A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation

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Mammalian SWI/SNF chromatin remodelling complexes exist in three distinct, final-form assemblies: canonical BAF (cBAF), PBAF and a newly characterized non-canonical complex (ncBAF). However, their complex-specific targeting on chromatin, functions and roles in disease remain largely undefined. Here, we comprehensively mapped complex assemblies on chromatin and found that ncBAF complexes uniquely localize to CTCF sites and promoters. We identified ncBAF subunits as synthetic lethal targets specific to synovial sarcoma and malignant rhabdoid tumours, which both exhibit cBAF complex (SMARCB1 subunit) perturbation. Chemical and biological depletion of the ncBAF subunit, BRD9, rapidly attenuates synovial sarcoma and malignant rhabdoid tumour cell proliferation. Importantly, in cBAF-perturbed cancers, ncBAF complexes maintain gene expression at retained CTCF-promoter sites and function in a manner distinct from fusion oncoprotein-bound complexes. Together, these findings unmask the unique targeting and functional roles of ncBAF complexes and present new cancer-specific therapeutic targets.

While the majority of mSWI/SNF gene mutations result in loss-of-function phenotypes, the SS18–SSX fusion hallmark to synovial sarcoma results in de novo, gain-of-function targeting of BAF complexes, which activates the unique synovial sarcoma gene expression signature. The incorporation of the SS18–SSX oncoprotein into BAF complexes results in protein-level destabilization of SMARCB1 (a feature shared with MRT), but this event is secondary and not required for the maintenance of synovial sarcoma gene expression or proliferation. Finally, genetic perturbation screens in cell lines bearing mutations in mSWI/SNF subunits that are part of paralog families (such as SMARCA4 and ARID1A) have unveiled synthetic lethal dependencies on residual complexes assembled with their rarely mutated partner paralogs (such as SMARCA2 and ARID1B). Collectively, these findings highlight subunit- and paralog-specific biological roles, such as those demonstrated in the context of development.

Defining the functional differences among the diverse array of complexes within the mSWI/SNF family represents a major goal for the field at large. In this study, we used biochemical and bioinformatic approaches to map mSWI/SNF complexes on chromatin, which revealed an unexpected, complex-specific localization of ncBAF complexes to CTCF and promoter-proximal sites. We find that cancers driven by core cBAF subunit perturbations, such as synovial sarcoma and MRTs, are uniquely dependent on ncBAF function. We further demonstrate that perturbation of...
ncBAF complexes is, unexpectedly, mechanistically distinct from perturbation of synovial sarcoma disease-driver SS18–SSX and that in both synovial sarcoma and MRT settings, ncBAF plays critical roles in maintaining gene expression at retained mSWI/SNF sites.

**Results**

Distinct function and genome-wide localization across mSWI/SNF complex families. Genes that encode proteins involved in similar biological pathways or protein complexes often exhibit coordinated fitness variation in genetic perturbation screens, such
as those performed across hundreds of human cancer cell lines. Specifically, we have previously established that mSWI/SNF complex families across the AFTPH locus.

Fig. 2 | Differential localization of the mSWI/SNF complexes, ncBAF, cBAF and PBAF on chromatin. a, Venn diagram of peaks from BRD9, GLTSCR1 and SMARCA4 (ncBAF) ChIP-seq experiments. b, Heatmap representing the correlations between normalized ChIP-seq reads (log2[reads per million mapped reads (RPM)]) over a merged set of all mSWI/SNF subunit peaks. ChIP was performed on n = 2 independent samples for each. c, Localization of ncBAF, BAF and PBAF complexes at the VEGFA locus. Chromatin immunoprecipitation was performed on n = 2 independent samples for each. d, Heatmap of CentriMo log-adjusted P values for top motifs returned by MEME-ChIP analysis for each ChIP-seq experiment. Experiments were performed on n = 2 independent samples for each. P values were calculated using a binomial test. e, Proportion of peaks from ChIP-seq experiments using indicated antibodies that overlap the CTCF ChIP-seq peaks in MOLM-13 and EoL-1 cell lines. f, The proportion of cBAF-, PBAF- and ncBAF-specific peaks overlapping with specified chromatin features (see also Supplementary Fig. 2). g, Example tracks demonstrating differential enrichment of mSWI/SNF complex families across the AFTPH locus. ChIP was performed in n = 2 independent samples for each.
Fig. 3 | Components of ncBAF are selective synthetic lethal dependencies in synovial sarcoma and MRT cell lines. a, Waterfall plots of CERES scores for mSWI/SNF subunits across n = 393 cell lines screened using CRISPR–Cas9 (Project Achilles). Synovial sarcoma (orange) and MRT (blue) cell lines are indicated. The dashed line represents the median dependency. b, BRD9 sensitivity across n = 387 cell lines in Project DRIVE (shRNA-based screening). The median z-score in each cancer type is plotted against the two-sided Fisher’s exact test −\log_{10}(P value) for BRD9 sensitivity (ATARIS score < −0.75). Annotations with false discovery rate (FDR) < 0.1 are coloured in red. c, Heatmap of CERES scores in SYO-1 (SS18-SSX-driven synovial sarcoma) and SW982 (histological synovial sarcoma mimic without SS18-SSX translocation) cells ranked by difference in dependency. d, Immunoblot of total cell lysates from SYO-1 cells ranked by dependency score and proliferation experiments in SYO-1 cells (treated with 500 nM dBRD9). Data points represent mean ± s.d.; point represents mean ± s.d.; left) and proliferation experiments in SYO-1 (bottom; n = 3 biologically independent experiments). See also Supplementary Fig. 7d. e, Representative colony formation assay performed on SYO-1 cells treated with dBRD9, BI-7273 or lenalidomide as a control (n = 3 biologically independent experiments).
BRD9 and GLTSCR1 peaks significantly overlapped one another (Fig. 2a and Supplementary Fig. 2b–d). Hierarchical clustering performed on ChIP-seq read density over the merged set of peaks across all ChIPs identified distinct, complex-specific enrichment on chromatin (Fig. 2b and Supplementary Fig. 2e), such as ncBAF complexes over the promoter (green), PBAF complex occupancy into the gene body (red) and cBAF complexes at distal sites (blue) at the VEGFA locus (Fig. 2c). PBAF and ncBAF complexes exhibited a distinct promoter-proximal distribution in comparison to cBAF, which were substantially more localized to distal sites (Supplementary Fig. 2f). In addition, at transcription start sites (TSSs), PBAF complexes were more enriched over gene bodies relative to ncBAF complexes (Fig. 2c and Supplementary Fig. 2g).

Motif analyses revealed a central enrichment of cBAF complexes over known transcription factor motifs, including FOS/JUN, AP-1, SPDEF and ETS (with PBAF complexes enriched at a subset of
ncBAF is not required for SS18-SSX1-mediated gene expression and primarily regulates fusion-independent sites. a, Immunoblot for ncBAF components in HA–SS18 and HA–SS18–SSX complex purifications (n = 1 biologically independent experiment). See also Supplementary Fig. 5h. b, Heatmap of significantly downregulated genes (adjusted P < 0.001 and log₂[fold change] < −0.59) in shSS18–SSX (7 d postinfection) and dBRD9 (3 d and 6 d postinfection) conditions in SYO-1 cells clustered into four groups according to the k-means. Benjamini–Hochberg adjusted Wald P values; n = 2 biological replicates for each RNA-seq experiment. c, Gene set enrichment analysis (GSEA) of RNA-seq data for shSS18–SSX and dBRD9 (Day 6) conditions in b. Specific pathways and gene sets are indicated. d, Immunoblot of CRL7250 whole cell lysates described in Supplementary Figure 5B (top; n = 1 biologically independent experiment) and heatmap of log₂[fold change] of gene expression in CRL7250 fibroblast cells treated with DMSO, dBRD9 (Day 6) or dBRD9 followed by lentiviral introduction of VS–SS18 or VS–SS18–SSX (bottom; n = 2 biological replicates for each RNA-seq experiment). The included genes were expressed (>1 reads per kilobase million (RPKM)) and had a log₂[fold change] of at least ±0.59 in at least one of the conditions. Genes were k-means clustered into two groups and samples were clustered hierarchically. See also Supplementary Fig. 7i. e, Heatmap of ChIP-seq read densities of SS18, BRD9 and H3K4me3 over SS18 sites in SYO-1 cells (shScr (control hairpin) and shSSX (targeting SS18–SSX)) conditions clustered into three groups. f, Gene expression of log₂[fold change] of genes closest to the fusion-dependent sites (n = 595) in shSS18–SSX and dBRD9 conditions. P values were calculated using two tailed t-test; n = 2 independent samples for each ChIP performed. Boxes represent the interquartile range (IQR); horizontal bars, the median; minima and maxima shown extend 1.5 x IQR from the box. g, Chromatin landscape (fusion-dependent, fusion-independent promoter and fusion-independent distal) of BRD9 peak nearest to the 500 most downregulated genes at day 6 of dBRD9 treatment. h, CERES scores of genes demonstrating significant expression changes (adjusted P < 0.001) in SYO-1 cells after 6 d of dBRD9 treatment. P value was calculated using a two tailed t-test. The violin plot represents the kernel density estimation with data quartiles shown as lines and the data median as a dot.

