Men who develop metastatic castration-resistant prostate cancer (CRPC) invariably succumb to the disease. Progression to CRPC after androgen ablation therapy is predominantly driven by deregulated androgen receptor (AR) signalling. Despite the success of recently approved therapies targeting AR signalling, such as abiraterone and second-generation anti-androgens including MDV3100 (also known as enzalutamide), durable responses are limited, presumably owing to acquired resistance. Recently, JQ1 and I-BET762 two selective small-molecule inhibitors that target the amino-terminal bromodomains of BRD4, have been shown to exhibit anti-proliferative effects in a range of malignancies. Here we show that AR-signalling-competent human CRPC cell lines are preferentially sensitive to bromodomain and extraterritorial (BET) inhibition. BRD4 physically interacts with the N-terminal domain of AR and can be disrupted by JQ1 (refs 11, 13). Like the direct AR antagonist MDV3100, JQ1 disrupted AR recruitment to target gene loci. By contrast with MDV3100, JQ1 functions downstream of AR, and more potently abrogated BRD4 localization to AR target loci and AR-mediated gene transcription, including induction of the TMPRSS2-ERG gene fusion and its oncogenic activity. In vivo, BET bromodomain inhibition was more efficacious than direct AR antagonism in CRPC xenograft mouse models. Taken together, these studies provide a novel epigenetic approach for the concerted blockade of oncogenic drivers in advanced prostate cancer.

The identification and therapeutic targeting of co-activators or mediators of AR transcriptional signalling should be considered as alternative strategies to treat CRPC. BRD4 is a conserved member of the BET family of chromatin readers, which includes BRD2, BRD3 and BRDT. BRD4 has a critical role in transcription by RNA polymerase II (RNA Pol II) by facilitating recruitment of the positive transcription elongation factor P-TEFb15,16. Similar to other BET-family proteins, BRD4 contains two conserved bromodomains, BD1 and BD2. Competitive binding of JQ1 or I-BET762 to the bromodomain pocket results in the displacement of BRD4 from active chromatin and the subsequent removal of RNA Pol II from target genes. Although most cancer cells express BET-family proteins, it is not clear why only a subset of cell lines from diverse cancers responds to BET inhibitors. Recently, BRD4 was shown to interact with sequence-specific DNA-binding transcription factors in a gene-specific manner. As the genetic and epigenetic landscape differs between tumour types, it is possible that distinct transcriptional regulators that associate with BRD4 might influence the action of BET inhibitors.

To discover new treatment options for CRPC, we treated a panel of five prostate cancer cell lines and one benign prostate cell line with JQ1, and found three of the AR-signalling positive cells to be sensitive to JQ1, although all six cell lines express high levels of its target proteins (Fig. 1a and Extended Data Fig. 1a, b). Next, knockdown of BRD2, 3 and 4 (Extended Data Fig. 1c) led to significant inhibition of cell proliferation and invasion, phenocopying JQ1 treatment (Extended Data Fig. 1d, e).

Furthermore, JQ1 treatment induced G0–G1 arrest, apoptosis and associated transcriptional downregulation of the anti-apoptotic protein BCL-xl (also known as BCL2L1) in AR-positive cells13,18 (Fig. 1b and Extended Data Fig. 1f–h). Similar to BCL2 downregulation by the BET inhibitor I-BET151 in leukaemia17, a reduction in BCL-xl by JQ1 could be explained in part by the observed loss of BRD2/3/4 recruitment to its promoter region (Extended Data Fig. 1j). Even at a relatively low 100 nanomolar (nM) concentration, long-term colony formation of AR-positive cells was severely inhibited by JQ1 (Extended Data Fig. 1k) with no apparent effect on JQ1 target proteins (Extended Data Fig. 1l, m).

As AR-positive cells were preferentially sensitive to JQ1, we examined whether JQ1 has an effect on AR target genes. VCaP human prostate cancer cells that harbour the TMPRSS2-ERG gene fusion and AR amplification showed a dose-dependent decrease in prostate-specific antigen (PSA) and ERG at the messenger RNA and protein levels upon JQ1 treatment (Fig. 1d, e). Similar effects were observed in LNCaP and 22Rv1 prostate cancer cells (Extended Data Fig. 2a, b). Furthermore, bortezomib did not reverse the JQ1-mediated PSA and ERG protein loss, indicating that these genes are regulated at the transcriptional level (Extended Data Fig. 2c).

We performed microarray analysis to examine changes in global gene expression upon JQ1 treatment. Gene set enrichment analysis (GSEA) using the AR gene signature revealed significant repression of these genes in AR-positive cells (Fig. 1i), suggesting a role of BET proteins in AR-mediated transcription. Additionally, we observed a loss of the MYC-associated gene signature in AR-positive cell lines upon JQ1 treatment (Extended Data Fig. 2d). MYC is a known transcriptional target of BET inhibition in haematological cancers. Interestingly, MYC levels were attenuated by JQ1 in cells that are AR positive and sensitive to JQ1 inhibition, but not in AR-negative cells (Extended Data Fig. 2e). Thus, high expression of MYC per se (Extended Data Fig. 1b) does not confer sensitivity to JQ1 in prostate cancer cells. Time-course experiments with JQ1 demonstrated loss of MYC (Extended Data Fig. 2f, g) and cycloheximide had no additional effect on MYC protein levels (Extended Data Fig. 2h, i), ruling out a post-translational mode of JQ1 action. Phenotypically, knockdown of MYC did not affect cell invasion (Extended Data Fig. 2j), whereas JQ1 treatment inhibited invasion (Extended Data Fig. 1e).

Furthermore, exogenous expression of MYC did not result in rescue of JQ1-mediated inhibition of cell growth (Extended Data Fig. 2k, l). Thus, although MYC levels may be repressed by JQ1 in AR-positive cells, and may have a role in proliferation, MYC does not seem to be the primary target for the antineoplastic effects of JQ1.

As BRD4 is known to engage sequence-specific DNA-binding proteins, we proposed that AR may interact directly with BRD4. We performed gel-filtration chromatography and found that AR and BRD4 predominantly co-eluted in a high-molecular-weight complex (Fig. 2a and Extended Data Fig. 3a). Moreover, RNA Pol II, a reported target for phosphorylation by...
**Figure 1** | Prostate cancer cell lines with intact androgen signalling are sensitive to BET bromodomain inhibition. 

- **a.** Half-maximum inhibitory concentration (IC₅₀) for JQ1 in each cell line is shown. 
- **b.** Induction of apoptosis in VCaP prostate cancer cells by JQ1. Cleaved PARP (cPARP) immunoblot analysis. GAPDH served as a loading control. 
- **c.** Quantitative reverse transcription polymerase chain reaction (qRT–PCR) analysis of indicated genes in VCaP cells treated with varying concentrations of JQ1 for 24 h. Data represent mean ± standard error of the mean (s.e.m.) (n = 3) from one of three independent experiments. 
- **d.** Immunoblot analyses of AR, PSA and β-Gal levels in VCaP cells treated with JQ1. 
- **e.** GSEA using AR target gene signature.

**Figure 2** | Physical association of the N-terminal domain of AR with BRD4 and its disruption by BET bromodomain inhibition. 

