Opposing effects of cancer-type-specific SPOP mutants on BET protein degradation and sensitivity to BET inhibitors

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It is generally assumed that recurrent mutations within a given cancer driver gene elicit similar drug responses. Cancer genome studies have identified recurrent but divergent missense mutations affecting the substrate-recognition domain of the ubiquitin ligase adaptor SPOP in endometrial and prostate cancers. The therapeutic implications of these mutations remain incompletely understood. Here we analyzed changes in the ubiquitin landscape induced by endometrial cancer–associated SPOP mutations and identified BRD2, BRD3 and BRD4 proteins (BETs) as SPOP–CUL3 substrates that are preferentially degraded by endometrial cancer–associated SPOP mutants. Conversely, prostate cancer–specific SPOP mutations resulted in impaired degradation of BETs, promoting their resistance to pharmacologic inhibition. These results uncover an oncogenomics paradox, whereby mutations mapping to the same domain evoke opposing drug susceptibilities. Specifically, we provide a molecular rationale for the use of BET inhibitors to treat patients with endometrial but not prostate cancer who harbor SPOP mutations.

Specific mutations in cancer-related genes can indicate whether a patient with cancer may or may not respond to a given drug. Generally, it is assumed that recurrent mutations within a specific gene have similar therapeutic implications, especially if the resulting amino acid changes occur within the same protein-coding domain. Genome studies have revealed recurrent point mutations mapping to the substrate-recognition domain of the ubiquitin ligase adaptor speckle-type POZ protein (SPOP) in 4–14% of prostate and endometrial cancers (Fig. 1a)2–6. In prostate cancer, SPOP mutations are confined to the amino acid residues of the substrate-binding cleft—a specific region within the substrate-recognition domain that is essential for substrate interaction and ubiquitin transfer. We and others have subsequently shown that these mutations act in a dominant-negative fashion to repress ubiquitination and degradation of oncogenic substrate proteins8–12. In contrast, recurrent amino acid substitutions in endometrial cancer and carcinosarcoma occur in an uncharacterized portion of the substrate-recognition domain (Fig. 1a and Supplementary Fig. 1a)1–6. Given the divergent mutation patterns in these tumor types, we speculated that SPOP mutations associated with endometrial cancer might differentially affect protein ubiquitination in comparison to prostate cancer–specific SPOP mutations, possibly resulting in distinct therapeutic opportunities.

RESULTS
Cancer-type-specific SPOP mutations have opposing effects on BET protein levels

To explore this hypothesis, we characterized changes in the ubiquitination landscape specific to endometrial cancer–associated SPOP mutants by mass spectrometry–based proteomics. To ensure that disease-relevant proteins were being expressed in our experimental setting, we chose human Ishikawa endometrial cancer cells, derived from a well-differentiated endometrioid cancer, for experimentation because their robustly expressed genes substantially overlapped with those found in SPOP-mutant tumor tissues (Supplementary Fig. 1b)6,13. Subsequently, we generated cells that stably overexpressed control vector, wild-type SPOP (SPOP-WT) or one of seven endometrial
cancer–specific mutated SPOP variants (E47K, E50K, E78K, S80R, M117V, R121Q and D140N; SPOP-MTs) (Supplementary Fig. 1c). In each case, we measured glycine–glycine remnants of ubiquitinated lysines (K-ε-GG) after trypsin digestion and stable isotope labeling of amino acids in cell culture (SILAC)-based mass spectrometry (Supplementary Fig. 1d). All K-ε-GG values (n = 17,239) were normalised to the corresponding protein ratios to account for ubiquitination-related changes in protein levels (Supplementary Table 1). K-ε-GG peptide values for cells expressing individual SPOP-MTs were compared to those from cells overexpressing SPOP-WT within experiments (Supplementary Fig. 2a,c,e,g).

Because protein ubiquitination is often linked to proteasomal degradation, we asked which differentially expressed K-ε-GG peptides showed an inverse correlation with protein abundance (Supplementary Table 1 and Supplementary Fig. 2b,d,f,h). We identified two patterns of ubiquitination and protein dysregulation in known and putative SPOP substrates pointing to possible private contact points between individual substrates and the mutant meprin and TRAF homology (MATH) domain (Fig. 1b,c and Supplementary Figs. 2 and 3). Peptides corresponding to tripartite-motif-containing 24 (TRIM24), anterior gradient protein 2 (AGR2), and nuclear receptor coactivator 3 (NCOA3)—all proteins with reported oncogenic properties—showed a decrease in K-ε-GG peptide abundance in cells expressing SPOP-MTs, followed by an increase in levels of the corresponding protein.

Similar dominant-negative patterns of substrate dysregulation by prostate cancer–specific SPOP mutants have been reported for TRIM24 and NCOA3 (refs. 8,9,17).
The most striking changes were found in proteins that exhibited elevated levels of BRD3 protein relative to those of controls, in agreement with the notion that endogenous SPOP was not able to degrade the degron variant (Fig. 2b and Supplementary Fig. 5b). To determine whether the motif mediated direct binding of SPOP to BRD3, we performed immunoprecipitation experiments in cells expressing either wild-type HA-BRD3 or the degron variant. SPOP protein was detectable after immunoprecipitation of wild-type HA-BRD3, whereas the substitutions in the BRD3 degron variant disrupted the BRD3–SPOP interaction (Fig. 2c). Thus, the SPOP-binding motif within BRD3 appears to be necessary for SPOP binding.

Next, we tested whether SPOP could ubiquitinate BRD3 as part of a cullin-3 (CUL3)–RING-box protein 1 (RBX1) ubiquitin E3 ligase complex. Knockdown of CUL3 increased HA-BRD3 levels and decreased BRD3 ubiquitination relative to control in 293T cells (Supplementary Fig. 5c). Furthermore, SPOP-WT, along with RBX1 and CUL3, directly ubiquitinated wild-type HA-BRD3 in vitro and in vivo, whereas the degron variant of HA-BRD3 remained unaffected (Fig. 2d and Supplementary Fig. 5d). Additional CUL3-dependent substrate adaptors (kelch-like family member 9 (KLHL9), KLHL13, KLHL21) failed to ubiquitinate BRD3 in vitro, verifying the specificity of SPOP toward BRD3 (Supplementary Fig. 5d).

To determine whether BRD3 ubiquitination induces its proteasomal degradation, we cultured SPOP- and BRD3-expressing 293T cells in the presence or absence of the proteasome inhibitor MG132. Short-term MG132 treatment increased levels of ubiquitinated HA-BRD3 (Fig. 2d). Prolonged proteasomal inhibition increased HA-BRD3 and degraded by endometrial cancer–specific SPOP mutants. Notably, similar changes were also found in human HEC-151 and RL95-2 endometrial cancer cells and in human 22Rv1 prostate cancer cells (Supplementary Fig. 3c–e).