Finally, ncBAF complexes were most enriched at CTCF sites, particularly CTCF sites co-localized with H3K4me1 (Supplementary Fig. 2). These CTCF co-localized sites comprised a greater portion of all ncBAF peaks relative to cBAF and PBAF peaks. Thus, although the localization and biological roles for mSWI/SNF complexes have been most extensively explored at enhancers15–18, these results imply specialized roles for ncBAF and PBAF complexes at CTCF sites and promoters, respectively, and demonstrate mSWI/SNF complex-specific chromatin localization.

Genome-scale fitness screening reveals cancer-specific dependencies on ncBAF complexes. To determine whether ncBAF
ncBAF is required for the maintenance of gene expression and retains co-localization with promoters and CTCF in SMARCBI-deficient cancers.

**Fig. 6** ncBAF is required for the maintenance of gene expression and retains co-localization with promoters and CTCF in SMARCBI-deficient cancers.

**a.** Venn diagram of BRD9 and SMARCA4 ChIP-seq peaks in TTC1240. **b.** Proportion of SMARCA4 peaks that overlap with a BRD9 peak in synovial sarcoma, MRT and mSWI/SNF-intact hematopoietic cancer cell lines. AML/T-ALL, acute myeloid leukaemia/T-acute lymphoblastic leukaemia. The P value was calculated using a two tailed t-test; n = 2 independent ChIPs.

**c.** Proportion of MRT-specific super-enhancers 1416 in TTC1240 that overlap a BRD9 peak. Fold change (log2) in SMARCA4 occupancy versus the mean occupancy between DMSO- and dBRD9-treated TTC1240 cells. Occupancy changes with FDR < 0.05 are highlighted; FDR values are multiple test-corrected Wilcoxon test P values; n = 2 biological replicates per ChIP.

**d.** Spike-in normalized heatmap of SMARCA4 and BRD9 ChIP occupancy across lost SMARCA4 sites in TTC1240 following dBRD9 treatment ranked by log2[fold change] of differentially expressed genes within 100 kb of a lost SMARCA4 peak.

**e.** H3K27ac ChIP occupancy in TTC1240 cells at SMARCA4 sites lost or retained following dBRD9 treatment. Blue dots indicate genes with a TSS within 100 kb of a lost site. MRT disease-associated genes with a TSS less than 100 kb.

**f.** Gene expression changes in TTC1240 with seven-day dBRD9 treatment. Blue dots indicate genes with a TSS within 100 kb of a lost site. MRT disease-associated genes with a TSS less than 100 kb.

**g.** Venn diagram of BRD9 and SMARCA4 ChIP-seq peaks in TTC1240 at hallmark ncBAF promoters and CTCF sites. Chemical or biological ncBAF disruption results in the loss of gene expression maintenance.

**h.** Genome-wide change (log2[fold change]) in SMARCA4 occupancy across SMARCA4 peaks in TTC1240 and MOLM-13 cells after dBRD9 treatment. **i.** Relative density of SMARCA4 peaks.

**j.** MRT and synovial sarcoma (SMARCB1-deficient) core promoters and other regulatory regions. ncBAF disruption results in the loss of gene expression maintenance.

**k.** Model for ncBAF dependency in cancers driven by cBAF perturbations. Perturbations in the core cBAF functional module (SMARCBI, SMARCE1 and ARID1A/B, with the exception of the ATPase subunits) result in the loss of cBAF gene regulatory function and a reliance on ncBAF for gene expression maintenance at hallmark ncBAF promoters and CTCF sites. Chemical or biological ncBAF disruption results in the loss of gene expression maintenance. AML/T-ALL, acute myeloid leukaemia/T-acute lymphoblastic leukaemia.
subunits are uniquely required for the proliferative maintenance of any cancer types, we analysed CRISPR–Cas9-based screens performed across 387 cancer cell lines26 and performed screens in three new synovial sarcoma cell lines (Supplementary Fig. 3a; Achilles 18Q3 dataset, https://cds.team/depmap/#). These screens identified significant, selective sensitivity of both synovial sarcoma and MRT cell lines to perturbations of the ncBAF complex subunits BRD9, GLTSCR1 and SMARCD1 (Fig. 3a). These dependency profiles were specific to synovial sarcoma and MRT, both of which are sarcomas, and not to other soft-tissue malignancies (Supplementary Fig. 3b). To corroborate these results, we analysed shRNA-based fitness screens from Project DRIVE26 and again found that synovial sarcoma (n = 5) and MRT (n = 4) cell lines were selectively sensitive to BRD9 suppression (Fig. 3b and Supplementary Fig. 3c). We further confirmed that ncBAF dependency was specific to SS18–SSX fusion oncprotein-positive synovial sarcoma, as a synovial sarcoma histological mimic cell line, SW982 (lacking the SS18–SSX fusion) was insensitive to ncBAF component perturbation (Fig. 3c).

Both synovial sarcoma and MRT exhibit perturbations to the cBAF core functional module26. Synovial sarcoma is uniformly characterized by the t(X;18) chromosomal translocation, which produces the SS18–SSX fusion oncprotein, a stable and dedicated mSWI/SNF complex subunit that destabilizes SMARCB118–20, whereas MRT and atypical teratoid/rhabdoid tumour (AT/RT) cell lines are driven by the bi-allelic loss of the SMARCB1 gene (encoding the SMARCB1/BAF47/hSNF5/INI1 subunit18–20; Supplementary Fig. 3d). In synovial sarcoma, the loss of proliferative fitness as a result of ncBAF subunit perturbation was comparable to SS18 perturbation, the driver of disease (Fig. 3a). Both synovial sarcoma and MRT cell lines exhibited higher sensitivity to the loss of BRD9 than acute myeloid leukaemia cell lines, which have previously been reported to be sensitive to BRD9 knockdown18,21 (Fig. 3a and Supplementary Fig. 3c). We found that acute myeloid leukaemia cell lines exhibited near uniform sensitivity to depletion of a wide range of mSWI/SNF complex subunits, and not uniquely to depletion of ncBAF subunits (Supplementary Fig. 3e). In synovial sarcoma and MRT, SMARCB1 (destabilized and deleted in these cancers, respectively) and other cBAF and PBAF subunits, such as SMARCD1, ARID1A and BRD7, did not score as dependencies (Fig. 3a and Supplementary Fig. 3c), thus highlighting the selective sensitivity to ncBAF subunit disruption in these cancer types.

To validate these findings, we depleted BRD9, both biologically and chemically, using shRNA and dBRD9 chemical degradation21 strategies. The suppression of BRD9 in SYO-1 synovial sarcoma cells significantly attenuated their proliferation in comparison to either control shRNA or shRNA directed against SMARCE1, a structurally essential component of cBAF and PBAF complexes that is not a part of ncBAF, thus further confirming the screening results (Fig. 3d and Supplementary Table 2). Synovial sarcoma cells treated with dBRD9 exhibited near complete depletion of BRD9 from whole-cell lysates and proliferative attenuation, approaching that which results from SS18–SSX oncprotein knockdown (Fig. 3e,f, Supplementary Fig. 3f,g and Supplementary Table 2). Knockdown of GLTSCR1 in SYO-1 cells also attenuated proliferation, providing support for the role of ncBAF complexes in synovial sarcoma cell maintenance (Supplementary Fig. 3h). Cell cycle analysis that was performed on cells treated with dBRD9 revealed a decrease in cells in the S phase and an increase in cells in sub-G1, relative to dimethylsulfoxide (DMSO) vehicle control, and annexin V staining demonstrated an increase in apoptotic cells (Supplementary Fig. 3i,j). Furthermore, global transcriptional profiling revealed similar effects on gene expression between dBRD9 and shBRD9 treatments, whereas shSMARCE1 resulted in discordant changes and minimal transcriptional effects (Fig. 3g). Lastly, dBRD9 treatment of SMARCB1-deficient MRT cell lines TTC1240 and G401 resulted in reduced proliferation (Fig. 3h, Supplementary Fig. 3k and Supplementary Table 2), whereas dBRD9 treatment in a SMARCB1-intact epithelioid sarcoma cell line ESX did not (Supplementary Fig. 3i). As mSWI/SNF complexes in synovial sarcoma and MRT/AT/RT epithelioid sarcoma disease settings exhibit the shared feature of cBAF perturbation by SMARCB1 (BAF47) loss or destabilization, these results unmask a selective ncBAF dependency in two aggressive and intractable cBAF-mutant cancer types.

Finally, we compared total degradation of BRD9 (dBRD9 degrader) with BRD9 bromodomain inhibition using BI-7273. Colony formation assays in SYO-1, HSSYII and Aska synovial sarcoma cell lines demonstrated that dBRD9 treatment was substantially and reproducibly more potent than BI-7273 (or treatment with lenalidomide control; Fig. 3i and Supplementary Fig. 3m,n). Moreover, these treatments in cell lines lacking BAF complex perturbations did not result in the inhibition of colony formation (Supplementary Fig. 3o–q). Knockout of BRD9 in HEK-293T cells resulted in destabilized ncBAF complexes (Supplementary Fig. 3r), perhaps underlying the increased potency observed with dBRD9 relative to BI-7273. Together, these data suggest that the total loss of BRD9 is more potent than bromodomain inhibition, thus supporting the possibility that other domains of BRD9 may be important for BRD9 subunit function.