- **a.** VCaP nuclear extracts were fractionated on a Superose-6 column and AR, BRD4 and RNA Pol II were analysed by immunoblotting. 
- **b.** Endogenous association of AR and BRD2/3/4. 
- **c.** VCaP and LNCaP nuclear extracts were subjected to immunoprecipitation using an anti-AR antibody. Immuno precipitates (IPs) were analysed for the presence of BRD2/3/4 by immunoblotting (IB; top). 
- **d.** Co-transfection with various His–BRD4 deletions and Halo–AR constructs. 
- **e.** As in d but with the indicated salt concentrations. 
- **f.** Representative sensograms from three independent experiments for AR–BRD4 (BD1–BD2) by an OctetRED biosensor showing direct interaction. Real-time binding was measured by immobilizing biotinylated AR protein on the super streptavidin biosensor and subsequent interaction with varying concentrations of BRD4 (BD1–BD2) protein. 
- **g.** Schematic of BRD4 and AR constructs used for co-immunoprecipitation experiments. CTD, C-terminal domain; ET, extraterminal domain; BD, DNA-binding domain; LBD, ligand-binding domain. 
- **h.** NTD domain of AR interacts with BD1 of BRD4. Equal amounts of in vitro translated proteins were combined and immunoprecipitated using Halo beads followed by immunoblot analysis with anti-GST antibody. JQ1 disrupts AR–BD1 interactions. Varying concentrations of JQ1 were incubated with AR–BD1, NTD1b–BD1 and AR–BD2 complexes before immunoprecipitation followed by immunoblot analysis.
BRD4 (ref. 21), also co-eluted in the same complex, suggestive of a large multi-protein complex composed of AR, BRD4 and RNA Pol II. Immunoprecipitation experiments further confirmed an endogenous association between AR and BRD4 (Fig. 2b). Additionally, we observed an interaction between AR and BRD2/3 (Fig. 2b), implicating a common region in BRD2, 3 and 4 proteins responsible for AR interaction. To map the region mediating this interaction, we tested the ability of different deletion variants of BRD4 to pull-down AR in 293T cells (Fig. 2c). A BRD4 variant containing the BD1 and BD2 domains maintained the ability to pull-down AR even at high salt concentrations (Fig. 2d, e). To determine whether the BD1–BD2 domains directly interact with AR, we carried out quantitative assessment of the binding affinity using the OctetRED system. We applied varying concentrations of BD1–BD2 protein to biosensors with immobilized AR and found that BRD4 interacts with AR in a concentration-dependent fashion, with an estimated dissociation constant ($K_d$) of 70 nM, supporting a high-affinity interaction (Fig. 2f and Extended Data Fig. 3b, c). To fine map this interaction we created a series of Halo-tagged AR and glutathione S-transferase (GST)-tagged BRD4 constructs for in vitro pull-down studies and demonstrated that the BD1, and to a lesser extent the BD2 domain, bind directly to the N-terminal domain (NTD) domain of AR, which was further mapped to a 38-amino-acid region—NTD1b—of AR (Fig. 2g and Extended Data Fig. 3d–f). Subsequently, we observed the disruption of BD1–AR and BD1–NTD1b interactions by JQ1 (Fig. 2h), as well as loss of endogenous BRD4–AR interactions (Extended Data Fig. 3g). Together, these data indicate that BET protein inhibition leads to disruption of the AR–BRD4 interaction, which probably explains the preferential activity of JQ1 in AR-positive prostate cancer cells.

The ubiquitously expressed BRD2, 3 and 4 proteins are suggested to have overlapping functions$^{10,13,22}$ and, consistent with this notion, we observed AR interactions with all three. Because BET inhibitors, such as JQ1 and I-BET762, have high affinity for the BD1 and BD2 domains of BRD2/3/4 proteins$^{10,11,13}$, we proposed that BET inhibitors may affect genome-wide recruitment of all three BET proteins. We performed chromatin immunoprecipitation followed by sequencing (ChIP-seq) with antibodies against BRD2/3/4 in VCaP cells treated with JQ1 or I-BET762 (Extended Data Fig. 4a) and observed a high genome-wide overlap between BRD2, 3 and 4 (62–86% peak overlap) (Extended Data Fig. 4b, c). JQ1 or I-BET762 treatment led to a reduction in the recruitment of all three proteins to chromatin (Extended Data Fig. 4d). Moreover, the reduced BRD2/3/4 recruitment was equally distributed for regions with or without AR (Extended Data Fig. 4e).

Binding of androgen (dihydrotestosterone (DHT)) to AR leads to its translocation from the cytoplasm to the nucleus, where it binds to regions of DNA harbouring androgen-responsive elements (AREs) and results in subsequent recruitment of proteins involved in transcriptional activation or suppression in a gene-specific manner. BRD4 interacts with acetylated histones as well as DNA-binding transcription factors, leading to context-dependent transcriptional activation or inhibition of target genes$^{13,19,22}$. As the AR–BRD4 interaction is disrupted by JQ1 (Fig. 2), we next explored whether AR localization is affected in a genome-wide context. We performed ChIP-seq with antibodies against AR, BRD4 and RNA Pol II in cells that were either starved, treated with DHT or DHT plus JQ1 (Extended Data Fig. 4a). Two anti-androgens, bicalutamide and MDV3100, were included for comparison. As expected, the average ChIP-seq signal for AR was highly enriched in DHT-treated cells (Fig. 3a and Extended Data Fig. 5a, b). Recruitment of AR to target loci was markedly attenuated by MDV3100 and less so by bicalutamide. Interestingly, JQ1 blocked AR recruitment almost as effectively as MDV3100 (Fig. 3a and Extended Data Fig. 5c–e). Furthermore, we observed a co-recruitment of AR and BRD4 at 2,031 sites. The strongest association was observed within promoters of AR-regulated genes (502 promoters, $P = 4 \times 10^{-57}$), and for the highest AR peaks (1,112 sites, $P = 1 \times 10^{-58}$) (Fig. 3b). Limiting our evaluation to AR and BRD4 coincident peaks, we observed that DHT-mediated AR recruitment to these loci was inhibited by MDV3100 and to a lesser extent by JQ1 (Fig. 3c). By contrast, the robust recruitment of ERG by JQ1 has important implications, as JQ1 almost completely abrogated DHT-induced BRD4 recruitment to the AR–BRD4 shared loci (Fig. 3d). Examples of gene tracks for AR and BRD4-associated genomic regions such as enhancers and super-enhancers$^{22}$ and the effects of different treatments on their levels are shown in Fig. 3e and Extended Data Fig. 5f. Corroborating the ChIP-seq data, gene expression analysis in VCaP and LNCaP cells showed more efficient repression of DHT-induced AR-target genes by JQ1 than by MDV3100 or bicalutamide (Extended Data Fig. 5g, h).
as the TMPRSS2-ERG gene fusion product is the oncogenic driver in 50% of prostate cancers. To investigate the effect of JQ1 on ERG-mediated transcription, we performed ERG ChIP-seq in cells treated with JQ1 for 12 hours—a time window in which significant expression levels of select ERG target genes (Extended Data Fig. 6e, f). As expected, the ERG-activated genes were downregulated and the ERG-repressed genes were de-repressed by JQ1 (Extended Data Fig. 6g, h). To evaluate BET inhibitor repression of ERG-mediated oncogenic function in an isogenic setting, we overexpressed ERG in RWPE and PC3 cells (Extended Data Fig. 7a, b). Treatment with JQ1 or I-BET762 led to an attenuation of ERG-mediated invasion (Extended Data Fig. 7c) and GSEA demonstrated a significant negative enrichment for ERG target genes upon BET inhibitor treatment (Extended Data Fig. 7d). Furthermore, we found that ERG was highly enriched on the known distal enhancer of MYC that was reduced upon JQ treatment (Extended Data Fig. 8a, b). Likewise, ETV1 occupies the same distal-enhancer region in ETV1 fusion-positive LNCaP cells. Knockdown of ERG or ETV1 along with AR led to MYC downregulation, implicating MYC regulation by ETS proteins in fusion-positive prostate cancer cells (Extended Data Fig. 8c–e). Notably, ChIP-seq analysis of AR and RNA Pol II enrichment on the MYC locus presented an interesting pattern in which DHT treatment led to increased AR and reduced RNA Pol II binding on the MYC distal enhancer and gene body, respectively, which was reinstated in the presence of MDV3100 or bicalutamide but not JQ1 (Extended Data Fig. 8f). This observation is consistent with the concomitant reduction in MYC expression upon DHT treatment that was de-repressed in the presence of MDV3100 but not JQ1 (Extended Data Fig. 8g–i). Lack of de-repression of MYC by JQ1 in this setting could be explained by the fact that both AR and ERG are absent from the MYC distal enhancer, leading to net loss of MYC expression. These data also suggest a mechanism by which CRPC patients become resistant to anti-androgen therapy by maintaining expression of the MYC oncogene.

Next, we sought to compare the efficacy of JQ1 and MDV3100, a direct AR antagonist used clinically to treat advanced CRPC. Before embarking on the in vivo experiment we tested them on VCaP cells in vitro for 8 days and observed marginal cell death by MDV3100 versus suppression of cell growth at sub-micromolar concentrations of JQ1 (Extended Data Fig. 9a). To rule out the possibility of JQ1 being a generic anti-androgen we confirmed that JQ1 had no effect on physiological androgen-regulated processes; however, JQ1 reduced testes size in mice, as reported previously. Treatment of VCaP tumour-bearing mice with JQ1 led to a significant reduction in tumour volume (Fig. 4a, b and Extended Data Fig. 10a), whereas MDV3100 had a less pronounced effect.