Because prostate cancer–specific SPOP mutants have been found to impair ubiquitination of substrates in a dominant-negative manner, we speculated that these mutants might have the opposite effect on BET protein levels of those identified in endometrial cancer. Indeed, overexpression of recurrent prostate cancer–specific mutations increased BET protein levels in human Ishikawa endometrial cancer cells, human 22Rv1 prostate cancer cells, human LHMAR prostate epithelial cells and mouse prostate epithelial cells and in mouse prostate epithelial cells (Fig. 1d,e and Supplementary Fig. 3f–h).

In aggregate, our findings suggest that BET proteins might represent SPOP substrates that are differentially ubiquitinated and degraded by endometrial cancer– and prostate cancer–specific mutants irrespective of cellular lineage. In support of these findings, nuclear levels of BET proteins correlated inversely with recurrent SPOP mutations in human endometrial cancer tissue analyzed by immunohistochemistry, whereas a positive correlation was noted in primary human prostate cancer tissues (Fig. 1f,g and Supplementary Fig. 4).
Cancer-type-specific SPOP mutants induce differential ubiquitination of BET proteins

To test whether the functional properties of endometrial cancer–specific SPOP mutants may translate into reduced BET protein levels in comparison to SPOP-WT when these mutants are expressed at endogenous levels, we identified the human EN endometrial cancer cell line that harbors a recurrent SPOP mutation (p.R121Q) in the Cancer Cell Line Encyclopedia (CCLE) (Supplementary Fig. 6a). We compared BET protein levels in EN cells to those in Ishikawa cells expressing equivalent levels of SPOP-WT (Fig. 3a). We found that EN cancer cells exhibited lower levels of BET proteins despite having higher levels of the corresponding mRNAs (Fig. 3a and Supplementary Fig. 6b). In line with the notion that enhanced protein degradation by SPOP-R121Q leads to reduced BET protein levels in EN cells, we found more significant increases in BET protein levels upon SPOP depletion or short-term proteasome inhibition in EN cells than in Ishikawa cells (Fig. 3b and Supplementary Fig. 6c–e). In addition to and in accordance with our observations above, endogenous SPOP-R121Q bound more efficiently to BET proteins in EN cells, in which we noted more pronounced degradation of BET proteins after inhibition of protein synthesis with cycloheximide (Fig. 3c and Supplementary Fig. 6f,g). Of note, endogenous SPOP levels were also increased after prolonged proteasome inhibition and reduced after inhibition of protein synthesis, indicating proteasomal turnover of SPOP itself (Figs. 2e and 3b,c).

To test whether the different BET protein binding and degradation kinetics in human EN and Ishikawa cells were a result of the specific amino acid substitutions within SPOP, we analyzed the activity of different SPOP species side by side in 293T cells. Endometrial cancer–associated SPOP mutants (E50K and R121Q) bound more strongly to HA-BRD3 than SPOP-WT did in vivo and in vitro, whereas the interaction of the prostate cancer–specific SPOP mutants W131G and F133L was reduced (Fig. 3d and Supplementary Fig. 6h). In line with this observation, ubiquitination of HA-BRD3 was increased with the endometrial cancer–specific SPOP mutants and decreased with the prostate cancer–specific mutants (Fig. 3e and Supplementary Fig. 6i). We next investigated whether the increase in ubiquitination mediated by the endometrial cancer–specific SPOP mutants was dependent on an intact degron on BRD3. Indeed, SPOP-E50K failed to ubiquitinate the degron variant of BRD3 (Fig. 3f) and to reduce BRD3 protein levels (Supplementary Fig. 6j). These results suggest that BET protein levels are at least in part affected by differential interactions between SPOP mutants and the BET degron.

Sensitivity to BET inhibitors is altered by cancer-type-specific SPOP mutations

Some cancer cells depend on the presence of BET proteins for tumor growth and survival\(^{18,21}\). Therefore, we sought to determine whether enhanced degradation of BET proteins in the context of endometrial cancer–specific SPOP mutants might create specific vulnerabilities; we speculated that endometrial cancer cells with low BET protein levels might become particularly susceptible to further reduction of BET protein levels. Indeed, EN cells were susceptible to individual knockdown of the BET proteins, which resulted in decreased growth (Supplementary Fig. 7a). In contrast, to achieve a similar effect on growth in Ishikawa cells, individual BET proteins had to be knocked down in the context of the SPOP-R121Q mutant. These data suggest that endometrial cancer cells with low levels of BET proteins in the context of endometrial cancer–specific SPOP mutants are

and endogenous BET protein levels in the presence of SPOP-WT overexpression (Fig. 2e and Supplementary Fig. 5e). SPOP was also detectable after immunoprecipitation of endogenous BET proteins (Supplementary Fig. 5f). Moreover, SPOP knockdown increased BET protein levels without concomitant changes in mRNA levels and impaired protein degradation after inhibition of protein synthesis with cycloheximide (Fig. 2f and Supplementary Fig. 5g,h). In aggregate, these data are consistent with a model in which ubiquitination of BET proteins promotes their proteasomal degradation.

Figure 3 BET proteins are differentially ubiquitinated and degraded by endometrial cancer– and prostate cancer–specific SPOP mutants. (a) Representative western blot (n = 4) of BET proteins and SPOP in Ishikawa and EN human endometrial cell lines. Statistical significance was determined by unpaired, two-tailed Student’s t-test (n.s., not significant). Error bars, s.e.m. (b) Representative western blot of the indicated proteins in Ishikawa and EN cells with or without 3 h of MG132 treatment (n = 3). (c) Representative western blot of the indicated proteins after the specified duration of cycloheximide (CHX) treatment in Ishikawa and EN cells (n = 3). (d) Interaction of HA-BRD3 with SPOP-WT, endometrial cancer–associated SPOP mutants (E50K, R121Q) and one prostate cancer–associated SPOP mutant (W131G). HA immunoprecipitation (top) and WCE (bottom) are shown from transiently transfected 293T cells overexpressing HA-BRD3 and the indicated SPOP constructs (n = 3). (e) Effects of SPOP-WT and SPOP mutants on in vivo ubiquitination of HA-BRD3 (n = 3). (f) In vivo ubiquitination of HA-BRD3-WT or HA-BRD3-degron-MT by SPOP-E50K (n = 3). Western blots shown in a–f are representative. ACTB was used as loading control. In each legend, n indicates the number of independent experiments performed.