CRISPR guide RNA tiling experiments define the required domains on GLTSCR1 and BRD9 ncBAF subunits. We next sought to identify specific regions on BRD9 as well as other ncBAF components that uniquely underlie the synthetic lethality in synovial sarcoma. We performed CRISPR tiling screens with an array of guide RNAs against the exonic sequences of several subunits and their paralogs in the SYO-1 synovial sarcoma cell line (Fig. 4a and Supplementary Table 2,3). Guides targeting regions of the disease driver SS18, part of the SS18–SSX fusion, dropped out as expected (Supplementary Fig. 4a,b). Consistent with the CRISPR and RNAi screening results above (Fig. 3), cBAF/PBAF-specific subunits SMARCB1 and SMARCE1 did not drop out (Supplementary Fig. 4c,d) and ncBAF–specific paralogs SMARCD1 and SMARCC1 exhibited more dropout relative to SMARCD2/3 and SMARCC2, respectively, paralogs that do not assemble into ncBAF (Supplementary Fig. 4e–h). Interestingly, guides targeting the GLTSCR domain of GLTSCR1 and most of the coding region of BRD9, including the bromodomain and the DUF3512, exhibited significant fitness dropout (Fig. 4b,c). In contrast, guides targeting the bromodomain and DUF3512 of PBAF-specific BRD7, the paralog of BRD9, did not result in reduced fitness (Fig. 4d). These data highlight the synthetic lethal specificity for ncBAF components and demonstrate the importance of the GLTSCR and DUF3512 domains of GLTSCR1/1L and BRD9, respectively, in ncBAF function in synovial sarcoma.

To understand the roles of the GLTSCR and DUF3512 domains in ncBAF complexes, we assessed the evolutionary conservation of these regions (Fig. 4e,f). The most evolutionarily conserved region of the GLTSCR1/1L paralogs is the GLTSCR domain, which suggests that it could possibly serve an important structural role. Indeed, immunoprecipitation followed by immunoblotting of mammalian GLTSCR1 N- and C-terminal truncation mutants demonstrated that this domain is required for interaction with ncBAF complexes and thus serves as a ncBAF-specific binding region (Fig. 4g and Supplementary Fig. 4i). In contrast, although the bromodomain and DUF3512 regions are evolutionarily conserved between BRD9 and BRD7 homologs across species (Fig. 4f), the mammalian BRD9 and BRD7 paralogs incorporate into ncBAF and PBAF complexes, respectively. To determine whether the DUF3512 domain is involved in complex-specific binding of the BRD9 and BRD7 subunits, we performed domain swapping experiments in which we fused the C-terminal DUF-containing region of BRD9 to the N terminus of BRD7 and vice versa (Fig. 4h). Swapping of BRD9 and
ncBAF is not required for SS18–SSX fusion-mediated gene expression and primarily regulates retained fusion-independent sites. SS18 is a subunit of both cBAF and ncBAF complexes (Fig. 1) and the SS18–SSX fusion protein is a dedicated and stable subunit in cBAF complexes in synovial sarcoma21. To understand the sensitivity of synovial sarcoma cells to ncBAF complex depletion, we investigated whether SS18–SSX incorporates into ncBAF complexes. Complex purifications via haemagglutinin (HA)-tagged wild-type SS18 and SS18–SSX1 revealed that, although ncBAF subunits co-purify with SS18–SSX, relative to SMARCA4 they are less robustly captured by purification of SS18–SSX1 than SS18 (Fig. 5a).

Given that the SS18–SSX1 fusion protein destabilizes SMARCB1, a core subunit in cBAF complexes but not present in ncBAF complexes, we sought to determine whether fusion-containing ncBAF complexes can drive oncogenesis and the hallmark gene expression phenotypes of synovial sarcoma tumours. Thus, we performed RNA-seq on SYO-1 synovial sarcoma cells treated with either an shRNA targeting SS18–SSX (shSSX) or dBRD9. Intriguingly, although treatment with dBRD9 resulted in proliferative attenuation similar to knockdown of the disease-driver SS18–SSX (Fig. 3), few genes were concordantly affected by both treatments (Fig. 5b and Supplementary Fig. 5a). Specifically, although both BRD9 and SS18–SSX perturbations similarly affected cell cycle pathways consistent with proliferative attenuation, we found discordant effects on mesenchymal stem cell differentiation, neural differentiation and bivalent polycomb target genes, gene sets that are hallmarks of the synovial sarcoma-specific oncogenic signature, suggesting different underlying mechanisms (Fig. 5c)22,23.

To determine whether BRD9 (and hence, ncBAF complexes) were required for de novo SS18–SSX-mediated gene activation, we performed RNA-seq in CURL250 human fibroblasts in which we expressed either wild-type V5–SS18 or V5–SS18–SSX1 fusion with or without 24h pre-treatment with dBRD9, followed by sustained dBRD9 treatment (Supplementary Fig. 5b). Despite full degradation of BRD9 protein, dBRD9 treatment did not attenuate SS18–SSX-mediated gene activation and polycomb target genes associated with H3K27me3-mediated repression were equally activated irrespective of dBRD9 treatment (Fig. 5d and Supplementary Fig. 5c). These data in the SYO-1 and CURL250 settings suggest that the function of ncBAF complexes is distinct from that of SS18–SSX-bound cBAF complexes known to oppose polycomb at cancer-specific sites on the genome21. In addition, ncBAF is not required for the de novo activation of synovial sarcoma-specific gene signatures driven by the SS18–SSX fusion protein, pointing to a distinct mechanism underlying ncBAF dependency in synovial sarcoma.

We next sought to define the divergent gene regulatory effects between SS18–SSX1 and BRD9 perturbation. In synovial sarcoma, the SS18–SSX fusion protein directs targeting of BAF complexes to a cancer-specific set of sites on chromatin which are crucial for oncogenesis25 (Fig. 5e). To assess whether the SS18–SSX fusion hijacks BRD9 to such cancer-specific sites, we performed ChiP-seq for BRD9 before and after SS18–SSX knockdown and found that BRD9 is minimally re-targeted by the SS18–SSX fusion to broad-peak fusion-dependent sites (Fig. 5e). Additionally, fusion-independent sites (sites retained irrespective of SS18–SSX knockdown) are largely marked by H3K4me3 and CTCF (Fig. 5e), two hallmarks of ncBAF complex targeting (Fig. 2), whereas fusion-dependent sites are not.

We found that the genes closest to fusion-dependent sites were strongly downregulated by SS18–SSX knockdown35, but the expression of these genes did not change with BRD9 degradation (Fig. 5f and Supplementary Fig. 5d). Instead, the most downregulated genes following dBRD9 treatment were closest to fusion-independent sites (Fig. 5g and Supplementary Fig. 5e). This result is consistent with the lack of requirement for BRD9 in mediating de novo activation of fusion-dependent genes in CURL250 fibroblasts and the divergent transcriptional effects between shSS18–SSX and dBRD9 treatments in SYO-1 synovial sarcoma cells. Finally, we compared changes in gene expression following dBRD9 treatment with gene dependency scores derived from CRISPR screening and found that genes downregulated by dBRD9 treatment were significantly enriched for sensitivities (Fig. 5h). However, dBRD9 treatment in a BAF-intact cancer cell line, such as MOLM-13, did not result in preferential downregulation of genes that are enriched for dependencies (Supplementary Fig. 5f). Together, these results support a model in which BRD9/ncBAF complexes are important for the regulation of gene expression at fusion-independent sites. We propose that ncBAF complexes, which preferentially associate with wild-type SS18 and are, in contrast to cBAF complexes, less perturbed by the incorporation of the fusion protein, are critical for maintenance of essential genes at fusion-independent sites in a setting in which SS18–SSX has further targeted cBAF complexes away from these sites.

ncBAF is required for the maintenance of gene expression via retained co-localization with CTCF in SMARCB1-deficient cancers. In the absence of SMARCB1 in MRT cell lines, residual SMARCA4-marked mSWI/SNF complexes are substantially more localized to promoter-proximal sites and are deficient in enhancer targeting22,24. Previous studies that used Brg1 (Smarca4) conditional knockout in mouse models identified that Smarcb1-deficient cells are still dependent on Smarca4 for survival35 and these data have been more recently corroborated in large-scale dependency screens in cancer cell lines. Thus, we investigated whether these residual mSWI/SNF complexes in MRT would primarily represent intact ncBAF complexes. We performed ChiP-seq for BRD9 in the MRT cell line TTC1240 and found that BRD9 localizes to a large proportion of SMARCA4 sites (Fig. 6a). In contrast, ncBAF complexes co-localize with approximately one-third or fewer of all SMARCA4 sites in mSWI/SNF-intact settings, such as MOLM-13 and Jurkat cells, and in MRT TTC1240 cells in which SMARCB1 has been rescued (Fig. 6b). Thus, a large percentage of residual mSWI/SNF complexes that are required for proliferative maintenance in SMARCB1-deficient cell lines represent ncBAF complexes.

