Transcriptional addiction in cancer cells is mediated by YAP/TAZ through BRD4

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Cancer cells rely on dysregulated gene expression. This establishes specific transcriptional addictions that may be therapeutically exploited. Yet, the mechanisms that are ultimately responsible for these addictions are poorly understood. Here, we investigated the transcriptional dependencies of transformed cells to the transcription factors YAP and TAZ. YAP/TAZ physically engage the general coactivator bromodomain-containing protein 4 (BRD4), dictating the genome-wide association of BRD4 to chromatin. YAP/TAZ flag a large set of enhancers with super-enhancer-like functional properties. YAP/TAZ-bound enhancers mediate the recruitment of BRD4 and RNA polymerase II at YAP/TAZ-regulated promoters, boosting the expression of a host of growth-regulating genes. Treatment with small-molecule inhibitors of BRD4 blunts YAP/TAZ pro-tumorigenic activity in several cell or tissue contexts, causes the regression of pre-established, YAP/TAZ-addicted neoplastic lesions and reverts drug resistance. This work sheds light on essential mediators, mechanisms and genome-wide regulatory elements that are responsible for transcriptional addiction in cancer and lays the groundwork for a rational use of BET inhibitors according to YAP/TAZ biology.

An emerging paradigm in cancer biology relates to the concept of 'transcriptional addiction': it posits that, to support their uncontrolled proliferation or other needs, tumor cells set high demands on transcriptional regulators, including chromatin regulators and even the basal transcriptional machinery1,2. The molecular mechanisms underlying the transcriptional dependency of cancer cells are poorly understood. Yet, it is an appealing concept, as general chromatin regulators or transcriptional cofactors are amenable to inhibition with small molecules3. The emblematic example is the antitumor activity of bromodomain and extraterminal domain (BET) inhibitors in various xenograft model systems and clinical trials4–6. BET inhibitors oppose the activity of BET coactivators (that is, bromodomain-containing protein 4 (BRD4) and its related factors BRD2 and BRD3)7. Although BET proteins have been proposed to serve as general regulators of RNA polymerase II-dependent transcription, genome-wide studies have instead shown that BET inhibitors display selective effects on gene expression7. In particular, BET inhibitors have been reported to have a disproportional effect on a set of highly expressed genes associated with super-enhancers8. The molecular basis of the transcriptional addiction associated to super-enhancers in cancer cells, as well as the determinants of the selectivity of BET inhibitors remain undefined8.

The transcriptional coactivators YAP (Yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif) are ideal candidates to mediate cancer-specific transcriptional addictions. In fact, YAP/TAZ are genetically dispensable for homeostasis in many adult tissues9–11, whereas YAP/TAZ activation is a hallmark of many human malignancies12–19. Here, we show that tumor transcriptional dependencies in fact overlap with tumor reliance on YAP/TAZ.

Results

BRD4 interacts with YAP/TAZ. With this background in mind, we started this investigation by carrying out chromatin immunoprecipitation with mass spectrometry (ChIP–MS) for endogenous YAP/TAZ, a procedure that allows the study of the composition of the native protein complexes entertained by YAP/TAZ and, in particular, nuclear interactions20. We detected some well-known nuclear partners of YAP/TAZ, including TEAD (the main YAP/TAZ DNA-interacting partner) and activator protein 1 (AP-1) family members21 and several subunits of the SWI/SNF complex22. YAP/TAZ protein complexes were also enriched in chromatin readers or modifiers, such as BRD4, histone acetyltransferases (HATs; p300 and p400) and the histone methyltransferase KMT2D (also known as MLL2) (Supplementary Table 1). The roles of p300, SWI/SNF and the H3K4 methyltransferase complexes in the context of YAP-dependent transcription have been previously described23–25. The association with BRD4 attracted our attention, as this hinted to a connection between YAP/TAZ-regulated gene expression and the transcriptional addiction of cancer cells.

To validate the interactions detected by ChIP–MS, we performed co-immunoprecipitation (co-IP) of endogenous proteins, revealing the presence of BRD4 and TEAD1 in YAP and TAZ immunocomplexes, and of YAP, TAZ and TEAD1 in BRD4 immunocomplexes (Fig. 1a). By proximity ligation assays (PLAs), we validated that...
**Fig. 1** BRD4 associates to YAP/TAZ and is a required cofactor for YAP/TAZ transcriptional activity. 

**a.** Interaction of endogenous YAP/TAZ, TEAD1 and BRD4 in MDA-MB-231 cells. Each co-IP experiment was performed three times with similar results. 

**b.** Endogenous YAP, TAZ or TEAD1 and exogenous BRD4 in MDA-MB-231 cells. The expression values (in RPKM) were determined by RNA-seq and are presented as box-and-whiskers plots (whiskers extend from the 10th to the 90th percentile; the box extends from the 25th to the 75th percentile; the line within the box represents the median). ****P<10⁻¹⁰ (one-tailed Mann–Whitney U-test). 

**c.** Recombinant BRD4 (rBRD4) is pulled down by the GST-YAP fusion protein. GST pulldown was repeated three times with similar results. WB, western blot. 

**d.** Genes activated by YAP/TAZ (n=2,073) are more likely to be inhibited by BET inhibitors than the not YAP/TAZ target genes. The CDK inhibitors and RG-108 do not display such effects. 

**e.** Fold change in gene expression of high-confidence YAP/TAZ direct targets (n=616) versus not YAP/TAZ targets (n=771) upon treatment with JQ1 or BET protein depletion. The y axis shows the fold change in the transcript levels versus DMSO-treated cells or cells transfected with siCO. Data are presented as box-and-whiskers plots, as in d. ****P<10⁻¹⁰ (one-tailed Mann–Whitney U-test). The expression level of all YAP/TAZ-activated genes (n=2,073) in MDA-MB-231 cells treated with DMSO (vehicle), BET inhibitors (JQ1 or OTX015), CDK inhibitors (flavopiridol or THZ1) or RG-108 (a DNA methyltransferase inhibitor, here used as a negative control to assess the effect of a compound targeting an epigenetic function that is not related to YAP/TAZ-dependent transcription). The expression levels were determined by RNA-seq and are presented as z-scores. Individual genes and their mean (black line) are presented. x axis is represented as a black dotted line. The group of not YAP/TAZ targets represents genes that are not significantly affected by YAP/TAZ depletion (FDR >0.05) in our RNA-seq data set. Data are presented as box-and-whiskers plots, as in d. ****P<10⁻¹⁰ (one-tailed Mann–Whitney U-test). The expression level of all YAP/TAZ-activated genes (n=2,073) in MDA-MB-231 cells treated with DMSO (vehicle), BET inhibitors (JQ1 or OTX015), CDK inhibitors (flavopiridol or THZ1) or RG-108 (a DNA methyltransferase inhibitor, here used as a negative control to assess the effect of a compound targeting an epigenetic function that is not related to YAP/TAZ-dependent transcription). The expression levels were determined by RNA-seq and are presented as z-scores. Individual genes and their mean (black line) are presented. x axis is represented as a black dotted line. The group of not YAP/TAZ targets represents genes that are not significantly affected by YAP/TAZ depletion (FDR >0.05) in our RNA-seq data set. Data are presented as box-and-whiskers plots, as in d. ****P<10⁻¹⁰ (one-tailed Mann–Whitney U-test).
this interaction occurs in the nucleus (Fig. 1b). Furthermore, by co-IP, transfected FLAG-tagged YAP co-purified endogenous BRD4 and BRD2 (Supplementary Fig. 1a). Importantly, the association between YAP or TAZ and BRD4 is direct, as attested by the interactions between purified recombinant proteins (Fig. 1c and Supplementary Fig. 1b). By using progressive carboxy-terminal deletion constructs, we mapped the minimal region sufficient for association with BRD4 between amino acids 108 and 175 of mouse TAZ (Supplementary Fig. 1c); notably, this region includes the WW domain21. However, removal of the sole WW domain from full-length TAZ did not impair its ability to associate with BRD4 (Supplementary Fig. 1d), indicating that another determinant for BRD4 association exists in the C-terminal transactivation domain.

Overall, the data indicate that YAP, TAZ, TEAD1 and BET proteins are part of the same nuclear multiprotein complex.

**YAP/TAZ mediate cancer transcriptional addiction.** To study the connection between YAP/TAZ and transcriptional addiction in cancer, we used MDA-MB-231 cells, a well-established model of triple-negative breast cancer (TNBC), a tumor type that requires high levels of uninterrupted transcription of a large set of genes to sustain its particularly aggressive nature22-24. Are YAP/TAZ causal to these dependencies? By comparing the transcriptional profiles (obtained by RNA sequencing (RNA-seq)) of control and YAP/TAZ-depleted cells (Supplementary Fig. 1e,f), we found that genes whose expression depends on YAP/TAZ were significantly more expressed than all the other genes (Fig. 1d); this conclusion was confirmed also when we restricted our analyses to high-confidence direct YAP/TAZ target genes, that is, genes dependent on YAP/TAZ whose promoters or enhancers contain YAP/TAZ-binding sites as assessed by ChIP followed by sequencing (ChIP–seq)1 (Supplementary Fig. 1g).

Moreover, genes whose biological function is associated with cell proliferation (~1,500 genes, as determined by Gene Ontology (GO) annotation) were transcribed at higher levels than the bulk of expressed genes (Fig. 1e); silencing YAP/TAZ with siRNAs led to a global downregulation of such a ‘growth program’ (Fig. 1e), in line with the previous report that MDA-MB-231 cells depleted of YAP/TAZ undergo growth arrest1. Indeed, 37% of the growth genes actively transcribed in these cells are YAP/TAZ targets (541 out of 1,449); their transcripts were particularly abundant, displaying a higher level of expression than non-YAP/TAZ targets associated with the same biological function (Supplementary Fig. 1h). Thus, the activation of essential growth genes in MDA-MB-231 cells relies on YAP/TAZ.