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Figure 4 Cancer-type-specific SPOP mutants alter BET inhibitor sensitivity in an opposing manner. (a) Response to JQ1 treatment by Ishikawa cells stably overexpressing SPOP mutants specific to endometrial cancer (E47K, E50K, E78K, S80R, M117V, R121Q, D140N; in grey) or prostate cancer (Y87C, F102C, W131G, F133L; in blue) in 3D semisolid cell culture (n = 3). Statistical analysis was performed using two-way ANOVA with Dunnett’s post test, 112 degrees of freedom (d.f.). (b) Correlation of IC50 (JQ1), shown in Supplementary Figure 7c, with BET protein levels, as quantified by mass spectrometry, in Ishikawa cells stably expressing recurrent endometrial cancer-specific SPOP mutants (R and P values were calculated using Spearman rank correlation). Each point represents the change in protein expression of individual SPOP mutants relative to that of SPOP-WT. (c) Response to JQ1 treatment (250 nM) by Ishikawa cells stably overexpressing SPOP-E50K and different degron-mutant constructs of the BET proteins (n = 3). (d) Effect of shRNA-mediated depletion of BRD2, BRD3 and BRD4 individually on JQ1 (200 nM) sensitivity in Ishikawa cells expressing SPOP-Y87C (n = 3). (e) JQ1 sensitivity of: Ishikawa cells expressing SPOP-WT, EN human endometrial cancer cells expressing SPOP-R121Q, NCI-H508 human large intestine cancer cells expressing SPOP-R121Q, NCI-H508 human large intestine cancer cells expressing SPOP-E47K and mutant VM-CUB1 human urothelial cancer cells expressing SPOP-E50K in 3D semisolid culture (n = 4). A representative immunoblot for the indicated proteins and cell lines is shown. ACTB was used as a loading control. P values in e are indicated above the compared bars (two-way ANOVA with Dunnett’s post test, 30 d.f.). Data represent mean ± s.e.m. Statistical significance was determined by unpaired, two-tailed Student’s t-test unless otherwise specified. *P < 0.05, **P < 0.01, ***P < 0.001. In each legend, n indicates the number of independent experiments performed.
particularly susceptible to further suppression of BET protein function. In support of this view, a functional overlap among BET proteins has been reported.\(^{22}\)

BET inhibitors are under clinical investigation as anticancer therapeutics, including for solid tumors.\(^{18,21,23,24}\) We anticipated that the susceptibility of cancer cells to these inhibitors might be influenced by differences in BET protein levels corresponding to SPOP mutants. Indeed, forced expression of endometrial cancer–specific SPOP mutants (shown to lower BET protein levels) sensitized Ishikawa cells to both pharmacologic BET inhibition across cell line models of human endometrial cancer\(^{20}\). We aimed to discover whether decreased levels of individual BET proteins related with a decrease in the half-maximal inhibitory concentration (IC\(_{50}\)) during JQ1 treatment (Fig. 4a). Functional overexpression of the BRD2, BRD3 and BRD4 degron variants reduced SPOP-E50K-mediated sensitization to JQ1 (Fig. 4c and Supplementary Fig. 7g). We then investigated whether increased BET protein levels in the context of prostate cancer might, on the contrary, induce resistance to BET inhibitors. Overexpression of prostate cancer–specific SPOP mutants in comparison to SPOP-WT rendered Ishikawa and 22Rv1 cells more resistant to JQ1 (Fig. 4a and Supplementary Fig. 7h,i), whereas individual (Fig. 4d and Supplementary Fig. 7j,l) or combined (Supplementary Fig. 7k) knockdown of BET proteins in the context of mutated SPOP-Y87C dampened this phenotype.

Next, we sought to determine whether recurrent SPOP mutations or decreased BET protein levels in general might predict sensitivity to pharmacologic BET inhibition across cell line models of human endometrial cancer. For this purpose, we assessed JQ1 sensitivity in 3D semisolid culture conditions across 12 different human cell lines, for which we determined BET protein levels in parallel. Decreased expression levels of BRD2, BRD3 and BRD4 were associated with sensitivity to JQ1 in many cases (Supplementary Fig. 8a–c); however,
Figure 6 Endometrial cancer–associated SPOP mutants sensitize cells to JQ1 treatment in vivo. (a) Tumor growth kinetics (left) and individual tumor weight (right) with \( n = 9 \) or without (control; \( n = 7 \)) JQ1 treatment in xenografts established from EN cells. (b) Tumor growth kinetics (left) and individual tumor weight (right) with \( n = 7 \) or without (control; \( n = 7 \)) JQ1 treatment in xenografts established from Ishikawa cells. (c) Representative histology \( (n = 7) \) (top) and quantification (bottom) of mitotic and apoptotic cells in EN and Ishikawa xenografts treated with vehicle (control) or JQ1. Scale bar, 50 \( \mu \)m. (d) Tumor growth kinetics (left) and individual tumor weight (right) with \( n = 7 \) or without (control; \( n = 6 \)) JQ1 treatment in xenografts established from Ishikawa cells stably overexpressing SPOP-E50K. (e) Tumor growth kinetics (left) and individual tumor weight (right) with \( n = 6 \) or without \( (n = 7) \) JQ1 treatment in xenografts established from Ishikawa cells stably overexpressing SPOP-SB8R. (f) Model showing the differential effects of cancer-specific SPOP mutations on both BET protein levels and sensitivity to BET inhibitors (BETi). Representative images of the indicated number of tumors \( (n) \) for each xenograft group are shown in a, b, d and e. Data in a, b, d and e shown as mean tumor volume + s.e.m.; midlines represent means. Statistical significance was determined by unpaired, two-tailed Student’s t-test. \(* P < 0.05, ** P < 0.01, *** P < 0.001.\)

we also found some notable exceptions to this rule, in agreement with the existence of other molecular mechanisms that regulate susceptibility to BET inhibitors\(^{27,28}\). Nevertheless, EN cells expressing the SPOP-R121Q mutant were sensitive to JQ1 inhibition, in line with our data generated in isogenic cell lines (Fig. 4a,e and Supplementary Figs. 7f and 8b). This finding led us to search for additional cell lines with recurrent endometrial cancer–associated SPOP mutations at the endogenous locus. We identified colorectal (NCI-H508) and urothelial (VM-CUB1) cancer cell lines in the Catalogue of Somatic Mutations in Cancer (COSMIC) Cell Lines Project that harbored SPOP mutations encoding p.E47K and p.E50K, respectively. Both cell lines were particularly sensitive to SPOP and displayed low BET protein levels that were responsive to proteasomal inhibition (Fig. 4c and Supplementary Fig. 8d). Thus, endometrial cancer–associated SPOP mutations may be more broadly associated with sensitivity to BET inhibitors.