Recently, several studies described the pro-metastatic effects of MDV3100 in pre-clinical models. To test whether MDV3100 treatment leads to spontaneous metastasis in our VCaP xenograft model, we isolated femur, liver and spleen from MDV3100-treated mice and found evidence of metastases in femur and liver (Extended Data Fig. 10b, c), whereas MDV3100 had a less pronounced effect. Taken together, these pre-clinical studies suggest that the use of MDV3100 in clinically localized prostate cancer may potentiate the formation of micro-metastases, unlike BET inhibitors. Consistent with previous reports, JQ1 and MDV3100 were both well tolerated by mice (Extended Data Fig. 10d). Although VCaP cells were originally derived from a patient with CRPC, VCaP tumour xenografts respond to castration in mouse models. We found that JQ1 still had a growth inhibitory effect in castration-resistant VCaP tumour xenografts and observed a 50% reduction in castration-resistant tumours by JQ1-treatment (Fig. 4c and Extended Data Fig. 10e).

Maintenance of AR signalling is the most common resistance mechanism that patients with advanced prostate cancer develop after conventional hormonal treatments. AR amplification, mutation and alternative splicing have all been suggested as potential resistance mechanisms to anti-androgen treatments. Over half of CRPC patients have at least one of these aberrations in the AR pathway. As BET inhibitors function ‘downstream’ of AR (Fig. 4d), our data indicate that these compounds may be effective in the context of AR-mediated resistance, including...
compensatory mechanisms involving related steroid hormone receptors that are also likely to require BET bromodomain function. By functioning downstream of AR, BET inhibition is less likely to be affected by acquired resistance associated with AR antagonists, including the recently identified F876L mutation of AR. Although both MDV3100 and JQ1 block AR recruitment to target loci on a genome-wide scale (the ‘AR cistrome’), we found that JQ1 probably has an enhanced inhibitory effect by further abrogating co-recruitment of BRD4, which is required for mobilization of the transcriptional machinery.

A recent study demonstrated that BET inhibition leads to preferential loss of BRD4 at super-enhancers and consequent transcriptional elongation defects. These super-enhancers were often associated with key oncogenic drivers in a variety of cancers. Tumour cells are thought to become addicted to select oncogenes and hence usually reliant on their high expression, which may explain the preferential sensitivity of BET inhibition in cancer versus normal tissues. Although MYC and its association with multiple myeloma was highlighted as a super-enhancer-dependent expression, which may explain the preferential sensitivity of BET inhibition in cancer versus normal tissues. Therefore, this framework probably applies to key transcription factors involved in the development of CRPC, including AR, ETS and MYC (Fig. 4d). Taken together, these data indicate that clinical evaluation of BET inhibitors is warranted in CRPC, either as mono-therapy or in combination with second-generation anti-androgens.

METHODS SUMMARY

Gene expression profiling was performed using the Agilent Whole Human Genome Oligo Microarray following the manufacturer’s protocol. Proteins were extracted by lysing the cells in RIPA lysis buffer (Sigma) supplemented with protease inhibitor cocktail (Sigma). Immunoblotting was performed with standard protocols using polyvinylidene difluoride (PVDF) membrane (GE Healthcare), and signals were visualized with an enhanced chemiluminescence system as described by the manufacturer (GE Healthcare). The ChIP assays for BRD2, BRD3, BRD4, AR, RNA Pol II, ERG and H3K27ac with specific antibodies were performed using HighCell Chip kit (Diagenode) following the manufacturer’s protocol. All procedures involving mice were approved by the University Committee on Use and Care of Animals at the University of Michigan and conform to all regulatory standards.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Taylor, B. S. et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 18, 11–22 (2010).
2. Chen, C. D. et al. Molecular determinants of resistance to antiandrogen therapy. Nature Med. 10, 33–39 (2004).
3. Visakorpi, T. et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nature Genet. 9, 401–406 (1995).
4. Stein, M. N., Goodin, S. & Dipaola, R. S. Abbratorone in prostate cancer: a new angle to an old problem. Clin. Cancer Res. 18, 1848–1854 (2012).
5. Reid, A. H. et al. Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abbratorone acetate. J. Clin. Oncol. 28, 1489–1495 (2010).
6. de Bono, J. S. et al. Abbratorone and increased survival in metastatic prostate cancer. N. Engl. J. Med. 364, 1995–2005 (2011).
7. Mukherji, D., Pezaro, C. J. & De-Bono, J. S. MDV3100 for the treatment of prostate cancer. Expert Opin. Investig. Drugs 21, 227–232 (2013).
8. Scher, H. I. et al. Increased survival with abbratorone in prostate cancer after chemotherapy. N. Engl. J. Med. 367, 1187–1197 (2012).
9. Lockwood, W. W., Zeijnualah, K., Brader, J. E. & Varmus, H. Sensitivity of human lung adenocarcinoma cell lines to targeted inhibition of BET epigenetic signaling proteins. Proc. Natl Acad. Sci. USA 109, 19408–19413 (2012).
10. Dawson, M. A. et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 478, 529–533 (2011).
11. Delmore, J. E. et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cancer Cell 146, 904–917 (2011).
12. Dussault, P. et al. Targeting MYCN in neuroblastoma by BET bromodomain inhibition. Cancer Discov 3, 308–323 (2013).
13. Filippakopoulos, P. et al. Selective inhibition of BET bromodomains. Nature 468, 1067–1073 (2010).
14. Attard, G., Richards, J. & de Bono, J. S. New strategies in metastatic prostate cancer: targeting the androgen receptor signaling pathway. Clin. Cancer Res. 17, 1649–1657 (2011).
15. Jang, M. K. et al. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol. Cell 19, 523–534 (2005).
16. Yang, Z. et al. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol. Cell 19, 535–545 (2005).
17. Lovén, J. et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 153, 320–334 (2013).
18. Mertz, J. A. et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc. Natl Acad. Sci. USA 108, 16669–16674 (2011).
19. Wu, S. Y., Lee, A. Y., Lai, H. T., Zhang, H. & Chiang, C. M. Phospho switch triggers Brd4 chromatin binding and activator recruitment for gene-specific targeting. Mol. Cell 49, 843–857 (2013).
20. Tomlins, S. A. et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310, 644–648 (2005).
21. Davaiah, B. N. et al. BRD4 is an atypical kinase that phosphorylates serine2 of the RNA polymerase II carboxy-terminal domain. Proc. Natl Acad. Sci. USA 109, 6927–6932 (2012).
22. Belkina, A. C. & Denis, G. V. BET domain co-regulators in obesity, inflammation and cancer. Nature Rev. Cancer 12, 465–477 (2012).
23. Chen, Y. et al. ETS factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to PTEN loss. Nature Med. 19, 1023–1029 (2013).
24. Matzuk, M. M. et al. Small-molecule inhibition of BRD3 for male contraception. Cell 150, 673–684 (2012).
25. Lin, T. H. et al. Androgen receptor antagonist ASC-J9 versus anti-androgens MDV3100 (Enzalutamide) or Casodex (Bicalutamide) leads to opposite effects on prostate cancer metastasis via differential modulation of macrophage infiltration and STAT3–CCL2 signaling. Cell Death Dis. 4, e764 (2013).
26. Harris, W. P., Mostaghel, E. A., Nelson, P. S. & Montgomery, B. Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion. Nat. Clin. Pract. Urol. 6, 76–85 (2009).
27. Taplin, M. E. et al. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res. 59, 2511–2515 (1999).
28. Sun, S. et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J. Clin. Invest. 120, 2715–2730 (2010).
29. Grasso, C. S. et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 487, 239–243 (2012).
30. Balbas, M. D. et al. Overcoming mutation-based resistance to antiandrogens with rational drug design. Elife 2, e00499 (2013).

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Author Contributions I.A.A. and A.M.C. conceived the study and the experiments. I.A.A. performed the experiments with assistance from V.L.D., R.M., S.D., C.E. and J.X.; J.W. carried out in vitro interaction studies; M.C., R.M. and I.A.A. analysed microarray data; J.E.-W., K.W.-R. and F.Y.F performed mouse xenograft studies; Y.-M.W. generated ChIP-seq libraries and X.C. performed the sequencing; M.C., R.Y., M.K.I. and Z.S.Q. performed ChIP-seq analysis with input from I.A.A.; S.W. provided compounds. I.A.A. and A.M.C. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Author Information Gene expression and ChIP-seq coordinates have been deposited in the Gene Expression Omnibus under accession number GSE55064. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.M.C. (arul@umich.edu).
METHODS

Cell culture. VCaP prostate cancer cells were grown in DMEM with Glutamax (Gibco); LNCaP, 22Rv1, DU145 and PC3 prostate cancer cells were grown in RPMI 1640; all were supplemented with 10% FBS (Invitrogen) in 5% CO₂ cell culture incubator. The immortalized benign prostate cell line RWPE-1 was grown in keratinocyte media with supplements (Lonza). All cell lines were tested and found to be free of mycoplasma contamination.