**Vulnerability of YAP/TAZ activity to BET inhibitors.** To assess whether the interaction with BRD4 is causal for the activation of YAP/TAZ transcriptional targets, we performed RNA-seq in cells treated with JQ1, the most established BET inhibitor. JQ1 occupied the bromodomain pockets of BET proteins in a manner that is competitive with the binding to acetylated histone tails, causing their displacement from chromatin1. Most YAP/TAZ-regulated genes (68%) displayed exquisite sensitivity to JQ1 (Fig. 1f and Supplementary Fig. 1i), and vice versa; genes that were most effectively downregulated by JQ1 were in fact YAP/TAZ dependent (Supplementary Fig. 1j). Indeed, treatment with JQ1 selectively decreased the transcript abundance of YAP/TAZ-dependent genes compared to all other active genes (Fig. 1g). The disproportional effect of JQ1 was confirmed when we restricted our analyses to high-confidence direct YAP/TAZ target genes (Fig. 1h).

The bias of JQ1 towards inhibition of YAP/TAZ-dependent genes (including direct targets) was also evident when restricting the analysis to genes regulating cell proliferation (Supplementary Fig. 1k–l); BET inhibition affected the expression of 604 genes associated to GO terms linked to cell proliferation and 428 of these (71%) were regulated by YAP/TAZ. Thus, the sensitivity of a broad number of growth-regulating genes to BET inhibition relies on YAP/TAZ. Effects similar to those of JQ1 were obtained with another BET inhibitor (OTX015; Fig. 1i,j and Supplementary Fig. 1l,o) and by knocking down BRD2/3/4 with two independent combinations of siRNAs (Fig. 1g,h). Moreover, the depletion of the sole BRD4 was sufficient, at least in part, to downregulate YAP/TAZ target genes (Supplementary Fig. 1m). We also found that endogenous YAP/TAZ remained nuclear upon treatment with BET inhibitors (Supplementary Fig. 1n), excluding the possibility that the compounds would indirectly cause YAP/TAZ cytoplasmic relocation.

YAP/TAZ transcriptional control has been connected to cyclin-dependent kinase 9 (CDK9)-induced elongation of nascent transcripts by Pol II17. Here, we find that, in stark contrast with BET inhibitors, inhibition of transcriptional CDKs with flavopiridol or THZ1 failed to display any bias towards inhibition of YAP/TAZ transcriptional targets (Fig. 1j and Supplementary Fig. 1o). Collectively, the data indicate that the physical association between YAP/TAZ and BRD4 is functionally relevant; BRD4 is a required cofactor for YAP/TAZ, conferring to YAP/TAZ target genes a specifically high dependency on BRD4 and vulnerability to BET inhibitors.

**BRD4 is recruited to chromatin by YAP/TAZ.** We next asked what underlies the disproportionate sensitivity of YAP/TAZ targets to BET inhibitors. To gain mechanistic insights into this connection, we performed ChIP–seq experiments to compare BRD4 and YAP/TAZ binding to chromatin. YAP/TAZ bind almost exclusively to enhancers13,23,27, whereas BRD4 binds to both active enhancers and active promoters (Supplementary Fig. 2a,b; see Methods for the definition of enhancers and promoters). We started our analysis from enhancer elements and found that BRD4 coverage was higher on enhancers containing YAP/TAZ-binding sites than on active enhancers not occupied by YAP/TAZ (Fig. 2a). We reasoned that differential BRD4 loading might correspond to differential responsiveness to JQ1; to verify this assumption, we performed BRD4 ChIP-seq in cells treated with JQ1. We found that JQ1 induced a preferential loss of BRD4 from YAP/TAZ-occupied enhancers compared to active enhancers without YAP/TAZ-binding sites (Fig. 2a and Supplementary Fig. 2c). Thus, the presence of YAP/TAZ peaks defines enhancers enriched of BRD4 and that are highly sensitive to BET inhibitors on the genome-wide scale. Do these elements correspond to super-enhancers? In fact, 80% of super-enhancers in MDA-MB-231 cells do contain YAP/TAZ peaks; yet, the vast majority (85%) of YAP/TAZ-occupied enhancers are by definition typical enhancers (Supplementary Fig. 2f,g). However, we observed that genes connected to YAP/TAZ-bound typical enhancers displayed a sensitivity to JQ1 that was strikingly similar to the much more restricted number of genes associated with super-enhancers (Supplementary Fig. 2h).

We then assessed whether the presence of YAP/TAZ was required for the engagement of BRD4 to chromatin, by performing BRD4 ChIP-seq in MDA-MB-231 cells depleted of YAP/TAZ. As shown by the average BRD4-binding profile and some representative enhancers in Fig. 2b,c, BRD4 recruitment to YAP/TAZ-containing enhancers was heavily reduced upon YAP/TAZ depletion, to an extent similar to JQ1 (see also Supplementary Fig. 2c–e). The determinants that drive the selectivity of BRD4 association to specific chromatin sites are unclear. BRD4 was reported to bind to some acetylated transcription factors through the bromodomain; however, arguing against this possibility in the case of YAP/TAZ, we found that their biochemical association with BRD4 is not affected by the presence of JQ1 or by mutations in the BRD4 bromodomains (Supplementary Fig. 2i). Thus, the comparable impoverishment of BRD4 recruitment to chromatin detected genome wide after YAP/TAZ depletion or JQ1 treatment reflects the need of a dual
Fig. 2 | YAP/TAZ are required for BRD4 recruitment to chromatin. a, Comparison of the BRD4 ChIP-seq signal (expressed as normalized read density, RPKM) in active enhancers with or without YAP/TAZ peaks (n = 5,169 and n = 30,281, respectively) in MDA-MB-231 cells treated with DMSO or JQ1 (1 µM, 6 h), or transfected with YAP/TAZ siRNAs (48 h). Data are presented as box-and-whiskers plots (whiskers extend from the 5th to the 95th percentile; the line within the box represents the median). **** P < 10\(^{-10}\) (one-tailed Mann–Whitney U-test), ++ + + + P < 10\(^{-10}\) (one-tailed Wilcoxon matched-pairs signed rank test). b, Average signal of BRD4 ChIP-seq reads in enhancers with YAP/TAZ peaks (n = 5,169) in a window of ±1 kb centered on the summit of YAP/TAZ peaks. c, Genome browser view of YAP-, BRD4- and H3K4me1-binding profiles at representative enhancers in MDA-MB-231 cells. Both JQ1 and YAP/TAZ siRNA induce a strong decrease in BRD4 binding. chr, chromosome. d, Box plot of the BRD4 ChIP-seq signal (RPKM) comparing the promoters of genes that are not activated by YAP/TAZ (n = 8,026) or of YAP/TAZ target genes (n = 616) in MDA-MB-231 cells (treated with DMSO). Data are presented as in a. **** P < 10\(^{-10}\) (one-tailed Mann–Whitney U-test). e, Treatment with JQ1 and YAP/TAZ depletion induce preferential loss of BRD4 at promoters of YAP/TAZ target genes (n = 616) versus promoters of not YAP/TAZ targets (n = 8,026). The fold change in BRD4 binding is calculated as RPKM	extsubscript{JQ1=DMSO}/RPKM	extsubscript{DMSO}. Data are presented as in a. **** P < 10\(^{-10}\) (one-tailed Mann–Whitney U-test). f, Heatmap showing BRD4 binding on the promoters of YAP/TAZ targets in MDA-MB-231 cells, in a window of ±1.5 kb centered on the TSS. g, Average signal of BRD4 ChIP-seq reads on the promoters of YAP/TAZ target genes (n = 616) in MDA-MB-231 cells, in a window of ±1.5 kb centered on the TSS. h, YAP-, BRD4- and H3K4me3-binding profiles at representative promoters of YAP/TAZ target genes (left) or not YAP/TAZ targets (right). The black arrows indicate BRD4 enrichment at the TSS. JQ1 and siYAP/TAZ induce a strong decrease in BRD4 binding on YAP/TAZ targets, whereas there is no overt variation of BRD4 binding on the TSS of not YAP/TAZ targets. GINS1 exemplifies direct YAP/TAZ target genes with YAP/TAZ-binding sites in both enhancers and the TSS; EZF3 exemplifies direct YAP/TAZ target genes regulated by YAP/TAZ exclusively from distal enhancers. i, YAP-, BRD4- and H3K4me3-binding profiles on a distal enhancer (left) and on the CDCAS5 promoter (right). JQ1 (1 µM, 6 h) and siYAP/TAZ (48 h) induce a strong decrease in BRD4 binding both on the enhancer, containing the YAP/TAZ peak, and on the TSS of CDCAS5.
association of BRD4 to YAP/TAZ and acetylated histones to keep BRD4 anchored to a large set of enhancers and super-enhancers, as such providing selectivity to BRD4 function.

We then surmised that the disproportionate sensitivity of YAP/TAZ targets to inhibition by JQ1 should be ultimately explained at the level of YAP/TAZ-regulated promoters. Focusing on high-confidence direct YAP/TAZ target genes (see Supplementary Table 2), we found that: (1) the transcription start site (TSS) of these genes exhibited higher BRD4 occupancy than the TSS of genes not activated by YAP/TAZ (Fig. 2d); (2) YAP/TAZ were required for BRD4 accrual on the promoters of their targets while marginally affecting the promoters of non-YAP/TAZ targets (Fig. 2e–g; see also Fig. 2h,i for the representative individual gene tracks and Supplementary Fig. 2) for validation by ChIP followed by quantitative PCR (ChIP-qPCR); and (3) JQ1 caused the preferential loss of BRD4 from YAP/TAZ-regulated promoters (Fig. 2e–g), matching the effects of JQ1 on gene expression. Importantly, as exemplified in Fig. 2h, JQ1 had only minor effects on BRD4 coverage on the promoters of genes that are not activated by YAP/TAZ, where it was in fact insufficient to induce a general downregulation of transcription (Fig. 1g,h). Thus, BRD4 levels at promoters closely reflect the dynamic of YAP/TAZ-mediated engagement of BRD4 at distant enhancers.