Moreover, we tested whether established SPOP substrates might either directly or indirectly influence responses to JQ1 through changes in BET protein levels. Neither knockdown nor overexpression of DEK, TRIM24, NCOA3 or ERG led to substantial changes in BET protein levels or the JQ1 response, further supporting the notion that SPOP mutants affect sensitivity to JQ1 directly through regulation of BET protein degradation (Supplementary Fig. 9).

Transcriptome analysis identifies FOSL1 as a determinant of JQ1 response

BET inhibitors bind to the bromodomains of BET proteins to displace them from the acetylated histone tails of transcriptionally active sites. Considering this function, we investigated transcriptional changes in response to overexpression of SPOP-WT or two recurrent endometrial cancer– and prostate cancer–associated SPOP mutants in Ishikawa cells (Supplementary Table 2). Unsupervised clustering and multidimensional scaling (MDS) analyses of gene expression revealed mainly opposing transcriptional changes in response to expression of the endometrial cancer– and prostate cancer–associated SPOP mutants, with SPOP-WT positioned in between the different types of SPOP mutants (Supplementary Fig. 10a,b). This result aligns well with the different BET protein levels observed across the cell lines (Supplementary Fig. 10c). Interestingly, the MDS analysis revealed a second feature that discriminated both types of mutants from SPOP-WT, possibly reflecting shared dysregulation of SPOP substrates such as TRIM24 or NCOA3 (Fig. 1c and Supplementary Fig. 10b)\(^{39}\).

Next, we interrogated transcriptional changes under JQ1 treatment in cells overexpressing endometrial cancer– versus prostate cancer–associated SPOP mutants and found a significant overlap between the differentially expressed genes and those with altered expression in the untreated conditions (Fig. 5a,b and Supplementary Fig. 10a–c). We identified 16 genes with altered expression across all conditions (untreated, 500 nM or 2 \( \mu \)M JQ1), including FOSLI, a reported target gene for BET proteins implicating in sensitivity to BET inhibitors\(^{29}\). FOSLI mRNA and FOSLI protein levels were reduced in cells that overexpressed endometrial cancer–specific, as compared to prostate cancer–specific, SPOP mutants, in accordance with the observed changes in BET protein levels and the transcriptome analysis (Fig. 5c). Notably, in human tumor tissues, FOSLI mRNA and FOSLI protein levels were also decreased in individuals with endometrial cancer harboring mutated SPOP, with the lowest mRNA levels...
observed in individuals who harbored the SPOP mutants shown to have the strongest effects on BET protein levels and sensitivity to JQ1 in our study (Fig. 5d,e and Supplementary Fig. 7c)\textsuperscript{30–33}.

Next, we investigated whether changes in BET protein levels in response to SPOP mutants and JQ1 treatment might decrease FOSL1 transcription, as triple occupancy of BRD2, BRD3 and BRD4 has been reported at the FOSL1 promoter (Supplementary Fig. 10d)\textsuperscript{24}. JQ1 treatment reduced FOSL1 expression levels in all conditions, whereas the relative expression levels remained the same between the different types of SPOP mutants (Fig. 5f). Knockdown of individual BET proteins decreased FOSL1 mRNA levels in JQ1-resistant Ishikawa cells overexpressing the prostate cancer–specific SPOP-Y87C variant (Fig. 4a and Supplementary Fig. 10e). Moreover, FOSL1 depletion itself directly lowered resistance to JQ1 in this setting, indicating functional involvement of this gene downstream of changes in BET protein levels and SPOP mutants (Fig. 5g). Taken together, these results suggest that BET protein level changes in response to SPOP mutants alter susceptibility to JQ1, at least in part, through transcriptional regulation of FOSL1.

**JQ1 treatment blocks tumor growth in xenografts expressing endometrial cancer–specific SPOP mutants in vivo**

Finally, we investigated whether our results showing altered sensitivity to JQ1 in response to SPOP mutants could be validated in an in vivo setting. For this purpose, we focused on expression of endometrial cancer–associated SPOP mutants because patients receiving a beneficial response from BET inhibitors may be identified in this setting. Indeed, JQ1 efficiently blocked the growth of tumor xenografts established from SPOP-mutant EN cells by reducing cell proliferation and inducing apoptosis, whereas Ishikawa-cell-derived tumors were largely resistant to JQ1 treatment (Fig. 6a–c). In accordance with the in vitro data, forced expression of SPOP-E50K or SPOP-S80R sensitized Ishikawa cells to JQ1 in vivo (Fig. 6d,e).

**DISCUSSION**

Recurrent missense mutations in SPOP—encoding a substrate receptor of a cullin–RING ubiquitin ligase—have been found in 5–10% of prostate and endometrial cancers in comprehensive genome sequencing studies\textsuperscript{3–6}. Surprisingly, the specific genetic alterations show no overlap between these tumor types, even though they are confined to the same substrate-recognition domain. Although the prostate cancer–associated mutations have been shown more recently to stabilize protein substrates relevant to prostate tumorigenesis\textsuperscript{8–12}, the therapeutic implications of both mutation types remain largely elusive.

Our study identifies the BET proteins (BRD2, BRD3 and BRD4) as bona fide SPOP substrates. Small-molecule inhibitors against this group of proteins are under clinical investigation in hematological and solid tumors because of the critical importance of these proteins in driving lineage-specific oncogenic transcriptional programs\textsuperscript{18,21,23,24}. We found that prostate cancer–associated SPOP mutations impair degradation of BET proteins, in line with the loss-of-function properties of these mutations reported previously\textsuperscript{8,9}, whereas endometrial cancer–associated SPOP mutations enhance BET protein degradation through a gain-of-function mechanism (Fig. 6f). The precise structural basis through which endometrial cancer–associated SPOP mutations enhance binding and ubiquitination of BET proteins and other substrates (for example, DEK) remains to be further elucidated. The altered BET protein levels in the SPOP-mutant setting influence the transcription of established target genes, such as FOSL1 (ref. 29), and thereby alter the susceptibility of cancer cells to BET inhibitors.

Of note in this regard, a recent report implicates enhanced FOSL1 activity as a mechanism of acquired resistance in ovarian cancer cells as well\textsuperscript{34}. Overall, our established model extends the list of previously reported mechanisms that influence BET inhibitor sensitivity\textsuperscript{27,35,36}.

BET inhibitors are currently under clinical development, and there is a critical need to identify patients who may respond to this treatment. Our preclinical study identifies SPOP mutations as a clinically detectable biomarker of BET inhibitor response. Thus, the detection of specific SPOP mutations may be used to select patients who may (endometrial cancer–associated SPOP mutations) or may not (prostate cancer–associated SPOP mutations) benefit from treatment with BET inhibitors.