Cell viability assay. Cells were normalized and IC₅₀ was calculated using GraphPad Prism software. For long-term colony formation assay, 10,000 cells per well were seeded in 6-well plates and treated with either 100 nM or 500 nM of JQ1 or DMSO. After 12 days, cells were fixed with methanol, stained with crystal violet and photographed. For colorimetric assays, the stained wells were treated with 500 μl 10% acetic acid and the absorbance was measured at 560 nm using a spectrophotometer.

Cell cycle analysis. Cells were grown in 6-well plates and treated with varying concentrations of JQ1. For cell cycle analysis, cells were washed 48 h post-treatment with PBS and fixed in 70% ethanol overnight. The cells were washed again with PBS, stained with propidium iodide and analysed by flow cytometry.

DNA isolation and quantitative real-time PCR. Total DNA was isolated from cells using RNeasy Mini Kit (Qiagen) and cDNA was synthesized from 1,000 ng total DNA using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). qPCRs were performed in duplicate or triplicate using Taqman assays (Applied Biosystems) or standard SYBR green reagents and protocols on a StepOnePlus Real-Time PCR system (Applied Biosystems). The target mRNA expression was quantified using the ΔΔCₚ method and normalized to GAPDH expression. All primers were designed using primer3 (http://rodro.wi.mit.edu/primer3) and synthesized by Integrated DNA Technologies. The primer sequences for the SYBR green and catalogue numbers for Taqman assays qPCR used are as follows: BRD2 qPCR fwd, CTGATCTTGGCAGAG CCT; BRD2 qPCR rev, GCCCTTCTCTGACGCTT; BRD3 qPCR fwd, CCTCCAGGAGTCTCATCC; BRD3 qPCR rev, ATGGTCTGTCTTCTCCTCC; BRD4 qPCR fwd, AGCCACGCAACACGATG TAG; BRD4 qPCR rev, CGCAGGATGATGAGTAGT GC; ERG qPCR fwd, CATATTCTTCCACCCGCACTCT; PSA (KLK3) qPCR fwd, ACCTGTCGAGGAGGGCGAAGG; PSA (KLK3) qPCR rev, GGCGGAGCAGCATGTTAC; BMP1B qPCR fwd, CCACCAT GTGAGAGACAG; BMP1B qPCR rev, GCAACCGAGATGACTCTCTT; MYC qPCR fwd, GCTGTCCTGAGAAGTG; MYC qPCR rev, GTTCAGATCGTCGAGTTACA; AR qPCR fwd, CGTGGATGCCTGCTTTAAT; AR qPCR rev, GGGAGCTTGAGGGCTGTAG; ET1 V5 qPCR fwd, GCAAGAGGGCTTCT; ET1 V5 qPCR rev, CCTCTCCGATCAGCTTCG; GAPDH qPCR fwd, TGCACCAACAACTGCTTAG; GAPDH qPCR rev, GGCTAGTGGCAGTGTG; GGTCaTG; MYC dis.enh ChIPPCR fwd, TGCAAGCTTCTCCTGGTGTA; MYC dis.enh ChIPPCR rev, CGGAGGGAGGAAGTCAT; MYC upstream ChIPPCR fwd, CCAGCAAGAATGACACCA; MYC upstream ChIPPCR rev, CGCCTGGCAAAATCATC; TaqMan primer probes TDRD1, catalogue no. Hs00229805 m1; CACNA1D, catalogue no. Hs00167753 m1; ARHGHD1 catalogue no. Hs00171288 m1; NDRG1, catalogue no. Hs00608387 m1; VGL, catalogue no. Hs00419715 m1; KR78, catalogue no. Hs0195539 g1; MALAT1, catalogue no. Hs00273907 s1; BCL-xL-qPCR, catalogue no. Hs00236329 m1; WNT2 qPCR, catalogue no. Hs00608224 m1; CRISp3 qPCR, catalogue no. Hs00195988 m1.

Antibodies and immunoblot analyses. Antibodies used in the immunoprecipitation (IP) and immunoblotting (IB) assays are AR IP, IB (Abcam catalogue no. ab74272); RNA Pol II IB (Abcam catalogue no. ab5408); BRD2 IB (Abnova catalogue no. PAB3245); BRD3 IB (SantaCruz catalogue no. sc-81020); BRD4 IB (Bethyl catalogue no. A301-985A); BCL-xl IB (Cell Signaling catalogue no. 2762); BRD3 IB (SantaCruz catalogue no. L-004936-00-005); BRD4 IB (SantaCruz catalogue no. sc-20720); MYC IB (Sigma catalogue no. PAB3245); BRD3 IB (SantaCruz catalogue no. L-004936-00-005); BRD4 IB (SantaCruz catalogue no. L-004936-00-005). The following antibodies were used for endogenous immunoprecipitation experiments: BCL-xL IB (Cell Signaling catalogue no. 3787); AR IP (Abcam catalogue no. ab2885); BRD2 IP (Abcam catalogue no. ab218); BRD3 IP (Abcam catalogue no. ab5408); BRD4 IP (Abcam catalogue no. ab218). The following antibodies were used for immunoblotting analyses: AR IP, IB (Abcam catalogue no. ab2885); BRD2 IP (Abcam catalogue no. ab218); BRD3 IP (Abcam catalogue no. ab5408); BRD4 IP (Abcam catalogue no. ab218).

Antibodies and immunoblot analyses. Antibodies used in the immunoprecipitation (IP) and immunoblotting (IB) assays are AR IP, IB (Abcam catalogue no. ab74272); RNA Pol II IB (Abcam catalogue no. ab5408); BRD2 IB (Abnova catalogue no. PAB3245); BRD3 IB (SantaCruz catalogue no. sc-81020); BRD4 IB (Bethyl catalogue no. A301-985A); BCL-xl IB (Cell Signaling catalogue no. 2762); cPARP IB (Cell Signaling catalogue no. 9541); GAPDH (141C10) IB (Cell Signaling catalogue no. 3683 s). All antibodies were used at dilutions suggested by the manufacturer.

Immunoprecipitations. For endogenous immunoprecipitation experiments, nuclear extracts were obtained from VCaP and LNCaP cells using NE-PER nuclear extraction kit (Thermo Scientific). Nuclear pellet was then lysed in IP buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% Triton-X 100, protease inhibitor) by sonication. Nuclear lysates (0.5–1.0 mg) were pre-cleaned by incubation with protein G Dynabeads (Life Technologies) for 1 h on a rotator at 4 °C. Five micrograms of antibody was added to the pre-cleared lysates and incubated on a rotator at 4 °C overnight before the addition of protein G Dynabeads for 1 h. Beads were washed three times in IP buffer and resuspended in 40 μl of 2× loading buffer and boiled at 90 °C for 10 min for separation of the protein and beads. Samples were then analysed by SDS–PAGE and western blotting as described earlier. For endogenous competitive assays, the VCaP cells were incubated with 5 or 25 μM JQ1 for 6 h before nuclear protein extractions.

For co-immunoprecipitation experiments in 293T cells, plasmids encoding various deletion mutants of BRD4 in pCDNA4c (Addgene) and full-length AR in pFN21 plasmid (Promega) were transfected using Fugene 6.0 HD (Roche) according to the manufacturer's instructions. Twenty-four hours after transfection, total proteins were extracted using IP buffer supplemented with protease inhibitor cocktail mix (Sigma) and the expressions of the corresponding proteins were analysed by immunoblotting.

Immunoprecipitation using Halo beads followed by immunoblotting with Anti-His antibody were performed as described earlier.
Cell-free protein–protein interaction studies. In vitro protein expression was carried out by cloning the desired expression cassettes downstream of a Halo or GST tag to produce fusion proteins. Briefly, AR and its subdomains were cloned into the pFN2K vector containing N-terminal GST sequence (catalogue no. G1891, Promega); BRD4 and its subdomains were cloned into the pFN19A vector containing N-terminal Halo sequence (catalogue no. G8461, Promega). After cloning, the fusion proteins were expressed using the cell-free transcription and translation system (catalogue no. LS0301, Promega) following the manufacturer’s protocol. For each reaction, protein expression was confirmed by Coomassie blue staining.