**Mechanisms of transcriptional addiction.** The data presented above indicate a model whereby YAP/TAZ bound to enhancers promote BRD4 overload on their target promoters, establishing higher expression levels of essential genes and, concomitantly, their vulnerability to BET inhibitors. In agreement with this prediction, treatment with BET inhibitors does not alter YAP/TAZ recruitment to chromatin (Supplementary Fig. 3a); yet, BET inhibitors block downstream gene expression, even in conditions in which YAP is overexpressed, consistent with YAP/TAZ acting upstream of BRD4 (Supplementary Fig. 3b). In line with this, in the absence of YAP/TAZ, even overexpressed BRD4 could not promote the expression of YAP/TAZ target genes (Supplementary Fig. 3c).

Next, we validated the functional interdependency between YAP/TAZ and BET proteins by overexpressing YAPSSA (a constitutively active version of YAP) in mammary epithelial cells (MCF10A), which normally display low YAP/TAZ activity. By ChIP, exogenous YAP is recruited at its cognate chromatin sites (Supplementary Fig. 3d); in turn, this leads to BRD4 recruitment at the same enhancer sites and associated promoters (Fig. 3a). BET inhibitors block BRD4 recruitment to these cis-regulatory elements, supporting the requirement of BRD4 binding to histones along with YAP/TAZ. By gene expression, exogenous YAP turns on its targets, but not in cells treated with JQ1 or depleted of BET proteins (Fig. 3b and Supplementary Fig. 3e), indicating that BRD4 operates downstream of YAP/TAZ. The same conclusion applies when we experimentally activated endogenous YAP/TAZ through inactivation of the Hippo pathway (Supplementary Fig. 3f).

We next investigated the mechanisms by which BRD4 accrual regulates transcription initiated at YAP/TAZ-target promoters. The role of BRD4 for transcriptional activation is best understood in terms of promotion of elongation through the recruitment of P-TEFBb. If so, we should expect that, upon YAP/TAZ depletion or JQ1 treatment, Pol II should remain paused, if not accumulate, on the promoters of YAP/TAZ targets. In contrast to our expectations, by ChIP–seq, Pol II loading was selectively decreased on the promoters of YAP/TAZ targets in YAP/TAZ-depleted MDA-MB-231 cells (Fig. 4a,b and Supplementary Fig. 4a–c). This implies that YAP/TAZ promote the recruitment of Pol II. Indeed, we detected an association between YAP and Pol II in endogenous complexes by co-IP (Supplementary Fig. 4d); intriguingly, this interaction was lost after experimental depletion of BRD4, at least suggesting that

![Fig. 3](https://example.com/fig3.png)
the latter serves as a key element in connecting YAP/TAZ-bound cis-regulatory elements with the transcriptional apparatus assembled on cognate promoters. In line with this, Pol II coverage on the TSSs of YAP/TAZ target genes was on average higher than all other expressed genes (Fig. 4a), proportional to BRD4 binding (Fig. 4c), and selectively reduced by JQ1 treatment (Fig. 4d). Representative gene tracks showing comparable effects of JQ1 and YAP/TAZ depletion on Pol II loading on the TSSs of YAP/TAZ target genes (but no substantial effects on non-YAP/TAZ targets) are presented in Fig. 4e and Supplementary Fig. 4e. Similar results were obtained after siRNA-mediated depletion of BRD2/3/4, as revealed by ChIP-qPCR at sampled promoters (Fig. 4f). This suggests that YAP/TAZ recruit Pol II by inducing BRD4 accumulation at the TSS.

BRD4 has been recently reported, and we have repeated, to display an intrinsic acetyltransferase activity, leading to acetylation of K122 in the globular domain of H3 (H2K122ac)28. Intriguingly, H3K122ac is associated with Pol II loading on promoters and transcriptional activation29; consistently, we discovered that the HAT domain of BRD4 is indeed crucial for the expression of YAP/TAZ target genes, as a HAT-defective BRD4 (ΔHAT) cannot substitute wild-type BRD4 (Supplementary Fig. 4f). We measured the levels of H3K122ac by ChIP-seq in control, YAP/TAZ-depleted or JQ1-treated cells. Strikingly, H3K122ac levels were significantly higher on the promoters of YAP/TAZ target genes (Fig. 4g), in line with the higher coverage of BRD4 (Supplementary Fig. 4g) and Pol II, and with transcriptional activation. This differential enrichment of H3K122ac was dependent on YAP/TAZ, as robust loss in H3K122ac was observed in YAP/TAZ targets upon YAP/TAZ depletion (Fig. 4h–k and Supplementary Fig. 4h). Paralleling BRD4 occupancy, H3K122ac on the promoters of YAP/TAZ targets was especially sensitive to JQ1 treatment (Fig. 4h–k and Supplementary Fig. 4h). Thus, we propose that YAP/TAZ promote the transcriptional activation of their target genes by favoring BRD4 overload on their promoters, therefore favoring Pol II recruitment through H3K122ac and association to Pol II.

**BET inhibition opposes YAP/TAZ pro-tumorigenic functions.**

To expand on the generality of the YAP/TAZ–BRD4 connection, we then asked whether YAP/TAZ transcriptional activity is especially sensitive to BET inhibitors in TNBC cell lines other than MDA-MB-231. For this, we measured the effects of YAP/TAZ depletion or JQ1 treatment on the expression level of a set of YAP/TAZ target genes and on cell proliferation. As shown in Fig. 5a–c, TNBC cells that are YAP/TAZ addicted are also sensitive to JQ1. Interestingly, we also found one cell line, BT-20, that is not YAP/TAZ addicted and is also resistant to BET inhibitors.

Next, we evaluated the effect of BRD4 silencing on YAP-induced cell transformation. For this, we performed a colony formation assay in soft agar with MCF10A cells, which are, per se, unable to seed colonies but acquire this capacity upon overexpression of YAPSSA. Downregulation of BRD4 (with shRNAs) reduced the number of colonies initiated by YAP-overexpressing cells (Fig. 5d and Supplementary Fig. 5a). In line with this, the addition of JQ1 to culture medium potently impaired initial colony formation (Fig. 5e), as well as the growth of established colonies (Fig. 5f), in agreement with the effects of JQ1 on the transcriptional and epigenetic effects of YAP expression.

Extending the translational significance of these findings, we assayed whether inhibition of BET proteins could blunt the growth of, if not cure, YAP/TAZ-addicted mammary tumors in vivo. Constitutive activation of the Wnt cascade in the mammary epithelium has been shown to induce TNBC-like tumors in mice30, a finding that parallels the high frequency of adenomatous polyposis coli (APC) epigenetic silencing in human TNBC31. YAP/TAZ are potently activated by aberrant Wnt signaling and, consistently, MMTV-cre; Apcfl/fl mice exhibited massive YAP stabilization (Fig. 5h).

By 8 weeks of age, MMTV-cre; Apcfl/fl mice displayed massive overgrowth of the mammary epithelium, with panductal and panlobular atypical hyperplasia and fibrosis (Fig. 5g and Supplementary Fig. 5d), expansion of the luminal layer and large discontinuities in the basal/myoepithelial layer (Fig. 5h) and Supplementary Fig. 5e), collectively configuring a preneoplastic/early neoplastic scenario. Strikingly, all of these lesions did not develop in MMTV-cre; Apcfl/fl mice also bearing Yap and Taz conditional alleles (Fig. 5g,h), indicating that YAP/TAZ are required for epithelial overgrowth and the development of these mammary lesions. We next tested whether established, already grown neoplastic lesions in MMTV-cre; Apcfl/fl mice could be treated by administration of BET inhibitors. For this, we treated 8-week-old female mice (that is, with an overt mammary gland phenotype) with a potent BET inhibitor (BAY-1238097 (ref. 32), 75 mg per kg per week) for 6 weeks. Strikingly, at the end of the treatment, lesions had greatly regressed due to cell death and epithelial remodeling with few remaining signs of mammary hyperplasia or fibrosis to an extent that the main mammary ducts returned to a normal appearance (Fig. 5i,j) and Supplementary Fig. 5f).

As a control, treatment of Apcfl/fl siblings (that is, lacking Cre expression)

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**Fig. 4 | YAP/TAZ and BRD4 regulate Pol II loading and H3K122ac on TSSs.**

**a.** Box-and-whiskers plots showing the distribution of the Pol II ChIP-seq signal (expressed as normalized read density, RPKM) in MDA-MB-231 cells comparing the promoters of genes that are not activated by YAP/TAZ (n = 8,026) or of YAP/TAZ target genes (n = 616) in control (DMSO) or YAP/TAZ-depleted cells. The box includes values within the 25th and 75th percentile (with the median highlighted by the line in the middle) and whiskers extend from the 5th to the 95th percentile. ** **** P < 10−10 (one-tailed Mann–Whitney U-test).

**b.** Heatmap showing Pol II loading on the promoters of YAP/TAZ targets in MDA-MB-231 cells, in a window of ±1.5 kb centered on the TSS.