More broadly, our results suggest a paradigm whereby mutations mapping to the same domain of a particular protein evoke opposing drug responses. Given the increasing use of cancer genome information in the clinical setting, caution may be needed when extrapolating therapeutic responses on the basis of similar mutations.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

*Note: Any Supplementary Information and Source Data files are available in the online version of the paper.*

**ACKNOWLEDGMENTS**

We thank M. Losa, M. Storz, P. Schraml, S. Dettwiller and F. Prutek for help with tissue handling and histology assistance. We thank Q. Zhong for his help with the next-generation sequencing bioinformatics pipeline. We thank all members of the IRB animal core facility for technical assistance and the animal work. We thank E. Samartizis and K. Dedes (University Hospital Zurich) for providing AN3CA, HEC1A, HEC1B, HEC116, SNG-II, EF184 and KLE cell lines. We thank the Oregon Health & Science University (OHSU) and the Cooperative Human Tissue Network (CHTN) for the tissue repository. We also thank all members of the laboratory for scientific discussions. J.-P.P.T. is funded by a Swiss National Science Foundation Professorship (PP00P3_150645) grant, a Swiss Cancer League (KSL-3654-02-2015) grant, a grant by the Jubiläumsstiftung Swiss Life AG and a grant by the Vontobel Stiftung. The Swiss National Science Foundation (310030B_160312/1), the European Research Council (268930), SystemsX IPHd (2013/128), Krebsforschungs Schweiz (KFS-3498-08-2014) and a GRL grant from the Korean government fund M.P. This work was also funded in part by a grant to P.J.W. provided by Oncosuisse (KLS-3384-02-2014-R) and the Foundation for Research in Science and the Humanities at the University of Zurich (SWF).

**AUTHOR CONTRIBUTIONS**

J.-P.P.T. originally developed the concept, further elaborated on it and designed the experiments together with H.J., G.E.T. and N.D.U. H.J., G.E.T., A.R., J.-P.P.T., N.D.U., T.S., S.N., A.U. and R.I.E. performed experiments and analyzed the data. H.J., G.C., G.E.T. and T.B. performed tumor xenograft experiments in immune-deficient mice. M.L., H.J. and J.-P.P.T. performed immunohistochemical experiments and analysis. P.J.W., P.S., H.M. and E.B. performed endometrial and prostate cancer samples with annotation for SPOP mutation status. D.G.M., M.E.C., A.B., B.I.N.W. and R.R.B. provided SPOP-mutant endometrial cancer samples. C.M.B., G.V.K., A.R. and L.C. analyzed genomic and RNA-seq data. J.-P.P.T., L.A.G., S.A.C., M.P., C.V.C., F.B. and P.J.W. provided funding and resources. J.-P.P.T., H.J. and G.E.T. interpreted the data and wrote the paper. H.J. and G.E.T. contributed equally to this work.

**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

SILAC labeling and cell culture. For SILAC experiments, human Ishikawa endometrial cancer cells were cultured in DMEM/F12 medium deficient in L-arginine and L-lysine and supplemented with 10% dialyzed FBS (Sigma-Aldrich), penicillin, streptomycin and l-glutamine (Invitrogen), and L-arginine (Arg-0) and L-lysine (Lys-0), L-arginine [13C6]HCl (Arg-6) and L-lysine [13C6,15N2]HCl (Lys-8) (Sigma-Aldrich) for 14 d (10 doublings). All media were supplemented with l-proline to prevent the conversion of arginine to proline37. Specifically, isogenic cell lines expressing vector control (C), wild-type SPOP (SPOP-WT) or mutants (MTs) were isotopically labeled with SILAC media and grouped into four experiments (Supplementary Fig. 1d). Each experiment included a cell line with overexpression of SPOP-WT for cell line comparison within and across experiments. The labeling for this cell line was switched to rule out labeling artifacts in the first three experiments. Approximately 100 million cells per condition were washed twice with PBS, harvested and snap frozen.

K-ε-GG profiling and proteome analysis by liquid chromatography mass spectrometry. Preparation of proteins for mass spectrometry analysis was completed as previously described14. Briefly, cell pellets were lysed in an ice-cold lysis buffer containing 8 M urea; 50 mM Tris-HCl, pH 8; 150 mM NaCl; 1 mM KCl; and 1 mM phenylmethylsulfonyl fluoride (PMSF); 50 μM PR-619; and 1 mM chloroacetamide. The lysate was cleared by centrifugation at 20,000 × g for 10 min. A bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific) was used to determine the protein concentration of each sample. Respective SILAC mixes were created by combining equal amount of protein per SILAC state. Proteins were reduced with 5 mM dithiothreitol (DTT) at room temperature (RT) and subsequently alkylated with 10 mM iodoacetamide at RT in the dark. Lysates were diluted 1:4 with 50 mM Tris-HCl, pH 8, and proteins were digested with sequencing-grade trypsin using an enzyme-to-substrate ratio of 1:50, overnight at 25 °C. Digestion reactions were quenched with trifluoroacetic acid (TFA), and the peptide solutions were cleared by centrifugation before desalting. Peptides were desalted using tC18 SepPak SPE cartridges (Waters) exactly as previously described14.

Peptides were fractionated offline by basic pH reverse-phase (bRP) chromatography, as previously described13,14. Input for each bRP separation was equivalent to 30 μg of starting protein material (10 μg protein per SILAC state) for replicate. Briefly, dried peptides were reconstituted in bRP buffer A (5 mM ammonium formate (pH 10.0)%25 acetonitrile). A Zorbax 300 Extend-C18 column (9.4 × 250 mm, 300 Å, 5 μm; Agilent) was used for separation. Using the gradient and flow-rate settings previously described14, a total of 96 2-ml fractions were collected across the entirety of the bRP separation. For proteome analysis, 5% of each fraction was taken and combined in a noncontiguous manner such that every 24th fraction was combined to create 24 final fractions. For K-ε-GG analysis, the remainder of each fraction was combined in a noncontiguous manner such that every eighth fraction was combined to create eight final fractions. Pooled fractions were dried completely using vacuum centrifugation.