BAF complex localization and function at enhancers and super-enhancers have been shown to be aberrant in MRT27,28. Thus, we examined BRD9 targeting to MRT-specific super-enhancers, defined by Chun et al. in primary tumours and cell lines compared to hESC lines and fetal brain tissue35. The TTC1240 cell line exhibits a strong overlap with these primary tumour-associated MRT-specific enhancers and super-enhancers (Supplementary Fig. 6a) and BRD9-marked ncBAF complexes localized to a large number of these MRT-specific super-enhancers, particularly those that encompass a TSS (Fig. 6c). To investigate the role of BRD9 at these genes in MRT, we treated TTC1240 cells with dBRD9 and performed ChiP-seq and RNA-seq. Treatment with dBRD9 resulted in a significant decrease in SMARCA4 occupancy, particularly at BRD9-marked sites genome-wide (Fig. 6d–f and Supplementary Fig. 6b). Consistent with overlap at super-enhancers, lost SMARCA4 peaks were highly enriched in H3K27ac relative to peaks that did not change (Fig. 6g). In addition, many of the downregulated genes had BRD9 occupancy at their promoters and genes that demonstrated significant changes had higher H3K27ac and BRD9 occupancy than active genes without significant changes (Supplementary Fig. 6c). Genes that were downregulated by dBRD9 and lost SMARCA4 occupancy were enriched for those genes overexpressed in MRT.
In characterizing the convergent mechanism of ncBAF dependency in synovial sarcoma and MRT, we unexpectedly found that although ncBAF complexes do incorporate the SS18–SSX fusion that drives synovial sarcoma, perturbation of BRD9 and disruption of SS18–SSX are mechanistically distinct. Non-canonical BAF complexes primarily regulate retained fusion-independent sites, linking to SMARCB1-deficient MRT in which ncBAF complexes comprise a large share of essential residual complexes that similarly maintain gene expression at retained mSWI/SNF sites. The retained sites in both of these disease settings have CTCF co-localization and promoter proximity in common, the two hallmarks of ncBAF complex localization. Thus, this work now provides a complex-specific basis for previously identified dependencies on SMARCA4 (BRG1) in SMARCB1-deficient cancers and highlights the importance of understanding subunit-specific contributions to complex assembly and function when designing strategies to target mSWI/SNF-perturbed cancers. Our data suggest the possibility that other cBAF-perturbed cancers, such as those with loss of ARID1A/B or SMARCE1, may exhibit similar ncBAF dependencies.

Online content

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References

1. Narlikar, G. J., GeetaL., Sundaramoorthy, R. & Owen-Hughes, T. Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. Cell 154, 490–503 (2013).
2. Clapier, C. R. & Cairns, B. R. The biology of chromatin remodeling complexes. Annu. Rev. Biochem. 78, 273–304 (2009).
3. Ho, I. et al. An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. Proc. Natl Acad. Sci. USA 106, 5181–5186 (2009).
4. Lessard, J. et al. An essential switch in subunit composition of a chromatin remodeling complex during neural development. Neuron 55, 201–215 (2007).
5. Lickert, H. et al. Ba690c is essential for function of BAf chromatin remodelling complexes in heart development. Nature 432, 107–112 (2004).
6. Priam, P. et al. SMARC2D subunit of SWI/SNF chromatin-remodeling complexes mediates granuloPOiesis through a CEP4 dependent mechanism. Nat. Genet. 49, 753–764 (2017).
7. Witzel, M. et al. Chromatin-remodelling factor SMARC2D regulates transcriptional networks controlling differentiation of neutrophil granulocytes. Nat. Genet. 49, 742–752 (2017).
8. Staahl, B. T. et al. Kinetic analysis of npBAF to nBAF switching reveals exchange of SS18 with CREST and integration with neural developmental pathways. J. Neurosci. 33, 10348–10361 (2013).
9. Yoo, A. S., Staahl, B. T., Chen, L. & Crabtree, G. R. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460, 642–646 (2009).
10. Yoo, A. S. et al. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476, 228–231 (2011).
11. Pedersen, T. A., Kowenz-Leutz, E., Leutz, A. & Nerlov, C. Cooperation between C/EBPα TBP/TFIIB and SWI/SNF recruiting domains is required for adipocyte differentiation. Genes Dev. 15, 3208–3216 (2001).
12. Pan, J. et al. Interrogation of mammalian protein complex structure, function, and membership using genome-scale fitness screens. Cell 6, 555–568 (2018).
13. Alpsoy, S. & Dykhuijen, E. C. Glioma tumor suppressor candidate region gene 1 (GLTSCR1) and its paralog GLTSCR1-like form SWI/SNF chromatin remodeling subcomplexes. J. Biol. Chem. 293, 3892–3903 (2018).
14. Wang, W. et al. Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev. 10, 2117–2130 (1996).
15. Kaeber, M. O., Aasland, A., Dong, M. Q., Yates, J. R. 3rd & Emerson, B. M. BRD7, a novel PRAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. J. Biol. Chem. 283, 32254–32263 (2008).
16. Kadoch, C. et al. Proteomic and bioinformatic analysis of mammalian SWI/ SNF complexes identifies extensive roles in human malignancy. Nat. Genet. 45, 592–601 (2013).
17. Shain, A. H. & Pollack, J. R. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. PLoS ONE 8, e55113 (2013).
18. Biegel, J. A. et al. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. Cancer Res. 59, 74–79 (1999).
19. Eaton, K. W., Tooko, L. S., Wainwright, L. M., Judkins, A. R. & Biegel, J. A. Spectrum of SMARCBI/INI1 mutations in familial and sporadic rhabdoid tumors. Pediatr. Blood Cancer 56, 7–15 (2011).
20. Versteege, I. et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature 394, 203–206 (1998).
21. Jones, S. et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science 330, 228–231 (2010).
22. Varela, I. et al. Exome sequencing identifies frequent mutation of the SWI/ SNF complex gene PBRM1 in renal carcinoma. Nature 469, 539–542 (2011).
23. McBride, M. J. et al. The SS18–SSX fusion oncoprotein hijacks BAF complex targeting and function to drive synovial sarcoma. Cancer Cell 33, 1128–1141 (2018).
24. Helming, K. C. et al. ARID1B is a specific vulnerability in ARID1A-mutant cancers. Nat. Med. 20, 251–254 (2014).
25. Hoffman, G. R. et al. Functional epigenetics approach identifies BRM/ SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. Proc. Natl. Acad. Sci. USA 111, 3128–3133 (2014).
26. Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. Nat. Genet. 49, 1779–1784 (2017).
27. Tsherniak, A. et al. Defining a cancer dependency map. Cell 170, 564–576 (2017).
28. Wang, T. et al. Gene essentiality profiling reveals gene networks and synthetic dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. Cell 170, 577–592 (2017).
29. Cowley, G. S. et al. Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies. Sci. Data 1, 140035 (2014).
30. Bell, A. C. & Felsenfeld, G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405, 482–485 (2000).
31. Bell, A. C., West, A. G. & Felsenfeld, G. The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell 98, 387–396 (1999).
32. Hark, A. T. et al. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature 405, 486–489 (2000).
33. Kenduri, C. et al. Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. Curr. Biol. 10, 853–856 (2000).
34. Alver, B. H. et al. The SWI/SNF chromatin remodelling complex is required for maintenance of lineage specific enhancers. Nat. Commun. 8, 14648 (2017).
35. Mathur, R. et al. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. Nat. Genet. 49, 296–302 (2017).
36. Wang, X. et al. SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. Nat. Genet. 49, 289–295 (2017).
37. Nakayama, R. T. et al. SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. Nat. Genet. 49, 1613–1623 (2017).
38. Kadoch, C. & Crabtree, G. R. Reversible disruption of mSWI/SNF (BAF) complexes by the SS18–SSX oncogenic fusion in synovial sarcoma. Cell 153, 71–85 (2013).
39. Clark, J. et al. Identification of novel genes, SYT and SSX, involved in the t(X;18)(p11.2; q11.2) translocation found in human synovial sarcoma. Nat. Genet. 7, 502–508 (1994).
40. Hohmann, A. F. et al. Sensitivity and engineered resistance of myeloid leukemia cells to BRD9 inhibition. Nat. Chem. Biol. 12, 672–679 (2016).
41. Martin, L. J. et al. Structure-based design of an in vivo active selective BRD9 inhibitor. J. Med. Chem. 59, 4462–4475 (2016).
42. Remillard, D. et al. Degradation of the BAF complex factor BRD9 by heterobifunctional ligands. Angew. Chem. Int. Ed. 56, 5738–5743 (2017).
43. Wang, X. et al. Oncogenesis caused by loss of the SNF5 tumor suppressor is dependent on activity of BRG1, the ATPase of the SWI/SNF chromatin remodeling complex. Cancer Res. 69, 8094–8101 (2009).
Methods