**c.** Linear correlation between BRD4 and Pol II occupancy on the TSSs of YAP/TAZ target genes (n = 616). R² was calculated using the linear regression analysis (F-test P < 0.0001).

**d.** Box-and-whiskers plots (defined as in a) showing the change in Pol II promoter occupancy in JQ1-treated cells versus control cells (DMSO), comparing the promoters of genes that are not activated by YAP/TAZ (n = 8,026) with the promoters of YAP/TAZ target genes (n = 616). **** P < 10−10 (one-tailed Mann–Whitney U-test).

**e.** Genome browser view of Pol II-binding profiles at representative promoters of YAP/TAZ target genes (left) or not YAP/TAZ targets (right). Pol II binding is reduced upon JQ1 treatment or YAP/TAZ depletion on the TSSs of YAP/TAZ targets.

**f.** ChIP-qPCR verifying Pol II binding to the promoters of established YAP/TAZ targets upon depletion of BET proteins. The GAPDH promoter represents a non-YAP/TAZ target. ChIP with pre-immune IgG displayed background signal (which was comparable in all samples). DNA enrichment was calculated as the fraction of input and is presented as the percentage of Pol II binding in control cells (siCTRL).

**g.** Box-and-whiskers plots of the H3K122ac ChIP-seq signal (RPKM), showing its enrichment on YAP/TAZ target genes (n = 616) compared with inactive promoters (n = 4,618) and not YAP/TAZ targets (n = 8,026) in MDA-MB-231 cells. Data are presented as in a. **** P < 10−10 (one-tailed Mann–Whitney U-test).

**h.** Box-and-whiskers plots showing the change in H3K122ac promoter levels in YAP/TAZ-depleted cells (siYAP) or JQ1-treated cells, both compared to control cells (DMSO). The preferential loss of H3K122ac at the promoters of YAP/TAZ target genes (n = 616) versus the promoters of not YAP/TAZ targets (n = 8,026) is shown. Data are presented as in a. **** P < 10−10 (one-tailed Mann–Whitney U-test).

**i.** Heatmap showing H3K122ac on the promoters of YAP/TAZ targets in MDA-MB-231 cells, in a window of ±1.5 kb centered on the TSS.

**j.** Average ChIP-seq profile of H3K122ac on the promoters of YAP/TAZ target genes (n = 616) in MDA-MB-231 cells, in a window of ±1.5 kb centered on the TSS.

**k.** Genome browser view of H3K122ac levels at representative promoters of YAP/TAZ target genes (right) versus not YAP/TAZ targets (left).
was overtly well tolerated and inconsequential for mammary gland homeostasis (Supplementary Fig. 5g,h). Thus, BET inhibition defines a vulnerability for YAP/TAZ-driven mammary tumors, in line with the results obtained in human TNBC cell lines.

We next explored the functional dependence of YAP/TAZ on BET proteins in tissues other than the mammary gland. For this, we opted for the mouse liver, a classic model system for the study of YAP/TAZ function in vivo.33,34. As previously reported35, YAP activation in hepatocytes of adult Albumin-creERT2; R26-LSL-rtTA; tet-O-YAPsiS127A mice (Supplementary Fig. 6a,b) promotes a pre-neoplastic condition, in which differentiated hepatocytes transdifferentiate into liver progenitor cells labeled by SOX9, a YAP/TAZ direct target in the liver (Fig. 6a); these cells then contribute to the generation of a ductular reaction, defined by small (‘oval’) cells infiltrating the liver parenchyma (Fig. 6b). Remarkably, treatment with a BET inhibitor (BAY-1238097) abolishes the appearance of both transdifferentiating cells (captured ‘in transition’ by the co-expression of SOX9 and the hepatocyte-specific HNF4-α) and ductular reactions (Fig. 6a,b and Supplementary Fig. 6c,d), without affecting the expression of the transgene (Supplementary...
Fig. 5 | Treatment with a BET inhibitor blunts YAP/TAZ-addicted breast tumors. a. Heatmap showing the regulation of YAP/TAZ target genes in TNBC cells after YAP/TAZ depletion (siYT1 and siYT2) or treatment with BET inhibitors (1µM, 24 h). The expression values are normalized to cells transfected with control (siRNA) and to GAPDH. b. Left: viability curves of TNBC cells treated with increasing doses of JQ1 (1nM to 100µM). Data are the mean of n = 8 independent wells (independently treated and evaluated). x axis is represented as a dashed line. Right: IC50 (half-maximum inhibitory concentration) of the listed cell lines. c. Proliferation of BT-20 cells is not impaired by YAP/TAZ depletion, whereas all cells sensitive to JQ1 are also affected by YAP/TAZ depletion. d. BRD4 downregulation by shRNAs impairs colony formation by YAPSSA-overexpressing MCF10A cells in soft agar. Data are presented as individual data points (n = 3 independent samples) ± average (bar), from one of three experiments, providing similar results. Similar results were obtained in MDA-MB-231 cells, whose colony-forming capacity depends on endogenous YAP/TAZ (see Supplementary Fig. 5b). e. Quantification of colonies formed by YAPSSA-overexpressing MCF10A cells in soft agar, upon treatment with 0.1µM or 1µM JQ1 for the entire experiment. Data are presented as in d. Similar results were obtained in MDA-MB-231 cells (see Supplementary Fig. 5c). f. Inhibition of the growth of colonies initiated by YAPSSA-overexpressing MCF10A cells in soft agar upon addition of JQ1 (1µM) to culture medium 8 or 15 days after seeding (treatment with JQ1 at day 1 is presented as reference for maximal inhibition). Data are presented as in d. g. Representative H&E staining of sections of mammary glands from MMTV-Cre; Apcfl/fl, MMTV-Cre; Apcfl/fl; Yapfl/fl, Tazfl/fl mice. Scale bar, 0.1mm. The same phenotype was observed in at least four mice per experimental group. h. Representative immunofluorescence pictures of mammary glands from the indicated mice, showing YAP accumulation in the nuclei of epithelial cells, the expansion of luminal cells (K8 positive) and discontinuities in the basal layer of K14-positive cells in MMTV-Cre; Apcfl/fl mice. The ducts of MMTV-Cre; Apcfl/fl, MMTV-Cre; Apcfl/fl; Yapfl/fl, Tazfl/fl mice display a normal morphology. Scale bar, 25µm. i. Representative immunofluorescence pictures of mammary glands from MMTV-Cre; Apcfl/fl mice treated with vehicle (n = 5) or the BAY-BET inhibitor (n = 5) for 6 weeks. Scale bars, 0.1mm. Note that the BAY-BET inhibitor has no effect on the histological appearance of mammary glands of Apcfl/fl (cre-negative) littermates (see Supplementary Fig. 5g). j. Representative immunofluorescence pictures of mammary glands from MMTV-Cre; Apcfl/fl mice, treated with vehicle (n = 5) or the BAY-BET inhibitor (n = 5) for 6 weeks, showing that treatment with the BET inhibitor restores the normal distribution of the luminal marker K8 and the basal marker K14 in the mammary ducts. Scale bars, 25µm. See Supplementary Fig. 5h for normal K8 and K14 staining in Apcfl/fl (cre-negative) mice treated with the BAY-BET inhibitor.
Fig. 6b. By reverse transcription qPCR (RT–qPCR) and in situ hybridization, the oval-cell marker Osteopontin (also known as Spp1) is induced in YAP transgenic livers but suppressed by concomitant treatment with a BET inhibitor (Fig. 6c and Supplementary Fig. 6e). Histologically, the BET inhibitor remarkably prevented the otherwise massive changes in the architecture of the liver parenchyma and reduced the appearance of proliferating Ki-67+ hepatocytes that are typically observed in vehicle-treated YAP transgenic livers (Fig. 6b and Supplementary Fig. 6h). Finally, liver overgrowth induced by YAP expression was inhibited by the BET inhibitor (Fig. 6d).

Next, we focused on another endoderm-derived tissue, the pancreas, testing the functional interdependency of YAP/TAZ and BRD4 in the early event of pancreatic tumor formation: acinar-to-ductal metaplasia (ADM)36. As recapitulated by ex vivo organoid cultures, transgenic expression of YAP in pancreatic acinar cells induces ADM, initially by turning acinar cells into ductal progenitors that only then start to proliferate37. Treatment with JQ1 opposes YAP-induced ADM in organoids and impairs ensuing cell proliferation (Fig. 6e,f and Supplementary Fig. 6i), as also validated by the expression levels of the ductal marker Krt19 and Ccnd1 (Supplementary Fig. 6j). These data complement the data shown above for the mammary gland and expand the generality of the YAP/TAZ–BRD4 interplay to diverse gene expression programs and in distinct tissue contexts both in vitro and in vivo.