For enrichment of K-ε-GG peptides, anti-K-ε-GG antibody from the PTMScan Ubiquitin Reminant Motif (K-ε-GG) kit was used (Cell Signaling Technology, cat. no. 5562). Prior to enrichment, the antibody was cross-linked to Protein A beads using dimethyl pimelimidate (DMP)14. Peptides were reconstituted in immunofinity purification (IAP) buffer, and enrichment was completed exactly as previously described14. Briefly, peptides were incubated with approximately 31 μg of anti-K-ε-GG antibody beads and incubated for 1 h at 4 °C with rotation. Beads were washed twice with 1.5 ml of ice-cold IAP buffer followed by three washes with ice-cold PBS. K-ε-GG peptides were eluted from the antibody with 2 × 50 μl of 0.15% trifluoroacetic acid (TFA). Peptides were desalted using StageTips. StageTips were conditioned by washing with 50 μl of 50% acetonitrile (MeCN) 0.1% formic acid (FA) followed by 2 × 50 μl of 0.1% FA. Peptides were then loaded on StageTips, washed twice with 50 μl of 0.1% FA and eluted with 50 μl of 50% MeCN/0.1% FA. Eluted peptides were dried completely using vacuum centrifugation.

Samples were reconstituted in 3% MeCN/0.1% FA. All samples were analyzed by nanoflow-ultra-performance liquid chromatography (UPLC)–higher-energy collisional dissociation (HCD)–MS/MS using a Q Exactive mass spectrometer (Thermo Fisher Scientific) coupled online to an Easy-nLC 1000 system (Proxeon). For K-ε-GG and proteome samples, 4/8 μl and 1/20 μl, respectively, were injected into the mass spectrometer. Samples were injected at a flow rate of 500 nM/min onto a PicoFrit column (360 μm (OD) × 75 μm (ID), 10-μM ID tip, 50-cm length (New Objective) self-packed with 24 cm of ReproSil-Pur 120-Ä, 1.9-μm C18-AQ beads). The nanoflow column was heated to 50 °C using a column heater (Phenix S8/7T). For liquid chromatography mass spectrometry (LC–MS/MS) analyses, the gradient and flow-rate settings were used as previously described4 and the MS acquisition time used for each K-ε-GG and proteome sample was 120 min. The Q Exactive was operated by acquiring an MS1 scan (R = 70,000) followed by MS/MS scans (R = 17,500) on the 12 most abundant ions. An MS1 and MS2 ion target of 3 × 106 and 5 × 104 ions, respectively, was used for acquisition. A maximum ion time of 10 ms and 120 ms was used for the MS1 and MS2 scans, respectively. The isolation width was set to 2.5 m/z, the HCD energy loss was set to 25, the dynamic exclusion time was set to 20 s, and the peptide-match and isotope-exclusion functions were enabled. A second round of bRP fractionation, K-ε-GG and MS analysis was completed for experiments 3 and 4 (6 mg per SILAC state for experiment 3 and 10 mg per SILAC state for experiment 4).

Mass spectrometry data analysis. Data were processed using the MaxQuant (version 1.2.2.5) software package. The human UniProt database including 248 common laboratory contaminants was used for searching. The enzyme specificity was set to trypsin, the maximum number of missed cleavages was set to 2 for proteome data and 4 for K-ε-GG data, the precursor mass tolerance was set to 20 ppm for the first search, and the tolerance was set to 6 ppm for the main search. Carbamidomethylation of cysteines was searched as a fixed modification, and oxidation of methionines and N-terminal acetylation of proteins were searched as variable modifications.

For K-ε-GG data, addition of glycine-glycine to lysine was also searched as a variable modification. For identification, the minimum peptide length was set to 7, and the false discovery rate for peptide, protein and side identification was set to 1%. The filter-labeled amino acid and peptide quantification functions were enabled. For proteome data, normalized ratios were obtained from the "proteinGroups" table. For K-ε-GG data, normalized SILAC ratios were obtained from the "GlyGly(K)Sites" table.

For K-ε-GG and proteome data sets, reverse and contaminant hits were removed from the analysis. Proteins were included in the data set if they were identified and quantified by two or more razor peptides per unique peptide in each SILAC triple-labeled experiment. K-ε-GG peptides were included in the final data set if the corresponding protein was quantified in the proteome data. To capture the ubiquitination changes associated with protein degradation, we normalized the K-ε-GG changes to their measured protein levels. The leading accession number was used to match the protein and K-ε-GG data. Quantitative, protein-normalized measurements were available for 17,239 K-ε-GG peptides. To assess and highlight which of the significantly deregulated K-ε-GG peptides were paralleled with opposing effects on total protein expression in the case of SPOP-MT versus SPOP-WT, protein-normalized SILAC ratios for K-ε-GG were multiplied by their corresponding protein-level ratio and also by −1 (Supplementary Fig. 2 and Supplementary Table 1).

Cell culture, transfection and infection. Ishikawa cells were purchased from Sigma; RL95-2, 22Rv1, MEF-962, VM-CUB1 and NCI-H508 cells were purchased from ATCC; EN cells were purchased from DSMZ; and HEC-151 cells were purchased from JCRB. AN3CA, HEC1A, HEC 1B, HEC116, SNG-II, EFE184 and KLE cells were kindly provided by E. Samartzis and K. Dedes (University Hospital Zurich). Ishikawa, RL95-2 and KLE cells were grown in F12/DMEM and KLE cells were kindly provided by E. Samartzis and K. Dedes (University Hospital Zurich). Ishikawa, RL95-2 and KLE cells were grown in F12/DMEM ( Gibco); MEF-962, HEC1-151, EN, HEC1B, HEC116, SNG-II, EFE184 and KLE cells were kindly provided by E. Samartzis and K. Dedes (University

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For stable knockdown experiments, cells were infected with pLKO-1 vectors (Sigma) and the following clones were used: SPOP: TRCN0000104341 (shSPOP); BRD2: TRCN0000031543 (shBRD2_1), TRCN0000305030 (shBRD2_2); BRD3: TRCN0000021376 (shBRD3_1), TRCN0000213777 (shBRD3_2); BRD4: TRCN0000021426 (shBRD4_1), TRCN0000021427 (shBRD4_2) and shBET (S’-TCCACTGTCAATAGTAACAT-3’); CUL-3: TRCN00000073345 (shCUL-3_1) and TRCN00000073344 (shCUL-3_2); DEK: TRCN000013104 (shDEK_1) and TRCN000013105 (shDEK_2); TRIM24: TRCN000021029 (shTRIM24_#) and TRCN00000194983 (shTRIM24_2); NCOA3: TRCN00000370320 (shNCOA3_1) and TRCN00000365253 (shNCOA3_2). After infection, cells were selected in the presence of puromycin (2 µg/ml). For overexpression, a derivative of the plX3 vector was used, throughout, in which the CMV promoter had been exchanged with a PGK promoter and the bacterial cistron cassette exchanged with mOrange or a puromycin-resistance cassette (plX.TR_C307, available at Addgene as plasmid 41392, pCW107). All open reading frames (ORFs) were cloned into pLX.TR_C307–mOrange using NheI and MluI (Thermo Fisher Scientific).