A total of 10 μl cell-free reaction containing Halo- and GST-tag fusion proteins were incubated in PBST (0.1% Tween) at 4°C overnight. Ten microliters of HaloLink beads (catalogue no. G931, Promega) were blocked in BSA at 4°C overnight. After washes with PBS, the beads were mixed with AR–BRD4 mixture and incubated at room temperature for 1 h. HaloLink beads were then washed with PBST four times and eluted in SDS loading buffer. Proteins were separated on SDS gel and blotted with anti-GST antibody (GE healthcare). For competitive assay, AR–BD1, NTD1b–BD1 and AR–BD2 mixture was incubated in the presence of varying doses of IQ1 compound.

AR–BRD4 direct interaction assays by OctetRED. The binding affinity between AR and BRD4 was determined by biolayer interferometry technology using the OctetRed system (Fortebio). Recombinant AR protein (catalogue no. RA-84686H, Creative Biomart) was biotinylated by EZ-Link NHS-PEG4 Biotinylation Kit (catalogue no. 21329, Thermo Scientific) following the manufacturer’s protocol, and any unincorporated biotin was removed from the reactions with Zeba 2 ml desalt columns. Biotinylated proteins (5 μg ml−1) were then incubated with super-streptavidin biosensors (catalogue no. 18-3057, Fortebio) in binding buffer (20 mM HEPES pH 7.4, 150 mM NaCl) and washed three times in binding buffer, and the AR–BRD4 association/dissociation was monitored by OctetRED for 10 min at 25°C. Non-specific binding was controlled by subtracting the signal obtained from AR–RN2F interactions from that of AR–BRD4 interactions and baseline signal shift was controlled by monitoring immobilized AR without BRD4. OctetRED analysis software was used to analyse the data.

Gene expression array analysis. VCaP, LNCaP, 22RV1 and DU145 cells were treated with 500 nM IQ1 for 24 h and total RNA was extracted using RNeasy Mini Kit (Qiagen) for gene expression array analysis. For anti-androgen comparative study, VCaP and LNCaP cells were grown in media containing 10% charcoal-stripped serum for 48 h followed by pre-treatment with 500 nM IQ1, 10 μM MDV3100 or 25 μM bicalutamide for 6 h and stimulated with 10 nM DHT (androgen) for 18 h. Cells treated with only vehicle or 10 nM DHT served as controls. For determining the effect of BET inhibitors in isogenic ERG system, RWPE-ERG and PC3-ERG cells were treated with only vehicle or 10 nM DHT served as controls. For each reaction, protein expression was carried out by cloning the desired expression cassettes downstream of a Halo or GST (catalogue no. C8461, Promega) was serially diluted in binding buffer, and the binding buffer was used to analyse the data.

Protein expression was determined by Western blotting. The ERG gene signature was generated by extracting twofold upregulated genes from the respective replicate PC3 cells using ERG-complementary Lacz-expressing cells. GSEA was performed using this gene set on gene expression data obtained from the IQ1- and I-BET762-treated RWPE and PC3 cells. We also ran GSEA using a gene set that was not changed upon expression of ERG to exclude the possibility that treatment with IQ1 and I-BET762 may change gene expression in a non-specific fashion. All of the gene expression array data (total 48) can be found in the Gene Expression Omnibus under accession number GSE55064.

ChIP and ChIP-seq. The ChIP assays for BRD2, BRD3, BRD4, AR, RNA Pol II, ERG and H3K27ac were performed using HighCell Chip kit (Diagnode) according to the manufacturer’s protocol. The antibodies used for ChIP assay are AR PG-21 (Milscore catalog no. 06-680); RNA Pol II (Abcam catalog no. ab5408); BRD2 (Bethyl catalogue no. A302-583A); BRD3 (Bethyl catalogue no. A302-368A); BRD4 (Bethyl catalogue no. A301-985A); H3(acetyl K27) (Abcam catalogue no. ab4729) and IgG (Diagnode). For BRD2/3/4 ChIP-seq experiments with BET inhibitors, VCaP cells were treated with 500 nM IQ1 or I-BET762 for 12 h. For AR signalling ChIP-seq experiments, VCaP cells were grown in charcoal-stripped serum containing media for 48 h followed by 6 h pre-treatment with vehicle or 500 nM IQ1 or 10 μM MDV3100 or 25 μM bicalutamide and then stimulated with 10 nM DHT for 12 h. For ERG ChIP-seq studies, VCaP cells were treated with 500 nM IQ1 or vehicle for 12 h. Next, cells were crosslinked for 10 min with 1% formaldehyde. Crosslinking was terminated by the addition of 1/10 volume 1.25 M glycine for 5 min at room temperature following by cell lysis and sonication (Bioruptor, Diagenode), resulting in an average chromatin fragment size of 200 bp. Chromatin equivalent to 5 x 105 cells were used for ChIP using various antibodies. Chip DNA was isolated (iPure Kit, Diagenode) from samples by incubation with the antibody at 4°C overnight followed by washing and reversal of crosslinking. The ChIP-seq sample preparation for sequencing was performed according to the manufacturer’s instructions (Illumina). ChIP-enriched DNA samples (1–10 ng) were converted to blunt-ended fragments using T4 DNA polymerase. E.coli DNA polymerase I large fragment (Klenow fragment) and T4 polynucleotide kinase (New England BioLabs (NEB)). A single A base was added to fragment ends by Klenow fragment (3’ to 5’ exo minus) reaction followed by ligation of Illumina adaptors (Quick ligase, NEB). The adapter-modified DNA fragments were enriched by PCR using the Illumina Barcode primers and Phusion DNA polymerase (NEB). PCR products were size selected using 3% NuSieve agarose gels (Lonza) followed by gel extraction using QIAEX II reagents (Qiagen). Libraries were quantified with the Bioanalyzer 2100 (Agilent) and sequenced on the Illumina HiSeq 2000 Sequencer (100-nucleotide read length).

ChIP-seq analysis

ChIP-seq enrichment levels. ChIP-seq enrichment levels within each peak were calculated from the sequencing data as follows. First, reads were aligned to the HG19 reference genome using Bowtie2 with all default settings. Second, aligned reads were sorted using NovoSort and exact duplicates were removed using Samtools 3. Third, for each peak (site) overlapping reads were counted and this count was divided by the length of the peak or site. Fourth, to correct for differences in sequencing depth and alignment coverage the values are further normalized by the number of aligned reads per million.

ChIP-seq reproducibility plots. To assess the biological variability of AR and ERG ChIP-seq experiments, we calculated enrichment levels of their respective replicates for each replicate. We called peaks using MACS with all default setting against an IgG control. We excluded peaks within genomic regions prone to technical artefacts 36. For each replicate we defined a set of concordant peaks as those overlapping in both peaks and concordant peaks as those overlapping in both replicates. For each concordant peak, we calculated enrichment levels within the union of the two overlapping peaks. The scatter plots include all peaks with enrichment levels up to the 99th percentile.

Overlaps of bromodomain proteins. We compared the genome-wide distribution of BRD2, BRD3 and BRD4 peaks in DMSO-treated VCaP cells. First, we called peaks for each of the proteins using MACS with all default settings and IgG control. Because we were interested in peaks that are possibly biologically significant we used a moderately stringent cut-off value (MACS score >100). Next, we identified genomic regions that were enriched for at least one of the proteins. Specifically, we called ‘reduced’ all stringently peaks using GenomicRanges 37. For each of these regions we calculated enrichment levels using the number of overlaps. Drug-induced changes of bromodomain protein enrichment levels. We compared the genome-wide distribution of BRD2, BRD3 and BRD4 peaks in DMSO-treated VCaP cells. First, we called peaks for each of the proteins using MACS with all default settings and IgG control. Because we were interested in peaks that are possibly biologically significant we used a moderately stringent cut-off value (MACS score >100). Next, we identified genomic regions that were enriched for at least one of the proteins. Specifically, we called ‘reduced’ all stringently peaks using GenomicRanges 37. For each of these regions we calculated enrichment levels using the number of overlaps.
enrichment levels upon drug treatment (I-BET762, JQ1) relative to the levels in the DMSO control. First, peaks were called for all conditions and proteins as described earlier. Next, for each protein separately, we identified genomic regions that were enriched in any (union) of the treatment conditions (DMSO, I-BET762 or JQ1). Within those regions we quantified enrichment levels as described earlier for determining ChIP-seq enrichment levels. As enrichment levels of different proteins are not directly comparable, we normalize all enrichments to the median level of the DMSO control.