Cell lines and tissue culture. HEK-293T, G401, TTC1240, ESX, IMR-90, BJ fibroblast, CRL2750 and NCH-1437 cells were grown in DMEM (Gibco) supplemented with 10% FBS, 1% Glutamax (Gibco) and 1% penicillin-streptomycin (Gibco). ES-2 cells were grown in McCoy’s 5A media (Gibco) supplemented with 10% FBS, 1% Glutamax (Gibco) and 1% penicillin-streptomycin (Gibco). EoL-1 and MOLM-13 cells were grown in RPMI (Gibco) supplemented with 10% FBS, 1% Glutamax (Gibco) and 1% penicillin-streptomycin (Gibco). The human rhabdomyosarcoma (RD) cell line was cultured in DMEM (Gibco) supplemented with 10% FBS, 1% Glutamax (Gibco) and 1% penicillin-streptomycin (Gibco). Calu-6 cells were grown in McCoy's 5A media (Gibco) supplemented with 10% FBS. Calu-6 cells were washed with PBS and stained using 500 μl 0.005% crystal violet (w/v) solution a change of medium every five days. By Day 14, the medium was removed, cells were washed with PBS and stained using 500 μl 0.005% crystal violet (w/v) solution. Colony formation assays were performed in a 6- or 96-well plate, 50 μl of the corresponding solution was added to each well and incubated for 1h at 3,000 rpm and 4 °C and the cytisol-containing layer was discarded. The nuclear pellets were resuspended in high-salt buffer containing 50 mM Tris–HCl pH 7.5, 10 mM KCl, 1.5 mM MgCl₂, 1 mM dithiothreitol (DTT) and 1 mM PMSF and incubated on ice for 5 min. The suspension was centrifuged at 5,000 rpm for 5 min at 4 °C, the pellets were resuspended in five volumes of fresh hypotonic buffer containing a protease inhibitor cocktail and homogenized using glass Dounce homogenizer. The suspension was then layered onto hypotonic buffer supplemented with sucrose containing 30% sucrose (w/v), centrifuged for 1h at 5,000 rpm and 4 °C and the cytosol-containing layer was discarded. The nuclear pellets were resuspended in high-salt buffer containing 50 mM Tris–HCl pH 7.5, 300 mM KCl, 1 mM MgCl₂, 1 mM EDTA, 1 mM, 1% NP40, 1 mM DTT, 1 mM PMSF and a protease inhibitor cocktail. Homogenates were incubated on rotator for 1 h. The homogenates then were centrifuged at 20,000 × g (30,000 g) for 1 h at 4 °C using an SW32Ti rotor. The chromatin pellets were discarded and the high-salt nuclear extract was filtered through a 0.45 μm filter and incubated overnight with HA magnetic resin. HA beads were washed four times (1.5 h per wash) in high-salt buffer and eluted with high-salt buffer containing 1 mg ml⁻¹ HA peptide. The eluted proteins were then subjected to density gradient centrifugation or dialysis.

Protein extraction methods. Ammonium sulfate nuclear extraction was performed as reported previously. Pellets were resuspended in IP buffer (300 mM NaCl, 50 mM Tris–HCl pH 7.5, 1 mM EDTA and 1% Triton-X100, supplemented with fresh protease inhibitor, 1 mM DTT and 1 mM PMSF) for subsequent experiments.

For whole cell lysates, cells were washed with PBS and resuspended in approximately five volumes of extraction buffer (20 mM Tris and 1.5% SDS). Chromatin was solubilized through sonication and proteins were quantified using bicinchoninic acid assay (BCA).

Immunoprecipitation. Nuclear extracts were quantified using BCA and 1 mg protein (1 mgml⁻¹ in IP buffer supplemented with protease inhibitors) was used per immunoprecipitation. Proteins were incubated overnight with 2–5 μg of antibody or with 25 μl Pierce Anti-HA Magnetic Beads with rotation at 4 °C. The solution of nuclear extract + antibody was incubated with 30 μl Protein G Dynabeads (Thermo Fisher) for 2 h at 4 °C with rotation and washed five times with IP buffer. Immunoprecipitated proteins were eluted with sample buffer (2× NuPAGE LDS buffer with 100 mM DTT) and loaded onto 4–12% Bis-Tris NuPAGE Gels (Life Technologies). See the Reporting Summary for the antibodies used in this study.

Glyceral gradient. Linear 10–30% glyceral gradients were prepared in 14 mm × 89 mm polycralline centrifuge tubes (Beckman Coulter) by overlaying 10% glyceral solution in HEMG buffer on 30% glyceral solution with mixing by a Gradient Master. The nuclear extracts (500–1,000 μg) were resuspended in 200 μl HEMG (0% glyceral) and overlaid on the gradient. Purified protein complexes were loaded in their elution buffers. The gradients were centrifuged in an SW41 rotor at 40,000 r.p.m. for 16 h at 4 °C and 0.55 ml fractions were collected for analysis.

Mass spectrometry sample preparation and analysis. Purified complex elutions (BRD9, BRD7, Mock) or glycerol gradient fractions (DPF2, fractions 13–14) were concentrated using StrataClean beads, loaded onto 4–12% SDS–PAGE gels, allowing to migrate 2 cm into the gel and stained with colloidal blue (Invitrogen). Stained samples were excised and sent to Taplin Biological Mass Spectrometry Facility at Harvard Medical School for analysis. A heatmap displaying log[number of total peptides + 1] was created using Seaborn.
Protein SDS–PAGE. Proteins were run on 4–12% Bis-Tris NuPAGE gels (Life Technologies). For Western blotting, the proteins were wet transferred onto PVDF membranes at 300 mA for 2.5 h, blocked for 1 h with 10% milk PBS-T and visualized using LI-COR Odyssey CLX. For silver staining, gels were stained using SilverQuest Silver Staining Kit (Thermo Fisher) according to the manufacturer’s protocol.

Chromatin immunoprecipitation. Cells were fixed in 1% formaldehyde (Sigma Aldrich) for 10 min at 37 °C and quenched with 125 mM glycine for 5 min at 5 °C. The cells were then washed with cold PBS and stored at −80 °C until use. Ten million cells per ChIP were used for EoL-1, MOLM-13 and Jurkat cell lines, and five million cells per ChIP were used for SYO-1 and TTC1240 cell lines. Nuclei were extracted and chromatin was sonicated using the adaptive focused acoustics technology with a Covaris sonicator. Sonicated chromatin was used in overnight immunoprecipitation reactions with the indicated antibodies (see Reporting Summary), followed by capture using Protein G Dynabeads (Thermo Fisher). For ChIP-seq using spike-in chromatin, 15 ng of spike-in Drosophila chromatin (Active Motif) was added to each sample with 2 μg spike-in antibody (Active Motif). Captured antibody-chromatin complexes were washed, eluted and treated with RNase A (Roche) for 30 min at 37 °C and Proteinase K (Life Technologies) for 3 h at 65 °C. ChIP DNA was extracted using SPRI beads (Beckman Coulter Agencourt AMP Xpure), washed and eluted.

RNA-seq sample preparation. RNA was collected from 2 × 10^6 cells per condition, in biological duplicates, using the RNeasy Mini Kit (QIAGEN) according to the manufacturer’s protocol.

Library preparation and sequencing. Library preparation and sequencing of ChIP DNA and RNA was performed by the Molecular Biology Core Facilities at the Dana–Farber Cancer Institute (75 bp single end on Illumina Nextseq 500).

ChIP-Seq data alignment. The ChIP-seq data were aligned using Bowtie2, version 2.1.0.3 to mapreads to the hg19 human reference genome, using the parameter –k 1.

For spike-in normalization, Drosophila DNA was aligned to the dm3 genome using Bowtie2 version 2.1.0.1 with the parameter –k 1. Duplicated reads were removed using samtools rmdup with the –b option (SAMtools v1.3.1). As per the manufacturer’s instructions, the normalization ratios were calculated using the ratio of the total number of non-redundant mapped reads in each sample to the sample with the fewest non-redundant mapped reads.

ChIP-Seq data analysis. Data processing. MACS234 version 2.1.0.2 was used to call peaks against input with a cutoff of q = 0.001. In EoL-1, MOLM-13 and TTC1240 cells, narrow peaks were called for all SWI/SNF antibodies and CTCF whereas broad peaks were used for all histone marks. In SYO-1 cells, broad peaks were called for all antibodies. Peaks that fell in ENCODE blacklisted regions or were mapped to unmappable chromosomes (not chr1–22, X or Y) were removed. Quality control metrics are available in Supplementary Table 5. All of the downstream analysis was performed on bam files with duplicates removed using the samtools rmdup command with the –b option. ChIP-seq tracks were generated using the bedGraphToBigWig script downloaded from UCSC. Bedgraph files were generated with MACS2 using the –b –SPMR options. For the TTC1240 SMARCA4 tracks shown, the bedGraph files values were multiplied by the spike-in normalization ratios calculated as described above.