Dose-response curves and cell-growth assays. Cells were seeded (between 1 × 10^4 and 1 × 10^5 cells/well) in a 96-well plate. Cells were subsequently treated with serial dilutions of JQ1 or OTX-015 in media to determine dose-response curves or were left untreated for cell-growth assays. After 96 h of treatment in the case of dose-response curves and 6 d for cell-growth assays cells were washed with PBS and stained with 0.5% crystal violet solution in 25% methanol. Crystal violet was then solubilized with 10% acetic acid and absorbance (OD, 590 nm) was measured in a microplate reader.

Clonogenic assay in methylcellulose. Cells were seeded (between 5 × 10^3 and 1 × 10^5 cells) in methylcellulose (Methocult H4100, StemCell Technologies) in duplicate and treated with vehicle (0.1% DMSO) or drug (JQ1). Cells were incubated at 37 °C and 5% CO₂ for 7–14 d, colonies were stained with MTT solution at 37 °C overnight and absorbance (OD, 590 nm) was measured in a microplate reader.

Xenograft model. All animal experiments were carried out in female athymic nude mice (BALB/c nu/nu, aged 4–6 weeks) according to a protocol approved by the Swiss Veterinary Authority (no. TI-14-2014). 2 × 10^6 Ishikawa and EN cells were injected subcutaneously in the dorsal flanks of nude mice. Once tumors reached approximately 100 mm^3, cells were harvested and treated with vehicle (0.1% DMSO) or drug (JQ1). Cells were seeded (5 × 10^5 cells/well) in a 96-well plate. Cells were subsequently treated with vehicle (0.1% DMSO) or drug (JQ1). Cells were incubated at 37 °C and 5% CO₂ for 7–14 d, colonies were stained with MTT solution at 37 °C overnight and absorbance (OD, 590 nm) was measured in a microplate reader.

To detect interactions of SPOP and HA-BRD3, cells were lysed in 1% NP-40 buffer (50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1% NP-40) with 2× protease inhibitor cocktail (Complete, Roche) and sonicated; 3 mg of lysate was incubated overnight with 2 µg of anti-HA-tag antibody (9658, Sigma) or control mouse IgG (sc-2025, Santa Cruz Biotechnology) at 4 °C. Subsequently, antibodies were collected using 25 µl of Protein A/Magnetic beads (88803, Fisher Scientific) for 2 h, followed by two washing steps with 1% NP-40 buffer. Proteins were eluted by addition of 1× SDS sample buffer under reducing conditions at 95 °C for 5 min. Quantitative analysis of the western blots was performed with protein levels normalized to VCL or ACTB expression. Uncropped immunoblots are presented in Supplementary Figure 11.

Chemicals. MG132 and cycloheximide (CHX) were purchased from Sigma and used at 10 µM and 100 µM, respectively, in all experiments. (+)-JQ1 and OTX-015 were purchased from Selleckchem and used at the indicated concentrations.

In vivo ubiquitination assay. 293T cells were transiently transfected with the indicated plasmids: pCW107-BRD3-WT or BRD3-degron-MT (2 µg), pCW107-SPOP-WT or SPOP-MT (2 µg), and CMV-8×His-Ub (2 µg). 48 h later, cells were treated with MG132 or DMSO for an additional 3 h. Cells were then washed with PBS and collected by centrifugation. A small amount of cells were lysed in RIPA buffer and the rest were lysed in Buffer C (6 M guanidine–HCl, 0.1 M Na₂HPO₄/NaH₂PO₄, 10 mM imidazole; pH 8). The WCE was sonicated and incubated with 60 µl of Ni-NTA agarose (Sigma) overnight at 4 °C. Next, Ni-NTA beads were washed once with Buffer C, twice with Buffer D (1 volume of Buffer C, 3 volumes of Buffer E) and once with Buffer E (25 mM Tris-HCl, 20 mM imidazole; pH 6.8). Elution of bound proteins was performed through boiling in 1× SDS loading buffer containing 300 mM imidazole. Samples were loaded, separated by SDS–PAGE and detected by immunoblotting.

In vitro ubiquitination and binding assays. Wild-type and SPOP-binding-mutant (degron-MT) constructs of HA-tagged human BRD3 were purified from transiently transfected HEK-293T cells. Wild-type and mutant human SPOP species were cloned, expressed and purified as described previously, using a GST instead of an MBP affinity tag. KLHL9, KLHL13, KLHL21 and Cdc34b were cloned, expressed and purified from Escherichia coli as described previously. CUL3 and RBX1 were purified in a preassembled complex from insect cells and neddylated in vitro using purified components as described previously. In vitro ubiquitination reactions with a total volume of 15 µl were assembled as follows: 10^6 HA-BRD3-expressing HEK-293T cells were harvested and lysed by sonication in IP buffer (20 mM Tris-HCl, pH 7.4; 150 mM NaCl; 5% glycerol; 1 mM TCEP; 1× Roche protease inhibitor cocktail). Immunoprecipitates were prepared with 10 µl of anti-HA affinity gel (Sigma) and washed with IP buffer. For respective reactions, 2 µl of HA-BRD3 IP resin was used and supplemented with 0.3 µM SPOP, KLHL9, KLHL13 or KLHL21; 0.2 µM CUL3–Nedd8/RBX1; 0.7 µM Cdc34; 0.2 µM UbE1 (Boston Biochem); and 25 µM ubiquitin (BostonBiochem) in ubiquitination buffer (3 mM ATP, 10 mM MgCl₂, 50 mM Tris-HCl pH 7.6, and 0.5 mM DTT). Reactions were incubated at 37 °C for 45 min and stopped by addition of SDS sample buffer. Samples were separated by SDS–PAGE and visualized through chemiluminescence using anti-HA (Sigma, A2095), HRP-coupled goat anti-mouse IgG (Bio-Rad, 170-6516), Clarity Western ECL Blotting Substrate (Bio-Rad, 1705061) and Fusion FX imaging platform (Vilber Lourmat).