Beyond controlling tumor initiation and growth, YAP/TAZ endow cancer cells with the capacity to acquire resistance to chemotherapeutics and molecularly targeted drugs38. Melanoma cells bearing BRAF-activating mutations are a point in case. It has been recently shown that resistance to BRAF inhibitors (such as PLX4032 (also known as vemurafenib)) is rapidly installed in a YAP/TAZ-dependent manner in melanoma cells39,40. In line with this, YAP overexpression in BRAF-mutant but still vemurafenib-sensitive cells is sufficient to install chemoresistance41. We hypothesized that JQ1 could be used to revert YAP-induced drug resistance. Indeed, the growth of YAP-overexpressing BRAF-mutant melanoma cells was strongly inhibited by the combined exposure to vemurafenib and JQ1, which was, per se, poorly active (Fig. 6g and Supplementary Fig. 6k). The depletion of BET proteins in YAP-overexpressing cells or treatment with BET inhibitors impaired the expression of YAP direct target genes (Fig. 6h and Supplementary Fig. 6l); these genes include AXL42, which has been reported to be a pillar in resistance to BRAF inhibitors43, and the immune checkpoint PD-L144. Next, we wanted to verify whether JQ1 could re-sensitize cells that have spontaneously acquired resistance to vemurafenib after chronic exposure. We found that JQ1 could inhibit the activity of the TEAD luciferase reporter in vemurafenib-resistant cells (Supplementary Fig. 6m), and, in viability assays, the combined treatment with JQ1 sensitized resistant cells to low doses of vemurafenib and impaired tumor cell viability to an extent that neither vemurafenib nor JQ1 could achieve when used individually (Fig. 6i and Supplementary Fig. 6n). The effect of JQ1 was phenocopied by the combination of vemurafenib with two independent mixes of BRD siRNAs (Supplementary Fig. 6o). Collectively, these experiments indicate the requirement of BET proteins in maintaining YAP/TAZ-induced resistance to vemurafenib in BRAF-mutant melanoma cells and suggest that BET inhibitors might indeed prove useful to revert YAP/TAZ-dependent drug resistance in melanoma cells.

Our data imply that, in human tumors, a relevant fraction of the oncogenic functions of BRD4 and its associated transcriptional dependencies, may be in fact associated with the biology of YAP/TAZ. To put this idea to the test, we analyzed a large data set of patients with breast cancer45 containing clinical annotations and transcriptional profiling. We stratified patients according to the expression levels of BET-dependent genes, identified in MDA-MB-231 cells at the beginning of this study. Patients with a higher expression of this signature had a worse prognosis, as expected from the known oncogenic functions of BRD4 (Fig. 6j). Remarkably, however, when we split the list of BET target genes into YAP/TAZ-dependent and -independent signatures, we found that only common YAP/TAZ/BET target genes retained predictive value, whereas BET targets that were not YAP/TAZ-dependent did not (Fig. 6j). This implies that the BRD4 oncogenic properties substantially rest on YAP/TAZ transcriptional responses. Similarly, analysis of a data set of human hepatocellular carcinomas42 with signatures of shared YAP/TAZ/BET targets versus BET targets that were not YAP/TAZ targets (derived from analyses of YAP/TAZ-depleted or JQ1-treated HepG2 cells; see details in methods) indicated that only the shared signature was meaningful for tumor aggressiveness (Supplementary Fig. 6p). Finally, in a cohort of patients with BRAF-mutant melanoma treated with vemurafenib46, only the expression of a signature
of shared YAP/TAZ/BET target genes in the primary tumor was associated with early disease progression (Supplementary Fig. 6q).

**Discussion**

A large body of evidence from several investigators has addressed various means by which BRD4 interacts with transcriptional regulators to nuance transcription. The present discovery of a YAP/TAZ–BRD4 axis advances on this paradigm and may suggest new therapeutic opportunities for cancer and other diseases that rely on YAP/TAZ transcriptional programs.

We have advanced on the molecular mechanisms underlying transcriptional addiction in tumor cells, identifying YAP/TAZ as relevant players in this phenomenon. The underlying molecular event is the physical and functional association between YAP/TAZ and BRD4: YAP/TAZ-bound enhancers recruit BRD4, leading to BRD4 accrual on their target promoters. We show that the YAP/TAZ–BRD4 complex confers a transcriptional advantage to a broad number of YAP/TAZ target genes; such a transcriptional ‘edge’ can be targeted by BET inhibitors in different cellular and tissue contexts with tumor preventive and suppressive effects.

The present results advance on the molecular definition of enhancer elements that are responsible for transcriptional dysregulations in cancer. Major emphasis in this respect has recently been placed on super-enhancers, although the molecular identity of the key transcription factors underlying the properties of these regulatory elements remains unknown. We found that super-enhancers...
largely consist of YAP/TAZ-occupied enhancers; this raises questions on the fact that numerous cancer-specific super-enhancers may simply represent the more noticeable ‘tip of the iceberg’ of a larger set of YAP/TAZ-bound enhancers that nonetheless display super-enhancer-like properties, as defined by strong enrichment of BRD4, a higher expression level of regulated target genes and a higher than average sensitivity to BET inhibitors.

We show that the association of BRD4 to chromatin requires the dual interaction with YAP/TAZ and with acetylated histones. This is reminiscent of the previously proposed requirement of both bromodomain-dependent and -independent roles for BRD4 function\(^2\); such complex formation on chromatin probably involves cooperative associations akin to those postulated to stabilize distinct transcription factors bound to joined motifs at their modular cis-regulatory elements. We postulate that new therapeutics may be designed around the BET-YAP/TAZ interaction surfaces, including the YAP/TAZ WW domain, the so far poorly understood YAP/TAZ transactivation domains and the YAP/TAZ-interacting domains of BRD4, which remain here unexplored.

Drugging YAP/TAZ is clearly a very challenging yet exciting goal for cancer research\(^3\), given the widespread and pervasive functions of YAP/TAZ in cancer cells, contrasting their dispensability for healthy tissues; BET inhibitors may start to fulfill this unmet need. From the other perspective, BET inhibitors are promising anticancer drugs, although drug resistance and the identification of responsive patient subpopulations remain critical open issues\(^4\). Our results collectively indicate that the oncogenic effects of BET proteins are in close association to YAP/TAZ biology, potentially offering new perspectives on how to select patients who are more likely to receive benefit from BET inhibitors, alone or in combination with other drugs. Molecularly annotated data sets of patient cohorts treated with BET inhibitors are not yet available; however, here, we show that patients stratified according to YAP/TAZ classifiers might in fact display differential sensitivity to these drugs, as the oncogenic potential of BET proteins, as inferred from gene expression signatures, seems to be essentially contained within genes addicted to BRD4 through YAP/TAZ.

YAP/TAZ are critical for inducing cell-fate plasticity in normal and tumor cells alike\(^5\). For example, they reprogram normal differentiated mammary cells into mammary stem cells\(^6\), or more differentiated tumor cells into cancer stem cells\(^7\). The nature of the epigenetic barriers controlling these transitions remains unknown, but it is tempting to speculate that BRD4 availability, and potentially other factors assembled by YAP/TAZ on chromatin, may link YAP/TAZ function to permissive versus restrictive chromatin states, as such guiding cell reprogramming or barring it. Thus, the YAP/TAZ-BRD4 connection may hold relevance in contexts other than cancer in which YAP/TAZ play essential roles, such as in heart repair and tissue regeneration.

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Author contributions
F.Z., M.C. and S.P. designed the study, analyzed the data and wrote the manuscript. F.Z., G.B. and L.F. performed the experiments. M.Forcato and S.B. performed the bioinformatics analysis. L.A., E.Q., D.D.B., V.G. and M.Fassan performed the animal experiments and the histological analysis. P.L. and B.H. provided the reagents and advice for the animal experiments. A.M. performed the initial experiments of this study.

Competing interests
B.H. and P.L. are employees of Bayer AG. S.P. is a consultant for and received institutional grants from Bayer AG.

Additional information
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**Methods**

Reagents and plasmids. Doxycycline, OX1015 (SML1605), flaviprid (F3055), human insulin, hydrocortisone and cholera toxin were from Sigma. Human epidermal growth factor (EGF) was from Peprotech. Verumafenib (A3004) and THZ1 (A882) were from Eurofins. QI1 was from BPS Bioscience (27402). RG 108 (ab141013) was from Abcam.

pCDNA-FLAG-YAP vectors (wild type or SSA, siRNA insensitive) were described in ref. 20. FLAG-YAPSSA and FLAG-YAP wild type were subcloned in pBABE-retroviral plasmids. pBABE-blasti retroviral vectors were generated by replacing the puromycin resistance gene with the blasticidin resistance gene in pBABE-puro (Addgene plasmid 1764, a gift from E. Verdin) and FLAG-BRD4 WT (Addgene plasmid 90005) were a gift from K. Polyan.

The coding sequence of HAT-deficient mouse BRD4 was a gift from D. Singer (National Cancer Institute, NIH, US). Wild-type and HAT-deficient mouse BRD4 were subcloned in the CSII-CMV-MCS-IRE2-Bsd lentiviral plasmid. Green fluorescent protein (GFP) from PL-SIN-EOS-C3(+/+)EiP (Addgene plasmid 21313, a gift from J. Vousden) and GFP from pCMV6-Flag (Ambion) were subcloned in the pCW57.1 lentiviral plasmid to establish stable cell lines. pCW57.1 was a gift from D. Root (Addgene plasmid 41393) and RSV-Flag-Brd2 (Addgene plasmid 86614) was a gift from M. Garcia-Dominguez.

For GST pull-down experiments, wild-type full-length YAP was cloned in pGEX-4T-3. GST-TAZ constructs were described in ref. 21. pCMV-LacZ was from Clontech. All constructs were confirmed by sequencing.

Cell lines, treatments and transfections. MDA-MB-231 cells were from the ICLC. MDA-MB-231 cells were cultured in DMEM/F12 (Life Technologies) supplemented with 10% FBS, glutamine and antibiotics. For YAP overexpression, cells were transfected with pBABE-puro-FLAG-YAP. For BRD4 overexpression, cells were transfected with CSII-CMV-MCS-IRE2-Bsd lentiviral plasmid. Green fluorescent protein (GFP) from PL-SIN-EOS-C3(+/+)EiP (Addgene plasmid 21313, a gift from J. Vousden) and GFP from pCMV6-Flag (Ambion) were subcloned in the pCW57.1 lentiviral plasmid to establish stable cell lines. pCW57.1 was a gift from D. Root (Addgene plasmid 41393) and RSV-Flag-Brd2 (Addgene plasmid 86614) was a gift from M. Garcia-Dominguez.