Overlaps for ChIP Venn diagrams were created using the CHIPPeakAnno10 v3.10.1.1 biocorporate package, the peak files were read using the tgoRanges() command and values were determined using the getVennCounts() function with maxgap = 0. Data was visualized using matplotlib. The number of overlapping peaks displayed in pie charts, bar charts and heatmaps was determined using the pybedtools12 intersect function. Proportions were calculated by dividing the number of overlapping peaks by the number of total peaks.

Read counts across peak sets of interest were calculated by calling the Rsubread13 v1.26.1 biocorporate package function featureCounts() on duplicate removed bam files. These values were divided by the total number of mapped reads divided by one million, giving a normalized value of RPM for each interval in the input bed.

Peak distance from TSS elements was determined using BEDTools v2.26.0 closest function with the hg19 ref flat TSS annotation.

Determination of super-enhancers was performed using the default settings in ROSE14: the TTC1240 H3K27ac CHIP-seq file and TTC1240 H3K27ac peak files were used as inputs. MRT-specific super-enhancers were downloaded from Chun et al15 and merged using bedtools merge, as many of their published enhancers abutted one another.

Data analysis and visualization. Metagene plots and heatmaps were generated created using HTSeq16 v0.9.1. To account for the 200 bp average fragment length selected for in sonication, the fragment length was extended by 200 bp from the edge of each genomic interval. Total read counts for each interval were normalized to reads RPM. For each antibody, the resulting matrix has a width of the number of base pairs in the window (in this study primarily 5,000) and a height of the number of peaks in the indicated set. Strandness of the interval was not considered, except for the TSS metagene plot in Supplementary Fig. 2g.

Metagene plots show the average RPM at each position. Heatmaps were visualized using python. Heatmaps were ordered by the maximum value in each matrix row of the indicated antibody. Heatmaps were coloured such that the midpoint of the colour spectrum is equivalent to the median of the set of maximum values in each row. For heatmaps where multiple peak sets are shown, these colour values were calculated for each antibody across both sets together. For the spike-in normalized heatmap, all heat map data was calculated as described but then multiplied by the normalization factor, described above, before plotting.

In the heatmap of EoL-1 histone marks and CTCF sites in Supplementary Fig. 2i, the HTSEQ procedure described above was carried over peaks that had been split into 100 bins and 2,500 bp on either side of the peak. The resulting matrix was k-means clustered to four clusters. This was carried out over the merged set of all EoL-1 SWI/SNF/SNF peaks.

The SYO-1 differential heatmap (Fig. 5e) was ordered by the ratio of the row means for BRD9 in the +/- shSSX conditions. Any interval that had an increase in mean BRD9 ChIP occupancy of at least 25% following shSSX treatment was considered gained. Any interval that had at least 25% decrease in mean BRD9 ChIP occupancy following shSSX treatment was considered lost. Intervals that did not change more than 25% in either direction were considered retained.

Differential occupancy of SMARCA4 in TTC1240 following dBRD9 treatment was determined using the DiffBind v2.4.8.1 biocorporate package (https://bioconductor.org/packages/release/bioc/html/DiffBind.html), with all default settings. Peak files and duplicate bam files were provided for each condition by SMARCA4 sample in each condition, along with the bam files corresponding to the input in each condition. The package functions count(), contrast(), analyze() and report() were used in sequence.

Gene ontology of genes near lost SMARCA4 sites in TTC1240 (Supplementary Fig. 6d) was performed using the Genomic Regions Enrichment of Annotations Tool (GREAT)17.

Motif analysis. A fasta sequence for a region of 250 bp on either side of the centre of each peak was generated using the bedtools getfasta function. Motif analysis on these sequences was done using the MEME-ChIP suite18.

In Fig. 2d, the motif with the highest CentriMo log-adjusted P value in the indicated transcription factor family was selected for each antibody. These log-adjusted P values were used to make a heatmap using the Seaborns clustermap function, clustered by correlation.

Enrichment plots for the motifs are the average number of the CentriMo site counts for each antibody in the window around the indicated motif split into bins of 10bp.

RNA-seq data analysis. Data processing. RNA-seq data reads were mapped using default parameters to hg19 using STAR25 version 2.5.2a.

RPKM values were calculated using GOFold version 1.1.423. Unless otherwise noted, log[fold change] and Bonferroni-corrected P values were generated using DESEQ2 v1.16.1, with reads mapped using RSBREAD26 v1.26.1. Genes were considered significantly altered if they had an adjusted P < 0.001 and a log([fold change] of at least 0.59 (approximately 50% change). All RNA-seq experiments were performed with biological replicates. Quality control metrics are available in Supplementary Table 5.

RNA BigWig files were generated using the bamCoverage command from deepTools release 2.4.2 with all default settings.

Data analysis and visualization. The input for GSEA was created by calculating the log[fold change] between the mean RPKM of the replicates in each condition +1. Non-coding genes (small nuclear (sn)RNAs and micro (mi)RNAs) were determined using MetaScape27.

Genes associated with MRT super-enhancers and differential expression files of genes between MRT and normal tissues were downloaded from Chun et al15; genes that were overexpressed in MRTs with Bonferroni-adjusted P < 0.01 were considered overexpressed in MRTs.

CRISPR–Cas9 and shRNA synthetic lethal screening data analysis. DRIVE data is publicly available and can be downloaded from the Novartis DRIVE Data Portal28. Statistical analysis was performed using the scipy stats package.

Significance values for shBRD9 in tissue types were calculated using a Fisher’s exact test and FDR-corrected using the Benjamini–Hochberg procedure. An ATARIS score of −0.75 was used as the cutoff for sensitivity.

Principal components analysis of fitness data from Project Achilles. Datasets were obtained from the Project Achilles Data Portal (https://portals.broadinstitute.org/achilles/about). The CRISPR data (Avana-180Q1) and the RNA data (2.28.2) for BAF subunits were scaled across cell lines. In the RNAi dataset, cell lines were
omitted if fitness scores were not available for all RBF genes. The fitness scores from both datasets were concatenated and correlated across genes and principal components analysis was performed on the resulting correlation matrix (R pcomp, default settings). The first two principle components were plotted.

All heatmaps and plots were generated using matplotlib and/or seaborn. Unless otherwise noted, all default parameters were used for the seaborn clustermap function.

Statistics and reproducibility. All statistics performed in this manuscript are detailed above and statistical tests and their respective parameters are indicated in the Figure legends. Representative data are shown from independently repeated experiments with similar results.

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

Code availability. Code used in this paper is available from the corresponding author upon request.

Data availability

The ChIP-seq and RNA-seq datasets generated and/or analysed during the current study have been deposited in the Gene Expression Omnibus repository under accession number GSE113042 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113042). Other datasets that were previously published and used in this study have been deposited in the Gene Expression Omnibus repository under accession numbers GSE90634 and GSE108025 available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE90634 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108025, respectively. The fitness data were derived from Project Achilles through the Project Achilles Data Portal (https://portals.broadinstitute.org/achilles/about/). The dataset derived from this resource that supports the findings of this study is available at https://portals.broadinstitute.org/achilles/datasets/all. All proteomics/mass-spectrometry data are deposited at the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD011103.

References

49. Naka, N. et al. Synovial sarcoma is a stem cell malignancy. Stem Cells 28, 1119–1131 (2010).
50. Munoz, D. M. et al. CRISPR screens provide a comprehensive assessment of cancer vulnerabilities but generate false-positive hits for highly amplified genomic regions. Cancer Discov. 6, 900–913 (2016).
51. Mashtalir, N. et al. Autodeubiquitination protects the tumor suppressor BAP1 from cytoplasmic sequestration mediated by the atypical ubiquitin ligase UBE2O. Mol. Cell 54, 392–406 (2014).
52. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012).
53. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol. 9, R137 (2008).
54. Zhu, L. J. et al. ChiPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. BMC Bioinform. 11, 237 (2010).
55. Dale, R. K., Pedersen, B. S. & Quinlan, A. R. Pybedtools: a flexible Python library for manipulating genomic datasets and annotations. Bioinformatics 27, 3423–3424 (2011).
56. Liao, Y., Smyth, G. K. & Shi, W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 41, e108 (2013).
57. Loven, J. et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 153, 320–334 (2013).
58. Whyte, W. A. et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153, 307–319 (2013).
59. Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169 (2015).
60. McLean, C. Y. et al. GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. 28, 495–501 (2010).
61. Machanick, P. & Bailey, T. L. MEME-ChIP: motif analysis of large DNA datasets. Bioinformatics 27, 1696–1697 (2011).
62. Dobin, A. et al. STAR: ultrastar universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
63. Feng, J. et al. GFOLD: a generalized fold change for ranking differentially expressed genes from RNA-seq data. Bioinformatics 28, 2782–2788 (2012).
64. Ramirez, F., Dundar, F., Diehl, S., Gruning, B. A. & Manke, T. deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res. 42, W187–W191 (2014).
65. 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).
66. Tripathi, S. et al. Meta- and orthogonal integration of Influenza “OMICs” data defines a role for UBR4 in virus budding. Cell Host Microbe 18, 723–735 (2015).
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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).

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- 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
- Raw Illumina output was converted to fastq format using Illumina Bcl2fastq v2.18.

