027>
 20. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biology* 9(9):R137. <https://doi.org/10.1186/gb-2008-9-9-r137>
 21. Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550. <https://doi.org/10.1186/s13059-014-0550-8>
 22. Puissant A, Frumm SM, Alexe G, Bassil CF, Qi J, Chanthery YH, Nekritz EA, Zeid R, Gustafson WC, Greninger P, Garnett MJ, McDermott U, Benes CH, Kung AL, Weiss WA, Bradner JE, Stegmaier K (2013) Targeting MYCN in neuroblastoma by BET bromodomain inhibition. *Cancer Discovery* 3(3):308-323. <https://doi.org/10.1158/2159-8290.CD-12-0418>
 23. Winter GE, Mayer A, Buckley DL, Erb MA, Roderick JE, Vittori S, Reyes JM, di Iulio J, Souza A, Ott CJ, Roberts JM, Zeid R, Scott TG, Paulk J, Lachance K, Olson CM, Dastjerdi S, Bauer S, Lin CY, Gray NS, Kelliher MA,

- Churchman LS, Bradner JE (2017) BET bromodomain proteins function as master transcription elongation factors independent of CDK9 recruitment. *Molecular Cell* 67(1):5-18. <https://doi.org/10.1016/j.molcel.2017.06.004>
24. Kanno T, Kanno Y, LeRoy G, Campos E, Sun HW, Brooks SR, Vahedi G, Heightman TD, Garcia BA, Reinberg D, Siebenlist U, O'Shea JJ, Ozato K (2014) BRD4 assists elongation of both coding and enhancer RNAs by interacting with acetylated histones. *Nature Structural & Molecular Biology* 21(12):1047-1057. <https://doi.org/10.1038/nsmb.2912>
 25. Muhar M, Ebert A, Neumann T, Umkehrer C, Jude J, Wieshofer C, Rescheneder P, Lipp JJ, Herzog VA, Reichholf B, Cisneros DA, Hoffmann T, Schlapansky MF, Bhat P, von Haeseler A, Kockx CE, van IJcken WFJ, Vakoc CR, Ameres SL, Zuber J (2018) SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis. *Science* 360(6390):800-805. <https://doi.org/10.1126/science.aao2793>
 26. Ott CJ, Kopp N, Bird L, Paranal RM, Qi J, Bowman T, Rodig SJ, Kung AL, Bradner JE, Weinstock DM (2012) BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. *Blood* 120(14):2843-2852. <https://doi.org/10.1182/blood-2012-02-413021>
 27. Herrmann H, Blatt K, Shi J, Gleixner KV, Cerny-Reiterer S, Mullauer L, Vakoc CR, Sperr WR, Horny HP, Bradner JE, Zuber J, Valent P (2012) Small-molecule inhibition of BRD4 as a new potent approach to eliminate leukemic stem- and progenitor cells in acute myeloid leukemia (AML). *Oncotarget* 3(12):1588-1599. <https://doi.org/10.18632/oncotarget.733>
 28. Perez-Salvia M, Esteller M (2017) Bromodomain inhibitors and cancer therapy: from structures to applications. *Epigenetics* 12(5):323-339. <https://doi.org/10.1080/15592294.2016.1265710>
 29. Stathis A, Bertoni F (2018) BET proteins as targets for anticancer treatment. *Cancer Discovery* 8(1):24-36. <https://doi.org/10.1158/2159-8290.CD-17-0605>
 30. Bradner JE, Hnisz D, Young RA (2017) Transcriptional addiction in cancer. *Cell* 168(4):629-643. <https://doi.org/10.1016/j.cell.2016.12.013>
 31. Filippakopoulos P, Knapp S (2014) Targeting bromodomains: epigenetic readers of lysine acetylation. *Nature Reviews Drug Discovery* 13(5):337-356. <https://doi.org/10.1038/nrd4286>
 32. Devaiah BN, Case-Borden C, Gegonne A, Hsu CH, Chen Q, Meerzaman D, Dey A, Ozato K, Singer DS (2016) BRD4 is a histone acetyltransferase that evicts nucleosomes from chromatin. *Nature Structural & Molecular Biology* 23(6):540-548. <https://doi.org/10.1038/nsmb.3228>
 33. Loven J, Vakoc CR (2013) Enhancer- and super-enhancer-mediated control of oncogenic transcription. *Cold Spring Harbor Symposia on Quantitative Biology* 78:181-189. <https://doi.org/10.1101/sqb.2013.78.020156>
 34. Roe JS, Vakoc CR (2016) The essential transcriptional function of BRD4 in acute myeloid leukemia. *Cold Spring Harbor Symposia on Quantitative Biology* 81:61-66. <https://doi.org/10.1101/sqb.2016.81.031229>