**Differential AR–BRD4 enrichment and AR–BRD4 overlap.** HPeak, a hidden Markov model (HMM)-based peak-calling software designed for the identification of protein-interactive genomic regions, was used for ChIP-seq peak determination. For enrichment plots shown in Fig. 3a, c and d, identified peaks for each sample are centred by peak summit and average coverage per million was counted within 1,500 bp relative to the peak centre. The overlap of AR- and BRD4-enriched regions were calculated by BEDtools. The significance of overlap between AR and BRD4 binding was calculated using a hypergeometric test based on the derived number of associated genes. The heatmap for AR peak enrichment was generated using python-based script on raw data and visualized using JavaTreeView.

**Differential ERG enrichment.** We identified sites with significant differences in ERG levels between DMSO- and JQ1-treated cells. First we focused on concordant peaks (see ChIP-seq reproducibility plots) that were overlapping or in the ±5 kb proximity of annotated gene loci. We defined a gene locus as the union of all of its known transcripts (Ensembl Genes 73). We used DESeq2 to assess the statistical significance of differences in ERG enrichment levels. Although DESeq2 was originally developed for RNA-seq, its statistical model is well-suited to count data in general. We used the tools’ default multiple hypothesis correction method and report peaks with significant differences in ERG levels (adjusted P value < 0.1). To assess quantitative differences in ERG levels at significantly ‘gained’ (positive difference in ERG levels upon JQ1 treatment) and ‘lost’ (negative difference in ERG levels upon JQ1 treatment) we followed the same procedure as described earlier for determining ChIP-seq enrichment levels.

**Murine prostate tumour xenograft model.** Four-week-old male SCID C.B17 mice were procured from a breeding colony at University of Michigan maintained by our group. Mice were anaesthetised using 2% isoflurane (inhalation) and 2 × 10^6 VCaP prostate cancer cells suspended in 100 μl of PBS with 50% Matrigel (BD Biosciences) were implanted subcutaneously into the dorsal flank on both sides of the mice. Once the tumours reached a palpable stage (100 mm^3), the animals were randomized and treated with either 10 mg kg\(^{-1}\) body weight MDV3100 or 50 mg kg\(^{-1}\) JQ1 by oral gavage or intraperitonially, respectively, for five days a week. Highly hormone-responsive seminal vesicles attached to the prostate were harvested from mice after 4 weeks of injection. Prostate were fixed in formalin solution and processed for sectioning. Standard haematoxylin and eosin staining was performed on the formalin-fixed sections, which were used to image the different lobes of the gland. To determine testosterone levels, blood samples were collected by cardiac puncture from mice anaesthetized with isoflurane. The serum was separated from the blood and stored at −80 °C until assayed. Serum testosterone levels were measured by ligand assay at the University of Michigan-ULAM Pathology Cores for Animal Research.

31. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl Acad. Sci. USA 102, 15545–15550 (2005).
32. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357–359 (2012).
33. Li, H. et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
34. Pickrell, J. K., Gaffney, D. J., Glid, Y. & Pritchard, J. K. False positive peaks in ChIP-seq and other sequencing-based functional assays caused by unannotated high copy number regions. Bioinformatics 27, 2144–2146 (2011).
35. Lawrence, M. et al. Software for computing and annotating genomic ranges. PLOS Comput. Biol. 9, e1003118 (2013).
36. Qin, Z. S. et al. HPeak: an HMM-based algorithm for defining read-enriched regions in ChIP-Seq data. BMC Bioinformatics 11, 369 (2010).
37. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842 (2010).
38. Saldanha, A. J. Java Treeview—extensible visualization of microarray data. Bioinformatics 20, 3246–3248 (2004).
39. Tran, C. et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 324, 787–790 (2009).
40. Asangani, I. A. et al. Characterization of the EZH2-MMSET histone methyltransferase regulatory axis in cancer. Mol. Cell 49, 80–93 (2013).
41. van der Horst, E. H., Leuppold, J. H., Schubbert, R., Ulrich, A. & Allgayer, H. TaqMan-based quantification of invasive cells in the chick embryo metastasis assay. Biotechniques 37, 940–942, 944, 946 (2004).
42. Gao, L. et al. Androgen receptor promotes ligand-independent prostate cancer progression through c-Myc upregulation. PLoS ONE 8, e63563 (2013).
Extended Data Figure 1 | BET bromodomain inhibitor JQ1 blocks cell growth, induces apoptosis and transcriptionally suppresses anti-apoptotic factor BCL-xl without affecting BRD2/3/4 proteins. **a**, Cell viability curves for the six prostate lines treated with JQ1. N = 6 wells of a 96-well plate per condition. **b**, BET bromodomain proteins are ubiquitously expressed in prostate cell lines. AR and MYC protein levels are also shown. GAPDH serves as a loading control. **c**, Knockdown of BET bromodomain proteins attenuates cell proliferation and invasion. qRT–PCR analyses of BRD2, BRD3 or BRD4 in VCaP cells transfected with short interfering RNA (siRNA) against their respective transcript or non-targeting (NT) siRNA. Data show mean ± s.e.m. (n = 3) from one of three independent experiments. **d**, VCaP- and LNCaP-cell proliferation after indicated gene knockdown. 20,000 cells were seeded in 24-well plates 24 h post-transfection with siRNAs and counted on day 0, 2, 4 and 6 (n = 3) by Coulter counter. Data show mean ± s.e.m. **e**, VCaP- and LNCaP-cell invasion (n = 6) after indicated gene knockdown. JQ1 was used at 500 nM. **f**, Cell cycle analysis of JQ1-treated prostate cell lines (after 48 h treatment with JQ1). Data represent three independent experiments. **g**, Induction of apoptosis as determined by appearance of cleaved PARP (cPARP) in VCaP prostate cancer cells by JQ1. GAPDH served as a loading control. **h**, Immunoblot demonstrating an increase in cPARP and decrease in BCL-xl in all three AR-positive cell lines compared with AR-negative PC3 cells upon JQ1 treatment. **i**, Relative BCL-xl mRNA levels as determined by TaqMan qPCR in JQ1-treated cells. Data show mean ± s.e.m. (n = 3) from one of three independent experiments. **j**, ChIP-seq data depicting loss of BRD2/3/4 recruitment to the BCL-xl promoter upon JQ1 treatment in VCaP cells. The genome browser representation of BRD2/3/4 binding events on the BCL-xl promoter region. The y-axis denotes reads per million per base pair (r.p.m. bp$^{-1}$), the x-axis denotes the genomic position. The bottom panel depicts the H3K27ac mark on the same promoter region in VCaP cells. **k**, Colony formation assays of prostate cell lines. Cells were cultured in the presence or absence of 100 and 500 nM of JQ1 for 12 days followed by staining (top) and quantification (bottom; mean ± s.e.m. n = 6). Representative photographs of crystal violet stained colonies (except for VCaP) used for quantification are shown. **l**, Cell cycle analysis of JQ1-treated prostate cell lines (after 48 h treatment with JQ1). Data represent three independent experiments. **m**, Colony formation assays of prostate cell lines. Cells were cultured in the presence or absence of 100 and 500 nM of JQ1 for 12 days followed by staining (top) and quantification (bottom; mean ± s.e.m. n = 6). Representative photographs of crystal violet stained colonies (except for VCaP) used for quantification are shown. **n**, BET bromodomain inhibitor JQ1 does not affect its target proteins. qRT–PCR analyses of BRD2, BRD3 and BRD4 in prostate cancer cell line panel treated with two different concentrations of JQ1 for 24 h. Data show mean ± s.e.m. (n = 3) from one of the three independent experiments. **o**, Immunoblot analysis of BRD proteins in prostate cell line panel treated with JQ1 for 48hrs. GAPDH serves as a loading control. Asterisks in **b** and **m** indicate non-specific band. Representative blots shown are from triplicate biological experiment. NS, not significant; *P ≤ 0.01; **P ≤ 0.001 by two-tailed Student’s t-test.
Extended Data Figure 2 | Effect of JQ1 on AR target genes and on MYC transcription. a, qRT–PCR analysis of indicated genes in LNCaP and 22RV1 cells treated with varying concentrations of JQ1 for 24 h. Data show mean ± s.e.m. (n = 3) from one of two independent experiments. b, Immunoblot analysis of AR and PSA in a panel of prostate cancer cells after treatment with two different doses of JQ1. GAPDH serves as a loading control. c, ERG and PSA are transcriptional targets of JQ1. Proteasome inhibitor bortezomib does not rescue ERG and PSA levels in JQ1-treated VCaP cells. Immunoblot analyses of ERG and PSA in VCaP and PSA in LNCaP cells treated with JQ1 followed by incubation with bortezomib as indicated. MYC, known to be degraded by proteasome, was used as a positive control for bortezomib treatment. GAPDH serves as a loading control. d, GSEA showing loss of MYC signature (four-gene set) in AR-positive VCaP, LNCaP and 22RV1 cells but not AR-negative DU145 cells after JQ1 treatment. Size, number of genes in each set; NES, normalized enrichment score. P and false discovery rate (FDR) q values indicate statistical significance. e, qRT–PCR and immunoblot analysis of MYC in JQ1-treated prostate cancer cells. Data show mean ± s.e.m. (n = 3) from one of two independent experiments. f, g, Time-course qRT–PCR and immunoblot analysis of MYC in AR-positive VCaP, LNCaP and 22RV1 cells after JQ1 treatment. h, Cyclohexamide (translation inhibitor) treatment does not enhance JQ1-mediated loss of MYC protein, ruling out post-translational degradation of MYC by JQ1. Time-course immunoblot analysis of MYC in VCaP, LNCaP and 22RV1 cells treated with cyclohexamide or cyclohexamide plus JQ1 as indicated. Representative blots from two independent experiments are shown. i, GAPDH-normalized MYC protein levels are shown. Band intensities from d were determined by ImageJ and the plots were generated using GraphPad Prism. j, MYC knockdown does not affect cell invasion. Box plot shows invasion of VCaP cells transfected with siNT or siMYC. Inset shows the image of invaded VCaP cells (n = 6). Right, qRT–PCR of MYC upon siRNA transfection. Data show mean ± s.e.m. from one of three independent experiments. k, Exogenous MYC introduction does not rescue JQ1-mediated cell growth inhibition. Cells were infected with control adeno-LacZ or adeno-MYC virus. Equal numbers of cells were plated 24 h after infection and treated with 500 nM JQ1 or I-BET762. Cells were counted (n = 3 wells) and plotted; day 0 of drug treatment was set at 100%. Data show mean ± s.e.m. from one of four independent experiments. l, Immunoblot analysis depicts overexpression of MYC in adeno-MYC infected cells on day 0 and day 7 of the experiment. GAPDH serves as a loading control. *P < 0.05; **P < 0.005 by two-tailed Student’s t-test.
Extended Data Figure 3 | Physical association of AR with BRD4 and its disruption by BET bromodomain inhibitor. a, LNCaP nuclear extract was fractionated on a Superose-6 column and AR, BRD4 and RNA Pol II were analysed by immunoblot analysis. b, c, Representative sensorgrams for AR–RNF2, RAS–BRD4(BD1–BD2) and RNF2–BRD4(BD1–BD2) interactions by an OctetRED biolayer interferometry. Real-time binding was measured by immobilizing biotinylated AR, RAS or RNF2 proteins separately on a streptavidin biosensor and subsequent interaction with varying concentrations of analyte proteins (RNF2 or BRD4(BD1–BD2)) individually. Immobilized RAS or RNF2 biosensors did not bind with BRD4, indicating that the AR–BRD4 interaction is specific. Representative sensograms from 4–6 independent experiment are shown. d–f, In vitro binding analysis of AR and indicated domains of BRD4. Equal amounts of in vitro translated full-length Halo–AR protein and GST–BRD4 domains were combined and immunoprecipitated using Halo beads followed by immunoblot analysis with anti-GST antibody. g, JQ1 disrupts the endogenous AR–BRD4 interaction.
Extended Data Figure 4 | Changes in genome-wide enrichment profiles of BRD proteins in response to bromodomain inhibitors. a, Table showing high-throughput sequencing read information for ChIP libraries of BRD2, BRD3, BRD4, AR, RNA Pol II, ERG, H3K27ac and IgG performed for this study. b, ChIP-seq was performed using BRD2, BRD3 and BRD4 antibodies in VCaP cells treated with DMSO, JQ1 or I-BET762 for 12 h. Genome-wide distribution of BRD2, BRD3 and BRD4 enriched sites. Highly significant peaks (see Methods) show relatively high overlap. A large majority of sites are occupied by at least two BRD proteins. BRD2 and BRD3 have the most similar localization pattern. c, BRD proteins show varying degrees of overlap. Shown is the ratio of sites occupied by either protein alone (unique) or co-occupied with another BRD-family protein (overlap). BRD4 shows the largest number of unique peaks. d, BET inhibitors JQ1 and I-BET762 attenuate recruitment of BRD proteins from chromatin. Enrichment levels for each protein were normalized to the median enrichment in vehicle-treated cells. BRD2 and BRD3 proteins show similar responses to both inhibitors, whereas BRD4 is more potently evicted by JQ1. e, BET bromodomain inhibitors deplete target proteins from genomic regions with or without AR. Mean enrichment levels within each subpanel were normalized to the maximum mean enrichment in vehicle-treated cells.
Extended Data Figure 5 | Influence of JQ1 and anti-androgens on genome-wide recruitment of AR and their effect on DHT-induced AR target gene expression. a, Two independent biological replicates of AR ChIP-seq experiments in VCaP cells show high correlation of normalized enrichment levels (see Methods) in the majority of treatment conditions. R² values for each biological duplicate are shown. b, Mean enrichment (coverage) profiles are similar between biological replicates and different between treatment conditions, indicating that no adverse changes in enrichment levels are observed between the replicates. c, Bar graph showing total number of AR peaks for VCaP-treated cells. The genome-wide individual peaks for AR yielded the highest number of peaks for DHT (35,390) whereas vehicle control cells showed only 13,874 peaks. However, the number of peaks for AR was 23,961, 18,264 and 32,212 in the presence of JQ1, MDV3100 and bicalutamide, respectively. d, Heat map representation of AR binding peaks in different treatment groups. Genomic target regions are rank-ordered based on the level of AR enrichment at each androgen response elements (ARE) within −1 kb and +1 kb flanking the genomic region. e, Venn diagram illustrating the overlap of AR-bound genes between different treatment groups. f, AR–BRD4 binding on KLK3 and FASN upstream regions. Genome browser representation of AR and BRD4 binding events on a putative enhancer and super-enhancer of AR-regulated KLK3 and FASN gene, respectively. The y-axis denotes reads per million per base pair (r.p.m. bp −1), the x-axis denotes the genomic position with a scale bar on top right. g, Expression of AR target genes in the presence of JQ1, MDV3100 or bicalutamide. Heat maps for VCaP and LNCaP cells treated with DHT (10 nM), DHT plus JQ1 (0.5 μM), DHT plus MDV3100 (10 μM) and DHT plus bicalutamide (25 μM). Red arrows indicate well-characterized AR target genes. h, qRT–PCR analysis of AR-regulated genes in the VCaP and LNCaP treated cells. To directly compare JQ1 and MDV3100 in blocking AR signalling, cells were treated with varying concentrations of JQ1 or MDV3100 followed by DHT treatment and analysed for AR targets. The reduction in DHT-induced gene expression was observed for JQ1 even at 100–250 nM whereas MDV3100 showed a marginal reduction at 10 μM, demonstrating the higher efficacy of JQ1 in blocking AR target gene expression. Data show mean ± s.e.m. (n = 3) from one of two independent experiments.
Extended Data Figure 6 | Effect of JQ1 on the TMPRSS2-ERG loci and ERG-mediated transcription in VCaP cells.  