Data analysis
- All open source packages that were used to process and analyze data in this study are detailed below in the “Software” section of ChIP-seq, and include MACS2 version 2.1.0, bedGraphToBigWig from UCSC, ChIPPeakAnno v3.10.1, pybedtools, Rsubread v1.26.1, BEDtools v2.26.0, ROSE, HTSeq v0.9.1, the DiffBind v2.4.8, MEME ChIP suite, and Genomic Regions Enrichment of Annotations Tool (GREAT).
- Packages used to analyze RNA-seq data include STAR 2.5.2a, GFOLD version 1.1.4, DESEQ2 v1.16.1, Rsubread v1.26.1, deepTools release 2.4, Gene Set Enrichment Analysis (GSEA), and Metascape. Any scripts or code written by the authors are available upon request from the corresponding author.
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
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- A description of any restrictions on data availability

The sequencing data sets generated during the current study have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE113042 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113042). Other data sets that were previously published and used in this study have been deposited in the Gene Expression Omnibus (GEO) repository under accession numbers GSE90634 and GSE108025 available at (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE90634) and (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108025) respectively.

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Life sciences study design

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

| Sample size | N/A. No human or animal subjects necessitating sample size calculations were used in this study. |
| Data exclusions | No data were excluded from analysis or reporting. |
| Replication | All experiments were performed in at least n=2 biologically independent experiments. For proliferation curve experiments, n=3 independent biological samples were used, enabling statistical calculations. No replicates were excluded from analyses presented, and all attempts at replication were successful. |
| Randomization | Not applicable as human or animal subjects were not used in this study. |
| Blinding | Not applicable as human or animal subjects were not used in this study. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|-------------------|-------------------|
| n/a | n/a |
| □ | □ |

Involved in the study

- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- CHiP-seq
- Flow cytometry
- MRI-based neuroimaging

Antibodies

| Antibodies used |
|----------------|
| Antibody Clone# Company Cat# Application Dilution |
| SMARCA4 D1Q7F Cell Signaling Technology 49360 Western blot 1:1000 |
| SMARCA4 G-7 Santa Cruz sc-17796 Western blot 1:1000 |
| SMARCC1 H-76 Cell Signaling Technology 11956S Western blot 1:1000 |
| SMARCC1 D7F8S Santa Cruz sc-10756 Western blot 1:1000 |
| SMARCD1 23 Santa Cruz sc-135843 Western blot 1:1000 |
| SMARCB1 A-5 Santa Cruz sc-166165 Western blot 1:1000 |
| SMARCC2 D809V Cell Signaling Technology 127605 Western blot 1:1000 |
| SMARCC2 G-12 Santa Cruz sc-166237 Western blot 1:1000 |
| Antibody Clone# | Company | Cat# | Lot# | Amount |
|----------------|---------|------|------|--------|
| BRD9 N/A       | abcam   | ab137245 GR257571-14 | 3ug   |
| GLTSCR1 S-16 Santa Cruz SC-240516 | A2313 | 15ul |
| Eol-1 BRD9 N/A | abcam   | ab66443 GR144569-1 | 3ug   |
| Eol-1 GLTSCR1 | Santa Cruz sc-240516 | A2313 | 15ul |
| Eol-1 BRD7 D9K2T Cell Signaling Technology 14910 | Lot 1 | 15ul |
| Eol-1 DPF2 EPR9206(B) abcam ab134942 | Y031611CS | 3ug |
| Eol-1 SMARCC1 fx 2, 4-11 homemade | N/A 3/9/15 | 3ug |
| Eol-1 SMARCA4 EPNCIR111A abcam ab110641 | GR150844-12 | 5ul |
| Eol-1 CTCF D31H2 Cell Signaling Technology 34185 Lot 3 | 3ug |
| Eol-1 H3K27Ac N/A | abcam ab4729 GR238017-2 | 3ug |
| Eol-1 H3K4me1 N/A | abcam ab8895 GR159018-3 | 3ug |
| Eol-1 H3K4me3 15-10C-E4 Millipore | 05745R 2326998 | 3ul |
| MOLM-13 BRD9 N/A | abcam ab137245 GR257571-14 | 3ug |
| MOLM-13 GLTSCR1 S-16 Santa Cruz SC-240516 | A2313 | 15ul |
| MOLM-13 BRD7 D9K2T Cell Signaling Technology 14910 | Lot 1 | 15ul |
| MOLM-13 DPF2 EPR9206(B) abcam ab134942 | Y031611CS | 3ug |
| MOLM-13 SMARCA4 EPNCIR111A abcam ab110641 | GR150844-12 | 5ul |
| MOLM-13 CTCF D31H2 Cell Signaling Technology 34185 | Lot 3 | 3ug |
| MOLM-13 SYO-1 BRD9 N/A | abcam ab137245 GR257571-20 | 3ug |
| MOLM-13 SYO-1 CTCF D31H2 Cell Signaling Technology 34185 | Lot 3 | 3ug |
| MOLM-13 TTC1240 BRD9 N/A | abcam ab137245 GR257571-20 | 3ug |
| TTC1240 BRD9 N/A | abcam ab137245 GR257571-23 | 3ug |
| TTC1240 SMARCA4 EPNCIR111A abcam ab110641 | GR3208604-3 | 5ul |
| TTC1240 CTCF D31H2 Cell Signaling Technology 34185 | Lot 3 | 3ug |
| Aska BRD9 N/A | abcam ab137245 GR257571-20 | 3ug |
| Jurkat BRD9 N/A | abcam ab137245 GR257571-20 | 3ug |

**Validation**

All antibodies used in this study have been thoroughly validated by our laboratory to be specific (using IP-mass-spectrometry in wild-type and KO cell lines, and using immunoblot in wild-type and KO cell lines).

**Eukaryotic cell lines**

Policy information about cell lines

| Cell line Source |
|------------------|
| HEP-2 from Dr. Gerald Crabtree at Stanford |
| EoL-1, Gift from Dr. Jay Bradner at DFCI |
| MOLM-13, Gift from Dr. Jay Bradner at DFCI |
| TTC1240, Gift from Dr. Timothy Triche |
| G401, ATCC |
| ES-X, ATCC |
| IMR-90, ATCC |
| CRL2250, Gift from Drs. Berkeley Gryder and Javed Khan at NCI |
Authentication

All cell lines were routinely checked for mycoplasma contamination and confirmed negative. All cell lines were subjected to routine fingerprinting analyses to confirm identity.

Mycoplasma contamination

All cell lines used in the study tested negative for mycoplasma.

Commonly misidentified lines

(See ICLAC register)

No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

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.

The sequencing data sets generated and/or analyzed during the current study have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE113042 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113042).

Files in database submission

Fastq and bigWig files for the following ChIP-Seq samples were deposited in the Gene Expression Omnibus, they can be accessed using the link above:

- GSM3094366 EOL1_Input_Naive_ChIP-Seq
- GSM3094367 EOL1_SMARCA4_Naive_ChIP-Seq
- GSM3094368 EOL1_BRD9_137245_Naive_ChIP-Seq
- GSM3094369 EOL1_BRD9_66443_Naive_ChIP-Seq
- GSM3094370 EOL1_GLTSCR1_Naive_ChIP-Seq
- GSM3094371 EOL1_SMARCC1_Naive_ChIP-Seq
- GSM3094372 EOL1_BRD7_Naive_ChIP-Seq
- GSM3094373 EOL1_DPFF2_Naive_ChIP-Seq
- GSM3094374 EOL1_H3K27Ac_Naive_ChIP-Seq
- GSM3094375 EOL1_CTCF_Naive_ChIP-Seq
- GSM3094376 MOLM13_Input_DMSO_ChIP-Seq
- GSM3094377 MOLM13_SMARCA4_DMSO_ChIP-Seq
- GSM3094378 MOLM13_BRD9_DMSO_ChIP-Seq
- GSM3094379 MOLM13_BRD7_DMSO_ChIP-Seq
- GSM3094380 MOLM13_DPFF2_DMSO_ChIP-Seq
- GSM3094381 MOLM13_GLTSCR1_DMSO_ChIP-Seq
- GSM3094382 MOLM13_CTCF_DMSO_ChIP-Seq
- GSM3094383 EOL1_H3K4me1_Naive_ChIP-Seq
- GSM3094384 EOL1_H3K4me3_Naive_ChIP-Seq
- GSM3094385 SYO1_Input_shControl_ChIP-Seq
- GSM3094386 SYO1_SS18_shControl_ChIP-Seq
- GSM3094387 SYO1_BRD9_shControl_ChIP-Seq
- GSM3094388 SYO1_CTCF_shControl_ChIP-Seq
- GSM3094389 SYO1_Input_shSSX_ChIP-Seq
- GSM3094390 SYO1_SS18_shSSX_ChIP-Seq
- GSM3094391 SYO1_BRD9_shSSX_ChIP-Seq
- GSM3094394 JURKAT_Input_Naive_ChIP-Seq
- GSM3094395 JURKAT_BRD9_Naive_ChIP-Seq
- GSM3094396 JURKAT_CTCF_Naive_ChIP-Seq
- GSM3094397 TTC1240_BRD9_N106_Empty_ChIP-Seq
- GSM3094398 TTC1240_BRD9_N106_SMARCB1_ChIP-Seq
- GSM3326008 TTC1240_BRD9_dBRD9_ChIP-Seq
- GSM3326009 TTC1240_BRD9_DMSO_ChIP-Seq
- GSM3326010 TTC1240_CTCF_Empty_ChIP-Seq
- GSM3326011 TTC1240_Input_dBRD9_ChIP-Seq
- GSM3326012 TTC1240_Input_DMSO_ChIP-Seq
- GSM3326013 TTC1240_SMARCA4_Rep1_dBRD9_ChIP-Seq
- GSM3326014 TTC1240_SMARCA4_Rep1_DMSO_ChIP-Seq
- GSM3326015 TTC1240_SMARCA4_Rep2_dBRD9_ChIP-Seq
- GSM3326016 TTC1240_SMARCA4_Rep2_DMSO_ChIP-Seq
- GSM3326017 Aska_BRD9_shScr_ChIP-Seq
Methodology