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temperature and then blots were developed with chemiluminescent reagents. Images were acquired with Image Quant LAS 4000 (GE healthcare). In vitro experiments were performed three times with similar results.

Antibodies used for western blot: anti-YAP/TAZ (sc-101199), anti-HA (Y-11, sc-805), and anti-BRD2 (D-2, sc-51402) from Santa Cruz; anti-GFP (AB-489) and anti-BRD4 (HPA013505) from Sigma; and anti-H3 (ab1791) and anti-Pol II CTD repeat YSPTSPS antibody (ab187) from Abcam.

**Immunofluorescence of cultured cells.** Cells were cultured on glass slides and treated with 1μM QJ1 or OTX015 for 24h. Cells were fixed for 10 min at room temperature with 4% PFA in PBS, permeabilized for 10 min at room temperature with PBS + 0.3% Triton X-100, blocked in 10% goat serum in PBS + 0.1% Triton X-100 (PBST) for 1 h and then incubated with anti-YAP/TAZ (sc-101199; Santa Cruz) primary antibody diluted in 2% goat serum in PBS, overnight at 4 °C. After four washes in PBS, samples were incubated with secondary antibody (Alexa 488, 1:200 in 2% goat serum in PBS) for 2 h at room temperature. Nuclei were counterstained with ProLong-DAPI (Molecular Probes, Life Technologies). Images were acquired with a Leica TCS SP5 confocal microscope equipped with a CCD camera.

**In situ PLA.** HEK293T cells were seeded on fibronectin-coated glass chamber slides and transfected with pFlag-CMV2-BRD4, pc2-HA-BRD4 or empty pc52+ for 48h. Cells were fixed after 24h, cells were fixed in 4% PFA for 10 min at room temperature. In situ PLA was performed with DuoLink In Situ Reagents (Sigma) according to the manufacturer’s instructions. Primary antibodies used in the PLA were: mouse anti-HA (F-7, sc-7932, Santa Cruz), mouse anti-TEF1 (610923, BD Biosciences), rabbit anti-FLAG (F-7425, Sigma), rabbit anti-YAP1 (EP1674Y, Abcam), rabbit anti-WWTR1 (HPA007415, Sigma). Images were acquired with a Leica TCS SP5 confocal microscope equipped with a CCD camera; for each field, a Z-stack was acquired; images were processed using Velocity software (PerkinElmer). We verified that the fraction of nuclei with a positive PLA signal corresponded to the fraction of transfected cells (determined by immunofluorescence for FLAG or HA).

**Luciferase reporter assays.** Luciferase assays were performed in M229 and M229-R5 cells with the TEAD reporter 8xGTCIT-Lux. The luciferase reporter (25 ng per cm²) was transfected together with CMV-β-galactosidase (25 ng per cm²) to normalize for transfection efficiency with the CProng (Rogo) colorimetric assay. DNA content in all samples was kept uniform by adding a pBluescript plasmid up to 250 ng per cm². Cells were plated at 20% confluence (day 0) and the following day (day 1) transfected with DNA. After 6h, cells were treated with different doses of JQ1 (1 μM, 10 μM, 0.1 μM and 1μM). Cells were harvested after 24 h of treatment (day 2). Firefly luciferase activity was measured with an Infinite F200PRO plate reader (Tecan), using luciferin (Sigma) as a substrate. Eight wells were analyzed for each experimental condition; data are presented as mean + s.d. Data are presented as the % viability compared to control cells (treated with DMSO or transfected with control siRNA (siC0); absorbance at the beginning of treatment was set as 100% and absorbance at the end of experiment was set as 100%. Each experiment was performed at least twice, with similar results.

**Colony formation assay in soft agar.** MCF1A cells (10⁴) and MDA-MB-231 cells (3×10⁴) were resuspended in complete growth medium with 0.35% agarose (Invitrogen) and were layered onto 0.5% agar beads in six-well plates. Complete medium was added on top of cells and was replaced with fresh medium twice a week for 3 weeks. Complete medium contained 1 μg ml⁻¹ doxycycline to activate the expression of shRNAs, where necessary. The indicated doses of JQ1 were added to the culture, starting 24h after seeding (unless differently specified). Assays were conducted in triplicate. For MCF1A0 cells, all colonies in a well were counted. For MDA-MB-231 cells, colonies were counted in six fields of each samples and the average number of colonies per field was calculated for each sample. Experiments contained three independent samples for each condition and were performed three times, with similar results.

**Isolation and culture of pancreatic acini.** Primary pancreatic acini were isolated as described in Panciera et al. from rTmAM2, colVAP mice or from rTmAM2 littermates as control (both male and females, 6–8-weeks old). Acini were seeded in neutralized rat tail collagen type I (Cultrex)/acinar culture medium (1:1) and overlaid with acinar culture medium (Waymouth’s medium (Life Technologies) supplemented with 0.1% FBS (Life Technologies), 0.1% BSA, 0.2 mg ml⁻¹ soybean trypsin inhibitor, 1x insulin-transferrin-selenium-ethanolamine (Life Technologies), 50 μg ml⁻¹ BPE (Life Technologies), 1 μg ml⁻¹ dexamethasone (Sigma) and antibiotics) supplemented with 0.5 μg ml⁻¹ doxycycline and DMSO or 10μM JQ1, as indicated. ADM events were counted 2–4 days after seeding. For EdU incorporation, 20μM EdU was added to culture medium for 90 min, then collagen cushions containing acini or ducts were extensively washed in PBS and fixed in 4% PFA for 20 min at room temperature. EdU staining was performed with Click-it EdU Alexa Fluor 488 Imaging Kit (Thermo Fisher Scientific), according to the manufacturer’s instructions. Total RNA was extracted with TRIzol reagent (Invitrogen).

**Mice.** Animal experiments were performed adhering to our institutional guidelines as approved by the OPBA (University of Padua) and the Italian Ministry of Health. All experimental mice used in this study were mixed strains and more than 6-weeks old; for mammary gland experiments, we used exclusively female mice. Transgenic lines used in the experiments were kindly provided by: D. Pan (Yapflox/-; A. R. Clarke56 (Apoflox/-; E. Camargo) (tetO-YAP5127A); P. Chambon (Albumin-creERT2), Tazflox/- and double Yapflox/-, Tazflox/- conditional knockout mice were as described in ref. 5. MMTV-cre (stock 00353) and R26-LSL-rtTA (stock 005670) were purchased from The Jackson Laboratory. Yap, Taz and Apc conditional knockouts were generated by B. Manning (MMTV-cre, Apoflox/- (n=5) mice were administered the BAY-BET inhibitor (BAY 1238097) by intraperitoneal injection for 6 weeks, starting at 8 weeks of age (15 mg per kg, 5 injections per week). Control mice were injected with vehicle (0.9% NaCl, pH 4). Harvesting, processing and stainings on mammary glands were performed as in ref. 5.

For the induction of the recombination in the liver, control (Albumin-creERT2; R26-LSL-rtTA+/+) mice and Albumin-creERT2; R26-LSL-rtTA+/- mice received 1 intraperitoneal injection per day of 3 mg tamoxifen (Sigma) dissolved in corn oil (Sigma) during 2 consecutive days. After 2 weeks, mice were administered doxycycline in drinking water for 10 days, during which they also received the BAY-BET inhibitor (15 mg per kg, 5 injections per week) or vehicle by intraperitoneal injections, as indicated in the corresponding Figures.

For validation of the Albumin-creERT2 driver (Supplementary Fig. 6a), Albumin-creERT2; R26-LSL-YFP/+ mice were injected with 3 mg tamoxifen (Sigma) per day dissolved in corn oil (Sigma) during 5 consecutive days and were killed after 2 weeks.

**Immunohistochemical and Immunofluorescences of liver tissue.** Immunohistochemical staining was performed on formalin-fixed, paraffin embedded tissue sections as described in ref. 49. Primary anti-Ki-67 polyclonal antibody (clone SP6; M3062) was from Spring Bioscience.

**RNA in situ hybridization.** Tissue sections (formalin fixed, paraffin embedded) were processed for RNA in situ detection using the RNAseq Duplex Detection Kit (Quirine van der Velden, the manufacturer of the BALLS cell Diagnostics). RNAscope probe used was Sp51 (Osteopontin; NM_001204201.1, region 2-1079), which was detected using the Fast Red detection reagent.

**RT-qPCR.** Total RNA extraction from cells and tissue was performed with the NucleoSpin RNA Blood kit (Ruer) according to the manufacturer’s instructions. Nanozoozer Scanner 2.08R was from Hamamatsu.

RNA from tissue sections. Tissue sections (formalin fixed, paraffin embedded) were processed for RNA in situ detection using the RNAseq Duplex Detection Kit (Quirine van der Velden, the manufacturer of the BALLS cell Diagnostics). RNAscope probe used was Sp51 (Osteopontin; NM_001204201.1, region 2-1079), which was detected using the Fast Red detection reagent.

**RT-qPCR.** Total RNA extraction from cells and tissue was performed with the NucleoSpin RNA Blood kit (Ruer) according to the manufacturer’s instructions. Nanozoozer Scanner 2.08R was from Hamamatsu.