For coimmunoprecipitation experiments in vitro, 1 µM recombinant SPOP-WT or SPOP mutants and 1 µl of HA-BRD3–WT IP resin were incubated in 200 µl of IP buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 5% glycerol) for 1 h at 4 °C. Therefore, resin was washed twice in the same buffer and samples were separated by SDS–PAGE and visualized through chemiluminescence using anti-HA and anti-SPOP antibodies (see above).

qRT-PCR. RNA was extracted using the RNeasy kit (Qiagen) and processed by the Kapa SybrFAST One-Step qRT–PCR kit according to the manufacturer’s instructions. qPCR was undertaken on an Applied Biosystems StepOnePlus System. Target mRNA expression was quantified using the comparative Ct method (ΔΔCt method) and normalized to cyclophilin expression. The following primers were used: BRD2, forward 5’-CTACGTAAGAACCACCCGAGG-3’.
reverse 5-GCTTTTCTCAAGCCAAGCTT-3, BRD3, forward 5-CCCTAGGAGATGCTATCCA-3, reverse 5-ATGTCGTTAGTGGTGCCGCAG-3, BRD4, forward 5-CTCCTCTAAAGAAAGGACAAGA-3, reverse 5-GCCCGTCTTCTTTGACTTGGAG-3, TRIIM24, forward 5-CAGCGCAGAATGGCTTAAAGC-3, reverse 5-GTGGTGGACAGTGAAATCTCAG-3, SPOP, forward 5-GAAATTCGTGTGGCGAATGAAAC-3, reverse 5-GCCCGGATCCTACTTCCTTGGAGA-3, FOSLI1, forward 5-CCTCAGGGAGACGGTGCAACA-3, reverse 5-TCCGAGGTATTGGACAGATGG-3, cyclophilin, (PPIA), forward 5-CAGG TCTCGGC ATCTGTTGTC-3, reverse 5-TCGGCTCTCCTGGCACTTCC-3.

DNA and RNA sequencing of endometrial cell lines. Whole-exome sequencing was performed for all endometrial cancer cell lines profiled in the CCLE.42 This data set was used to determine mutation status for SPOP cell lines included in this study. In addition, RNA sequencing was performed at the Broad Institute using the Illumina TruSeq protocol for 17 CCLE cell lines. Reads were aligned to the human reference genome build hg19 using TopHat version 1.4, and mRNA expression levels were determined using RNA-SeQC. Reads per kilobase of transcript per million mapped reads (RPKM) values for each cell line were correlated with the median RPKM values of endometrial cancer tissues with recurrent SPOP point mutations identified in the TCGA portal.4 A threshold of 10 RPKM was used to determine the overlap of robustly expressed genes in the cell lines with the genes expressed in human tumor tissues.

For the analysis of transcriptional output changes in response to SPOP mutants, isogenic Ishikawa cells stably overexpressing either SPOP WT or an SPOP mutant (endometrial, ES65, E47K; prostate, Y87C, W131G) were generated. RNA-seq was performed on cells either untreated or treated with JQ1 (500 μM or 2 μM) for 4 h (Supplementary Table 2). Total RNA was extracted using the RNeasy kit (Qiagen), and sample quality was assessed using the Agilent RNA kit (Agilent). Libraries were labeled with the Ion Xpress Barcode Adapters 1-96 Kit (Thermo Fisher Scientific/Life Technologies) and sequenced on the Ion Library Quantitation Kit (Thermo Fisher Scientific/Life Technologies). Twenty to twenty-six libraries were multiplexed for template preparation and enrichment using the Ion PI Hi-Q OT2 200 Kit (Thermo Fisher Scientific/Life Technologies). Enriched samples were then loaded on an Ion P1 Chip v3 (Thermo Fisher Scientific/Life Technologies) and sequenced on the Ion Proton System using the Ion PI Hi-Q Sequencing 200 Kit (Thermo Fisher Scientific/Life Technologies). Sequencing run quality metrics were taken from the Torrent Suite Software (Thermo Fisher Scientific/Life Technologies) for each run. Sequencing data were then analyzed with Ion Reporter Software (Thermo Fisher Scientific/Life Technologies). We identified another SPOP-D140Y-mutated serous cancer, while the remaining cases did not harbor any additional recurrent mutations nor any other type of mutation in SPOP at an allelic fraction higher than 0.2. The study was approved by the scientific ethics committee at the Institute of Surgical Pathology, University Hospital Zurich (approval no. KEK-ZH-NR: 2010-0358).

Immunohistochemistry. For the detection of BET and FOSL1 proteins, slides were first dehydrated. For antigen retrieval, slides were incubated in a water bath at 98 °C for 20 min using a citrate buffer at pH 6 (BETs) or pH 9 (FOSL1) (Dianpath, T0050). For microarrays of prostate cancer tissue, the antigen retrieval for BRD2 and FOSL1 was extended to 40 min in total. Subsequently, slides were cooled to RT for 20 min and endogenous peroxidases were blocked for an additional 10 min with 3% H2O2 (VWR, 23615.248). After washing, slides were incubated for 10 min with a protein blocking solution (Dako, X0909). Then, slides were incubated with primary antibodies at the following concentrations for 1 h in antibody diluent reagent solution (Life Technologies, 003118): BRD2 (Abcam, ab13960; 1:500), BRD3 (Bethyl, A302-368A; 1:50), BRD4 (Abcam, ab128874; 1:400) and FOSL1 (Sigma-Aldrich, AV31377; 1:2,000). For microarrays of prostate cancer tissue, the BRD2 antibody was used at a dilution of 1:200. Thereafter, slides were washed and incubated with biotinylated anti-rabbit IgG (Vector, BA-1000) in PBS for 30 min at RT and subsequently washed and incubated another 30 min with Vectastain ABC kit at a dilution of 1:150 in PBS. Detections were performed using the ImmPACT DAB system (Vector, SK-4105) for 4 min at RT followed by nuclear staining with Mayer hematoxylin (Dialpath, C0303). Immunohistochemical staining was evaluated as follows for BET proteins: no detectable staining in more than 70% of tumor cell nuclei was referred to as negative, 30% or more tumor cell nuclei weakly stained (discernable nuclei) as weak and more than 30% of nuclei strongly stained (invisible nuclei) as strong.

Statistical analysis. GraphPad Prism version 7.00 (GraphPad Software) was used for analysis. Data are depicted as means ± s.e.m. unless otherwise specified. An unpaired, two-tailed independent Student’s t-test with unequal variance assumption was performed to analyze cell culture experiments. Two-way ANOVA (Dunnett’s post test) was used for multiple comparisons. Extra-sum-of-squares F-test was used to determine the statistical significance of dose-response curves. The Spearman correlation coefficient was used to compare RNA-seq expression data from SPOP-mutant human tumors and BET protein levels with endometrial cell line data. Kendall’s tau-b was used to test correlation of immunohistochemical staining with SPOP mutation status.

Data availability. CCLE data are available online at http://www.broadinstitute.org/ccle/home. The original mass spectrometry spectra have been deposited in the public proteomics repository MAssIVE and are accessible at ftp://MSV000080401@massive.ucsd.edu when providing the data set password “ubiquitin.” If requested, also provide the username “MSV000080401.” Sequencing data have been deposited in the NCBI public database and are accessible under BioProject PRJNA357942.
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