Replicates

For profiling subcomplex localization in AML, BRD9 ChIPs were done in biological duplicate with multiple independent antibodies in both MOLM13 and EOL-1. All other BAF subunit ChIPs were performed at least once in the independent cell lines EOL1 and MOLM13, with at least 2 antibodies targeting the each subcomplex. In the TTC1240 dBRD9 and DMSO treatment, SMARCA4 ChIP-Seq was performed in biological replicate and these replicates were used to determine differential localization. TTC1240 +SMARCB1 ChIP BRD9 ChIP was also done in biological replicate, however one sample was removed due to insufficient quality (low number of peaks). For synovial sarcoma experiments, fusion and non-fusion complexes were targeted with at least 3 independent antibodies.

Sequencing depth

All ChIP-Seq samples were single-end sequenced, the raw and mapped read number for each are below:

| Sample Name                        | Raw Reads  | Mapped Reads |
|------------------------------------|------------|--------------|
| EOL1_Input_Naive_ChIP-Seq          | 42699075   | 40669502     |
| EOL1_SMARCA4_Naive_ChIP-Seq        | 42341658   | 40326539     |
| EOL1_BRD9_137245_Naive_ChIP-Seq   | 43447352   | 40728707     |
| EOL1_BRD9_66443_Naive_ChIP-Seq    | 42280285   | 40193228     |
| EOL1_SMARCC1_Naive_ChIP-Seq       | 45761828   | 45205765     |
| EOL1_BRD7_Naive_ChIP-Seq          | 41626359   | 39680595     |
| EOL1_DPF2_Naive_ChIP-Seq          | 21669829   | 20808853     |
| EOL1_H3K27Ac_Naive_ChIP-Seq       | 74052071   | 66199250     |
| EOL1_CTCF_Naive_ChIP-Seq          | 40528536   | 39213478     |
| MOLM13_Input_DMSO_ChIP-Seq        | 93329733   | 89605195     |
| MOLM13_SMARCA4_DMSO_ChIP-Seq      | 5070615    | 54923551     |
| MOLM13_BRD9_DMSO_ChIP-Seq         | 63412810   | 59467899     |
| MOLM13_BRD7_DMSO_ChIP-Seq         | 45959144   | 47390911     |
| MOLM13_DPF2_DMSO_ChIP-Seq         | 31158535   | 29961390     |
| MOLM13_GLTSCR1_DMSO_ChIP-Seq      | 17825260   | 16942277     |
| MOLM13_CTCF_DMSO_ChIP-Seq         | 49435852   | 48148480     |
| EOL1_H3K4me1_Naive_ChIP-Seq       | 82356833   | 80809430     |
| EOL1_H3K4me3_Naive_ChIP-Seq       | 50558228   | 49324772     |
| SYO1_Input_shControl_ChIP-Seq     | 70319926   | 67773771     |
| SYO1_SS18_shControl_ChIP-Seq      | 50297551   | 49248273     |
| SYO1_CTCF_shControl_ChIP-Seq      | 50839866   | 49248273     |
| JURKAT_Input_Naive_ChIP-Seq       | 51476102   | 50131117     |
| JURKAT_BRD9_Naive_ChIP-Seq        | 24082851   | 22286692     |
| JURKAT_CTCF_Naive_ChIP-Seq        | 45704160   | 44472440     |
| TTC1240_BRD9_N106_Empty_ChIP-Seq | 40508291   | 39040020     |
| TTC1240_BRD9_N106_SMARCB1_ChIP-Seq| 3731904    | 35886274     |
| TTC1240_CTCF_Empty_ChIP-Seq       | 30451167   | 29859369     |
| TTC1240_CTCF_DMSO_ChIP-Seq        | 28267013   | 27586551     |
| TTC1240_BRD9_DMSO_ChIP-Seq        | 35453747   | 31754443     |
| TTC1240_SMARCA4_Repl1_DMSO_ChIP-Seq| 30274909   | 26965363     |
| TTC1240_SMARCA4_Repl2_DMSO_ChIP-Seq| 26794147   | 21150749     |
| TTC1240_INPUT_dBRD9_ChIP-Seq      | 30672294   | 29974187     |
| TTC1240_BRD9_dBRD9_ChIP-Seq       | 33762508   | 28773196     |
| TTC1240_SMARCA4_Repl1_dBRD9_ChIP-Seq| 28977197   | 22765324     |
| TTC1240_SMARCA4_Repl2_dBRD9_ChIP-Seq| 31275737   | 25903044     |
| Aska_BRD9_shScr_ChIP-Seq          | 46476740   | 44063069     |
| Aska_BRD9_shSSX1_ChIP-Seq         | 27453257   | 26243192     |

Antibodies

| Sample Name: Antibody               | Catalog Number |
|-------------------------------------|----------------|
| EOL1_SMARCA4_Naive_ChIP-Seq        | Abcam EPNCIR111A |
| EOL1_BRD9_137245_Naive_ChIP-Seq    | Abcam 137245   |
| EOL1_BRD9_66443_Naive_ChIP-Seq     | Abcam 66443    |
| EOL1_SMARCC1_Naive_ChIP-Seq        | Abcam CST 14910|
| EOL1_DPF2_Naive_ChIP-Seq           | Abcam ab134942|
| EOL1_CTCF_Naive_ChIP-Seq           | Abcam CST 34185|
| MOLM13_SMARCA4_DMSO_ChIP-Seq       | Abcam EPNCIR111A|
| MOLM13_BRD9_DMSO_ChIP-Seq          | Abcam ab137245|
| MOLM13_BRD7_DMSO_ChIP-Seq          | Abcam ab134942|
| MOLM13_DPF2_DMSO_ChIP-Seq          | Abcam ab134942|
| MOLM13_CTCF_DMSO_ChIP-Seq          | Abcam CST 34185|
**Peak calling parameters**

In EOL1, TTC1240, MOLM13 and Jurkat, narrow peaks were called for all BAF subunits and CTCF against an input using MACS2 ‘callpeak’ with a q-value cutoff of 1e-3 and the --nomodel option. Broad peaks were called for all histone marks using MACS2 ‘callpeak’ with a q-value of 1e-3, and the options --broad_cutoff --broad --nomodel --SPMR. In the synovial sarcoma cell lines SYO1 and Aska, histone marks and BAF subunits peaks were called using the broadPeak options above, while CTCF peaks were again called using the narrowPeak options.

**Data quality**

For all samples, peaks were called using a q-value cutoff of 1e-3 against an input. Excepting particular disease or experimental conditions (such as BRD9 peaks after BRD9 degradation with dBRD9) all samples had >6000 peaks, most far higher (20,000+), a full list of peak numbers for each sample is available in Supp.Table 6. Read coverage in peaks was calculated to ensure quality as well. For the TTC1240 DMSO and dBRD9 treatment, a spike-in control was done to normalize read levels between samples.

**Software**

ChIP-Seq Data was aligned using Bowtie2, version 2.1.0 to the hg19 reference genome with the parameter k -1. MACS2 version 2.1.0 was used to call peaks against input with a cutoff of q = .001. Duplicates were removed from reads files using samtools rmdup with the -b option. (SAMtools v1.3.1)

Overlaps for ChIP venn diagrams were created using the bioconductor package ChiPPeakAnno v3.10.1, peak files were read in using the toGRanges() command, values were determined using the getVennCounts() function with maxgap=0. Data was visualized using matplotlib. Read count across peak sets of interest were calculated by calling the Rsubread v1.26.1 bioconductor package function feature0.9eCounts() on duplicate removed bam files. Metagene plots and heatmaps were generated created using HTSeq v0.9.1. Peak distance from TSS elements was determined using bedtools closest function with the hg19 ref Flat TSS annotation. Intersection of peaks was determined using bedtools intersect. (bedtools v2.26.0)Determination of super enhancers was performed using ROSE. Differential occupancy of SMARCA4 in TTC1240 upon dBRD9 treatment was determined using the DiffBind v2.4.8 bioconductor package, with all default settings. Motif analysis on these sequences was done was done using the MEME-Chip suite.