**Gene expression analyses by RT-qPCR were carried out with QuantiStudio 5 thermal cycler (Thermo Fisher Scientific). Experiments were performed at least three times. Expression levels were calculated relative to GAPDH. Human primer pairs are:

- **AURKA**: GCCCCTCTGGGTTAAGAgAAAG, GCCGAAAGATTGGACTGTTAT; **AXL**: CACCCAGAAAGCCAGTTGCT, CGGTCTCGGAGTTAAGCTC; **BRD4**: GAGGGAGCACCACCAACTGG, CACGGAGGTTCGTCGAGC;
Articles

**RNA-seq.** Cells were harvested by the RNeasy Mini Kit (Qiagen) for total RNA extraction and contaminating DNA was removed by the RNase-Free DNase Set (Qiagen). RNA-seq libraries for deep-sequencing were prepared with the Illumina TruSeq Standard Total RNA with Ribo-Zero Gold kit and sequencing was performed with Illumina HiSeq 2500. About 40 M reads per sample were obtained. Raw reads were aligned using TopHat2 (version 2.0.5) (development version) to build version hg19 of the human genome. Counts for UCSC annotated genes were calculated from the aligned reads using HTSeq2 (version 0.6.0). Normalization and differential analysis were carried out using edgeR package62 and R (version 3.0.0). Raw counts were normalized to obtain counts per million mapped reads (CPM) and reads per kilobase per million mapped reads (RPKM). Only genes with a RPKM greater than one in at least two samples were retained for differential analysis. Genes were considered differentially expressed (DE) if the p-value was less than 0.05 and a fold change of ≥2 and ≤0.5. Genes with a fold change of ≥2 and ≤0.5 were clustered. Glycolysis and Metabolism (with GO:0005975: glycolysis, for example, GO:0006012: monosaccharide metabolism), DNA replication and repair (for example, GO:0006260: DNA replication, repair and recombination) were included in a single category named ‘cell proliferation’.

**GO analysis.** GO analyses were performed on all the genes expressed in MDA-MB-231 cells (RNA-seq RPKM ≥1) using Enrichr (http://amp.pharm.mssm.edu/Enrichr)4,44. Genes annotated with GO terms related to cell cycle (for example, GO:0007020: mitotic cell cycle and GO:0007283: cell proliferation), DNA replication and repair (for example, GO:0006260: DNA replication, repair and recombination) were included in a single category named ‘cell proliferation’. Genes annotated with GO terms related to glycolysis (for example, GO:0005975: glycolysis) and DNA replication and repair (for example, GO:0006260: DNA replication, repair and recombination) were included in a single category named ‘DNA replication and repair’. Genes expressed with a Benjamini–Hochberg false discovery rate (FDR) ≤1% and a fold change of ≥2 and ≤0.5 were clustered. For ChIP–seq data, raw reads were aligned using Bowtie2 (version 0.12.7) to build version hg19 of the human genome, retaining only uniquely mapped reads. Redundant reads were removed using SAMtools.

**Analysis of ChIP-seq data.** For ChIP–seq, the lists of monomethylation of H3K4 (H3K4me1)-, trimethylation of H3K27ac- and H3K122ac-enriched regions (peaks) in MDA-MB-231 cells were already described in ref. 13. For ChIP–seq data, raw reads were aligned using Bowtie2 (version 0.12.7) to build version hg19 of the human genome, retaining only uniquely mapped reads. Redundant reads were removed using SAMtools.

**Definition of promoters and enhancers.** To define promoter regions in the genome and to identify a list of 2-kb-wide regions centered on each TSS mapped in the build version hg19 of the human genome (downloaded from the UCSC genome browser27), we obtained a list of promoter regions by including only the TSSs overlapping with H3K4me3-enriched regions. Active enhancers were defined as promoter regions displaying enrichment for H3K4me1 and H3K27ac. For this, we first defined a list of 2-kb-wide regions centered on each TSS mapped in the build version hg19 of the human genome (downloaded from the UCSC genome browser27). We then obtained a list of promoter regions by including only the TSSs overlapping with H3K4me3-enriched regions. Active enhancers were defined as promoter regions displaying enrichment for H3K4me1 and H3K27ac. For this, we first defined a list of 2-kb-wide regions centered on each TSS mapped in the build version hg19 of the human genome (downloaded from the UCSC genome browser27). We then obtained a list of promoter regions by including only the TSSs overlapping with H3K4me3-enriched regions. Active enhancers were defined as promoter regions displaying enrichment for H3K4me1 and H3K27ac.
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Annotation of YAP/TAZ-binding regions to enhancers and target gene promoters. YAP/TAZ-bound enhancers were defined as active enhancers overlapping with both YAP and TAZ peaks. Similarly, YAP/TAZ-bound promoters were defined as promoters regions (as defined above) overlapping with both YAP and TAZ peaks. Gene promoters associated with YAP/TAZ-bound enhancers through DNA looping were defined as promoter regions associated with at least one YAP/TAZ-bound enhancer on the bases of Hi-C data (as in ref. 71). YAP/TAZ direct target genes were defined as those whose promoters are associated with YAP/TAZ-bound enhancers or are directly binding YAP/TAZ.

Calculating normalized read count and density at enhancers and promoters. ChIP-seq reads aligning to each cis-regulatory region (active enhancers and promoters for BRD4 ChIP-seq experiments and promoters only for Pol II and H3K122ac ChIP-seq experiments) were calculated using the BEDTools suite78. The total number of control samples (JQ1 and DMSO) for each cell line, subtracted of the normalized counts from IgG ChIP-seq (background) of the same cells. Briefly, we first ranked all active enhancers by increasing the total background, subtracted of the normalized read counts of BRD4 in units of total RPM (r-axis) and plotted the total background, subtracted of the normalized counts of BRD4 in units of total RPM (r-axis) (see Supplementary Fig. 4f). This plot revealed a clear point in the distribution of BRD4 at active enhancers where the occupancy signal began increasing rapidly. To geometrically define this point, we found the y axis point for which a line with a slope of 1 was tangent to the curve. We define enhancers above this point to be super-enhancers and enhancers below that point to be typical enhancers. Annotation of super-enhancers to target genes was carried out on the bases of Hi-C data as detailed before for all active enhancers. Genes were considered as associated to super-enhancers if their promoters were associated at least with one super-enhancer; all of the genes whose promoters are associated with active enhancers but with no super-enhancers were labeled as genes associated with typical enhancers.

ChIP-seq heatmaps and average profiles. Heatmaps and average signal profiles were generated using a custom R script, which considers a 1-kb window centered on TAZ peak summits falling on active enhancers or a 1.5-kb window centered on the TSSs of YAP/TAZ target genes. Normalized read density (RPDM) was calculated from pooled replicates using MACS79 (version 2.0.10) callpeak function with appropriate control samples (IgG for BRD4 and input DNA for Pol II and H3K122ac) and displayed using Integrative Genomics Viewer. Normalized read density was calculated with a resolution of 50bp. Each row in the heatmap represents a genomic region around a peak summit or TSS and rows are ranked according to TAZ or DMSO_BRD4 ChIP-seq signal intensity, respectively.

Generation of the signatures of BET-dependent genes. For the analysis of the breast cancer data set, we used the data generated for this study to identify a list of BET-dependent genes, defined as those whose expression was significantly downregulated (fold change ≤0.75; FDR ≤0.05) in both JQ1-treated and siBRD2/3/4-transfected MDA-MB-231 cells compared to control cells. Starting from this list, we then defined a list of common YAP/TAZ/BET target genes, defined as BET-dependent target genes that are also YAP/TAZ direct target genes whose expression is robustly downregulated (fold change ≤0.67) upon transfection with both YAP/TAZ siRNA mixes. We also defined a list of BRD-dependent but YAP/TAZ-independent genes in BC cells, composed by genes that are not downregulated (fold change >0.75) after transfection with either YAP/TAZ siRNA mixes. These two lists are of similar size: the YAP/TAZ/BET signature is composed of 220 genes and the BRD-dependent and YAP/TAZ-independent signature is composed of 228 genes.

For the analysis of the hepatocellular carcinoma data set, we made use of public gene expression data sets obtained from human hepatoma HepG2 cells treated with JQ1 (GSE51143)37 to identify BET target genes, defined as those whose expression was significantly downregulated (fold change ≤0.75; P ≤0.05) by JQ1 treatment compared with DMSO-treated cells. We then used data obtained by ref. 71 (GSE49384) by transfecting YAP/TAZ siRNAs in HepG2 cells also transfected with siRNAs against NF2 and LATS2, to identify YAP/TAZ-dependent genes in these cells. We used these data to identify common YAP/TAZ/BET target genes, defined as BET target genes that are significantly downregulated (fold change ≥0.75; P ≤0.05) after YAP/TAZ knockdown; conversely, BRD-dependent genes that are not significantly downregulated (fold change ≥1; P >0.05) after YAP/TAZ knockdown were used to define the signature of BET target genes that are YAP/TAZ independent. These two lists are of similar size: YAP/TAZ/BET signature is composed of 84 genes and the BET-dependent and YAP/TAZ-independent signature is composed of 89 genes.

For the analysis of the vemurafenib-resistant BRAF-mutant melanoma data set, YAP/TAZ/BET shared genes were defined as the JQ1-sensitive genes identified by Fontanals-Cierva et al.73 in two different melanoma cell lines that were also found significantly downregulated (fold change ≤0.5; FDR ≤0.01) after YAP/TAZ knockdown in two vemurafenib-resistant melanoma cell lines, according to the data from the data set GSE8859979. JQ1-sensitive genes that were not significantly downregulated (fold change ≥1.3; P >0.05) after YAP/TAZ knockdown were instead included in the signature of BET-dependent and YAP/TAZ-independent genes in melanoma cells. These two lists are of similar size: the YAP/TAZ/BET signature is composed of 41 genes and the BET-dependent and YAP/TAZ-independent signature is composed by 47 genes.