a, Genome browser representation of RNA Pol II binding events within the ERG gene body. The y-axis denotes reads per million mapped reads per base pair (r.p.m. bp$^{-1}$), the x-axis denotes the genomic position and the black arrow indicates the region involved in the TMPRSS2-ERG fusion.  
b, AR and BRD4 binding on the promoter of the ERG$^59$-fusion partner TMPRSS2 in VCaP cells. Note the reduced RNA Pol II and AR–BRD4 recruitment levels in DHT plus JQ1 tracks for the ERG gene body and TMPRSS2 promoter respectively.  
c, High reproducibility of ERG ChIP-seq experiments. Biological replicates of ERG ChIP-seq experiments show very high correlation of normalized enrichment levels (see Methods) in the JQ1- and DMSO-treated conditions.  
d, Significant changes in ERG levels upon JQ1 treatment at ERG-binding sites in the proximity of gene loci. Changes in ERG enrichment levels were assessed using DESeq2. Statistically significant differences were observed for ERG gain and ERG loss. Significant ERG gains are associated with quantitatively modest changes in enrichment level. On the other hand, significant ERG losses are associated with greater changes in enrichment levels. Individual number of peaks for each panel is shown.  
e, Genome browser representation of ERG-binding events on bona fide ERG-activating target genes.  
f, Genome browser representation of ERG-binding events on ERG-repressed target genes.  
g, TaqMan qRT–PCR analysis of ERG-activated genes in VCaP cells after JQ1 treatment.  
h, TaqMan QRT–PCR analysis of ERG-repressed genes in VCaP cells after JQ1 treatment. Data represent mean ± standard deviation (s.d.) ($n = 3$) from one of two independent experiments. *$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.0005$ by two-tailed Student’s $t$-test.
## Extended Data Figure 7 | BET bromodomain inhibitors reverse ERG-mediated functions in an isogenic cell line system.

**a, b**, qRT–PCR and immunoblot showing overexpression of ERG in RWPE and PC3 prostate cell lines. Data represent mean ± s.e.m. (n = 3). c, BET inhibitors block ERG-induced RWPE and PC3 cell invasion. RWPE and PC3 cells stably expressing either LacZ or ERG were treated with DMSO (n = 4), 500 nM JQ1 (n = 4) or I-BET762 (n = 4) for 24 h before plating in Matrigel-coated Boyden chambers. After 48 h cell invasion was quantified. Left, representative photomicrographs of invaded cells are shown with a 100 μm scale bar (lower Boyden chamber stained with crystal violet). Right, bar graph shows fold change in cell invasion, with DMSO-treated LacZ-expressing cells set to 1. Data represent mean ± s.e.m. from one of three independent experiments. d, BET inhibitors reverse ERG-induced gene transcription. GSEA of the ERG target gene signature (see Methods) in RWPE cells overexpressing ERG (RWPE-ERG) and PC3-ERG cells treated with JQ1 or I-BET762 (500 nM) for 24 h. ERG-induced genes are repressed by JQ1 or I-BET762 treatment. e, GSEA using a random gene set shows no significant positive or negative enrichment by JQ1 or I-BET762 treatment in RWPE-ERG and PC3-ERG cells. NS, not significant; ***P ≤ 0.0001 by two-tailed Student’s t-test.
Extended Data Figure 8 | JQ1 inhibits ETS (ERG/ETV1) factors that regulate MYC expression in VCaP and LNCaP cells. a, Genome browser representation of ERG- and ETV1-binding events on the MYC distal enhancer\(^4\). JQ1 treatment in VCaP cells reduces ERG enrichment, as shown in two independent ERG ChIP-seq experiments. The y axis denotes reads per million per base pair (r.p.m. bp\(^{-1}\)), the x axis denotes the genomic position. LNCaP ETV1 ChIP-seq data are based on data from ref. 23 (GEO accession code GSM1145322), and show ETV1 recruitment to the MYC distal enhancer. b, ChIP-PCR validation of loss of ERG recruitment after JQ1 treatment in VCaP cells. Data show mean ± s.d. (n = 3) from one of two independent experiments. c, Knock-down of AR or ETS factor reduces MYC gene expression in VCaP and LNCaP cells. qRT–PCR for AR, ETS and MYC expression data. h, Anti-androgen restores DHT-repressed MYC expression in VCaP cells treated with vehicle, DHT (10 nM), DHT plus JQ1 (500 nM), DHT plus MDV3100 (10 μM) or DHT plus bicalutamide (25 μM). Inability of JQ1 to de-repress MYC in this setting could be explained by the fact that both AR and ERG are de-recruited from the MYC distal enhancer, leading to net loss of MYC expression. i, MDV3100 and not JQ1 restores DHT-repressed MYC protein levels in VCaP cells. Immunoblot of MYC protein in VCaP cells pre-treated with vehicle, MDV3100 (10 μM) or JQ1 (500 nM) for 4 h followed by DHT (10 nM) for 20 h. Data show mean ± s.d. (n = 3) from one of two independent experiments. NS, not significant; *P ≤ 0.01; **P ≤ 0.001; ***P ≤ 0.0001 by two-tailed Student’s t-test.

### Table

| Probe ID | Gene   | DHT + JQ1 | DHT + MDV3100 | JQ1 | Vehicle |
|----------|--------|-----------|---------------|-----|---------|
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.26| 0.26    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.26| 0.26    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.04| 0.04    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.77| 0.77    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.67| 0.67    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.04| 0.04    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.67| 0.67    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.04| 0.04    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.77| 0.77    |
| A_23_P215956 | MYC   | 1.00      | 1.00          | 0.67| 0.67    |
Extended Data Figure 9 | JQ1 does not affect normal prostate growth and testosterone levels but reduces testis size in mice. **a**, Comparison of JQ1 and MDV3100 treatment on VCaP cell viability in vitro. N = 8 wells of a 96-well plate per condition. VCaP cells were treated with MDV3100 or JQ1 for 8 days and assayed for viability with Cell-titerGLO. **b**, Gross images showing highly hormone-responsive seminal vesicles attached to prostate gland (red and black arrows, respectively) from male mice treated for 30 days with vehicle, JQ1 (50 mg kg\(^{-1}\)) or MDV3100 (10 mg kg\(^{-1}\)). Vehicle or JQ1-treated mice show no change in the appearance of seminal vesicles. By contrast, MDV3100-treated animals show remarkable shrinkage of seminal vesicles. **c**, Mice treated with JQ1 do not show any adverse changes to anterior or ventral prostate morphology. The haematoxylin and eosin images show normal morphology of anterior and ventral prostate from vehicle- or JQ1-treated mice. MDV3100-treated mice show attenuated remnant glands of anterior or ventral prostate. **d**, Male mice (n = 3 per group) treated with vehicle or JQ1 for 30 days exhibit similar serum testosterone levels. Data represent the mean ± s.e.m. **e**, Gross analysis of testis from mice treated with vehicle or JQ1 for 30 days. **f**, Testis weight from vehicle control or JQ1-treated mice. Data represent the mean ± s.e.m. from n = 7 mice per group. NS, not significant; *P ≤ 0.0001 by two-tailed Student’s t-test.
Extended Data Figure 10 | In vivo effects of BET bromodomain inhibition in VCaP xenograft model. a, VCaP cells were implanted subcutaneously in mice and grown until tumours reached a size of approximately 100 mm³. Xenografted mice were randomized and then received vehicle, 50 mg kg⁻¹ JQ1 or 10 mg kg⁻¹ MDV3100 5 days a week as indicated. Calliper measurements were taken twice a week. Individual tumour volumes from different treatment groups at the end of the experiments with P values are shown. b, MDV3100 treatment leads to spontaneous metastasis. Mice bearing VCaP xenografts (subcutaneously engrafted) treated with vehicle (n = 6) or MDV3100 (n = 6) were assessed for spontaneous metastasis to the femur (bone marrow) and soft tissues such as liver and spleen. Genomic DNA isolated from these sites was analysed for metastasized cells by measuring human Alu sequence (by Alu-qPCR). MDV3100-treated mice showed spontaneous metastasis to femur and liver. Spleen did not show presence of human ALU sequences. c, As in a, for mice bearing VCaP xenografts treated with vehicle (n = 6), JQ1 (n = 6) or MDV3100 (n = 6), MDV3100-treated but not JQ1-treated mice showed metastasis to femur and liver. d, JQ1 or MDV3100 treatment does not affect animal weight. Mice from VCaP cell xenograft experiments treated with vehicle, 10 mg kg⁻¹ MDV3100 or 50 mg kg⁻¹ JQ1 were weighed at the time of calliper measurements. e, Individual tumour volume for vehicle- or JQ1-treated VCaP mouse xenograft (for data shown in Fig. 4c). Mean ± s.e.m. is plotted. Statistical significance was determined by two-tailed Student’s t-test.