Collection and processing of gene expression data. Breast cancer gene expression data were generated, normalized and annotated as described in ref. 5. Briefly, starting from a collection of 4,640 samples from 27 major data sets comprising microarray data and clinical information, we derived a compendium (meta-data set) comprising gene expression levels and clinical outcome for 3,661 unique samples from 25 independent cohorts. Gene expression data of 247 human hepatocellular carcinoma were downloaded from the Gene Expression Omnibus GSE14520 series matrix files and used as is after removing non-tumor and normal samples2,4. Transcriptional profiles of BRAF-mutant melanomas were obtained from GSE5050979. Briefly, starting from non-normalized data, we generated normalized gene expression values (MA450K probeset) MDA-MB-231 for samples from patients before commencing treatment with dabrafenib or vemurafenib (n = 27) using variance stabilization and quantile normalization of the lumi Bioconductor package8. All microarray data analyses were performed in R version 3.4.2 with the annotation packages of Bioconductor packages of Release 3.5.

Average signature expression and signature scores. Signature scores were obtained summarizing the standardized expression levels of signature genes into a combined score with zero mean8. The average signature expression was calculated as the standardized average expression of all signature genes in all samples and plotted as the mean ± standard error of the mean (s.e.m.).

Kaplan–Meier survival analysis. To identify two groups of tumors with either high or low signature, we used the classification rule described in ref. 79. Briefly, tumors were classified as ‘low’ if the combined signature score was negative and as ‘high’ if the combined signature score was positive. This classification was applied to the expression values of the breast cancer meta-data set and of GSE14520 hepatocellular carcinomas. To evaluate the prognostic value of the BRD-dependent signatures, we estimated the probabilities that patients would remain free of metastases or survive using the Kaplan–Meier method. To confirm these findings, the Kaplan–Meier curves were compared using the log-rank (Mantel–Cox) test. P values were calculated according to the standard normal asymptotic distribution. Survival analysis was performed in GraphPad Prism.

Statistical analysis. Statistical analysis for the generation of gene expression and DNA-binding data were performed in R and are described in the above paragraphs of Methods. Other statistical analyses were performed with GraphPad Prism 7.0. A one-tailed Mann–Whitney U-test was used for the pairwise comparison of gene expression levels between groups of genes (from RNA-seq experiments) or DNA binding (from ChIP–seq data); a one-tailed Wilcoxon matched-pairs signed rank test was used when comparing the same group of genes or binding sites in different experimental conditions. Linear regression analysis was used to study correlation. The log-rank (Mantel–Cox) test was used to compare survival curves.

In vivo studies, mice were randomly assigned to treatment groups; sample size was not pre-determined. A two-tailed unpaired Student’s t-test was applied for comparison between groups, when required; all analyzed samples were included for statistical analysis.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The RNA-seq and ChIP–seq data generated in this study have been deposited in the GEO database under accession GSE102409. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
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| ☒  | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒  | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒  | The statistical test(s) used AND whether they are one- or two-sided |
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| ☒  | A description of all covariates tested |
| ☒  | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☒  | A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
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| ☒  | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒  | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒  | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |
| ☒  | Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection | LAS AF software for Leica TCS SPS II was used to acquire confocal images. LASV4.4 software was used to acquire pictures of cell cultures with a Leica DM IRB microscope. NDPscan3.1 was used to acquire IHC images. ImageQuant LAS 4000 1.2 was used to acquire western blot images. QuantStudio Design & Analysis Software v1.4.3 was used to acquire qPCR data.

Data analysis | TopHat (version 2.0.5), HTSeq (version 0.6.0), edgeR package and R (version 3.0.0) were used to analyze RNA-seq data. Software used for the analysis of ChIP-seq data are described in the dedicated section of this Reporting Summary. GraphPad Prism 7.0d for Mac was used for statistical analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data from this study have been deposited in the GEO database under accession number GSE102409 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102409).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: Sample size was not predetermined. Generally accepted samples sizes were used, with reproducible differences between conditions indicating that the sample size is sufficient.

Data exclusions: All data were included.

Replication: All experiments were reproducible. Every figure states how many times each experiment was performed with similar results.

Randomization: For animal experiments, littermates were randomly allocated to treatment groups.

Blinding: Investigators were blinded for the evaluation of histological sections. Investigators were not blinded for analyses relying on unbiased measurements of quantitative parameters.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✖   | Unique biological materials |
| ☐   | Antibodies |
| ☐   | Eukaryotic cell lines |
| ✖   | Palaeontology |
| ✖   | Animals and other organisms |
| ☐   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq |
| ☑   | Flow cytometry |
| ☑   | MRI-based neuroimaging |

Antibodies

Antibodies used in ChIP-seq experiments were:
- anti-BRD4: A301-985A, Bethyl-Lab (lot A301-985A100-5)
- anti-PolII: ab817, Abcam (lot GR271062-1)
- anti-Histone H3 (acetyl K122): ab33309, Abcam (GR284790-3)
- normal rabbit IgG: I5006, Sigma

Antibodies used for all other experiments were:
- anti-BRD4: E2A7X, CST
- anti-YAP1: 13584-1-AP, Proteintech
- anti-WWTR1: HPA007415, Sigma
- normal rabbit IgG: I5006, Sigma
- anti-YAP/TAZ: sc-101199, Santa Cruz
Validation

All antibodies were validated by the producer. Moreover, the validation of BRD4 antibody for ChIP is reported in Supplementary figure 2a.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) MDA-MB-231 cells were from ICLC. HEK293T and MCF10A cells were from ATCC. SUM149PT and SUM-159PT were kindly provided by Dr. S. Ethier. M229 and M229-5S cells were a gift from Dr. JC Marine. WM3248 and WM3248-R6 cells, and SKMEL28 and SKMEL28-R2 were a gift from Dr. J.Kim.

Authentication MDA-MB-231, MCF10A and HEK293T were authenticated by DSMZ service.

Mycoplasma contamination All cell lines were routinely tested for mycoplasma contamination and were negative.

Commonly misidentified lines None of the cell lines used in this study is present in the database of commonly misidentified cell lines.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals All experimental mice used in this study were mixed strains and more than 6-8 weeks old at the beginning of experiments. For mammary gland experiments we used exclusively female mice; for other experiments, both male and female mice were used.

Wild animals No wild animals were used in this study.

Field-collected samples The study did not involve samples collected on the field.

ChIP-seq

Data deposition

☑ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102409

Files in database submission

raw files:
1-piccolo-160901_ep_TCAACCTT_L003_R1_001.fastq.gz
2-piccolo-160901_ep_AAGGCTCT_L003_R1_001.fastq.gz
3-piccolo-160901_ep_TCTCCCTT_L003_R1_001.fastq.gz
4-piccolo-160901_ep_GGATGTC_L003_R1_001.fastq.gz
9-piccolo-160901_ep_TCTCCCTT_L004_R1_001.fastq.gz
10-piccolo-160901_ep_GGAGGTCT_L004_R1_001.fastq.gz
11-piccolo-160901_ep_GGAGGTCT_L004_R1_001.fastq.gz
12-piccolo-160901_ep_AAGGCTCT_L004_R1_001.fastq.gz
17-piccolo-160901_ep_ATGGCGAT_L005_R1_001.fastq.gz
18-piccolo-160901_ep_AAGGCTCT_L006_R1_001.fastq.gz
19-piccolo-160901_ep_GTAGGTCT_L005_R1_001.fastq.gz
20-piccolo-160901_ep_TCTCCCTT_L006_R1_001.fastq.gz
5-piccolo-160901_ep_CATCCAAG_L003_R1_001.fastq.gz
6-piccolo-160901_ep_GTCAACAG_L003_R1_001.fastq.gz
| Genome browser session | n.a. |
|------------------------|------|
| Methodology            |      |
| **Replicates**         | For every experimental condition 2 biological replicates were produced. Sample processing (from cell seeding to chromatin immunoprecipitation) was performed independently for the 2 replicates. |
| **Sequencing depth**   | BRD and RNA Pol II: 40 millions of single-end reads per sample  
H3K122ac: 60 millions of single-end reads per sample |
| **Antibodies**         | BRD4: Bethyl laboratories, cat. A301-985A, lot A301-985A100-5; see validation of specificity by ChIP-qPCR with BRD4 siRNA in MDA-MB-231 cells in Supplementary Figure 2a.  
RNA polymerase II, clone 8WG16: Abcam, ab817 (ChIP grade), lot GR271062-1 (validation by supplier: http://www.abcam.com/ma-polymerase-ii-ctd-repeat-ysptsps-antibody-8wg16-chip-grade-ab817.html)  
Histone H3 (acetyl K122): Abcam, ab33309, lot GR284790-3 (used for ChIP-seq in human cells in PUBMED 23415232) |
| **Peak calling parameters** | Raw reads were aligned using Bowtie to build version hg19 of the human genome retaining only uniquely mapped reads. Indexes were retrieved from Illumina's iGenomes collection for UCSC hg19 genome. Redundant reads were removed using SAMtools.  
MACS callpeak function was used to generate bedGraph files for pooled replicates using the -B --SPMR parameters and appropriate control samples (IgG for BRD4, Input DNA for Pol2 and H3K122ac).  
BedGraph files were converted to bigWig format with UCSC bedGraphToBigWig utility.  
Peak calling was not performed in this study. |
| **Data quality**       | FastQC was used to assess raw sequence data quality. ngs.plot was used to check ChiP average profile around TSS and gene bodies. |
| **Software**           | CASAVA (version 1.8.4)  
FastQC (version 0.11.5, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/)  
Bowtie (version 0.12.7, Langmead et al., 2009)  
SAMtools (version 0.1.18, Li et al., 2009)  
ngs.plot (version 2.00, Shen et al., 2014)  
MACS2 (version 2.0.10, Zhang et al., 2008)  
UCSC bedGraphToBigWig (http://hgdownload.soe.ucsc.edu/admin/exe/linux.x86_64/). |