A role for SMARCB1 in synovial sarcomagenesis reveals that SS18-SSX induces canonical BAF destruction

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Abstract

Reduced protein levels of SMARCB1 (a.k.a. BAF47, INI1, SNF5) have long been observed in synovial sarcoma (SS). Here, we show that combined Smarcb1 genetic loss with SS18-SSX expression in mice synergized to produce aggressive tumors with histomorphology, transcriptomes, and genome-wide BAF-family complex distributions distinct from SS18-SSX alone, indicating a defining role for SMARCB1 in SS. Smarcb1 silencing alone in mesenchyme modeled epithelioid sarcomagenesis. In mouse and human SS cells, SMARCB1 was identified within PBAF and canonical BAF (CBAF) complexes, co-incorporated with SS18-SSX in the latter. Recombinant expression of CBAF components in human cells reconstituted CBAF sub-complexes that contained equal levels of SMARCB1, regardless of SS18 or SS18-SSX inclusion. In vivo, SS18-SSX expression led to whole-complex CBAF degradation, rendering increases in the relative prevalence of other BAF-family subtypes, PBAF and GBAF complexes, over time. Thus, SS18-SSX alters BAF subtypes levels/balance and genome distribution, driving synovial sarcomagenesis.
Statement of Significance

The protein level of BAF component SMARCB1 is reduced in synovial sarcoma, but plays a defining role, incorporating into PBAF and SS18-SSX-containing canonical BAF complexes. Reduced levels of SMARCB1 derive from whole-complex degradation of canonical BAF driven by SS18-SSX, with relative increases in the abundance of other BAF-family subtypes.
Introduction

Malignant transformation represents the arrival at a particular cellular state with the specific potential to adapt, proliferate, and develop into a tissue with deadly consequences for its host organism. Mechanisms by which normal cells regulate differentiation and stemness (the capacity for subsequent differentiation) are often repurposed or dysregulated in oncogenesis. Chromatin remodeling, particularly the functions performed by the SWI/SNF-family of complexes (also termed—and herein referred to generally as—BAF-family complexes in mammals, for BRG1 and Associated Factors), plays a major role in differentiation, stemness, and malignant transformation, in many cellular contexts\(^1\). Mutations in components of BAF-family complexes have been identified in a significant portion of cancers of many tissue origins\(^2\). A few malignancies appear to be driven at the genetic level primarily (if not solely) by BAF-family derangements\(^3\). One of these is malignant rhabdoid tumor (MRT), a pediatric cancer that consistently involves homozygous loss of function in the gene encoding the BAF component SMARCB1 (also called hSNF5, INI1, or BAF47). There are very few, if any, secondary alterations in the genomes of MRTs, suggesting that loss of \(SMARCB1\) is an independent driver of the MRT oncogenic program\(^4\). \(Smarcb1\) loss in the \(Mx1\) lineage in the mouse has also proven sufficient to induce tumors mimicking MRT\(^5\).

Another cancer, epithelioid sarcoma (EpS), also shares the homozygous loss of \(SMARCB1\) function at either the gene or protein level\(^6\). Unlike MRT, EpS rarely afflicts children and typically bears a more complex genome, with additional genetic aberrations from normal cells\(^7\). EpS shares with MRT particular cellular features, such
as rhabdoid bodies (paranuclear inclusions) in the cytoplasm and epithelioid morphology.

A third malignancy, synovial sarcoma (SS), involves a \( t(X;18) \) chromosomal translocation that creates a fusion to an SSX gene, from \( SS18 \), a gene whose product is a known BAF-family component. All three malignancies, MRT, EpS, and SS, share the unusual combination of mesenchymal origins (MRT in only a subset of cases) and the ultimate expression of some epithelial marker proteins. However, only SS takes this mesenchymal-to-epithelial transition a step further by generating histomorphologically distinct gland-like structures in some tumors.

SS also has a unique relationship with SMARC1B, with recognition in pathology comparative series that SS tissues have reduced levels of nuclear immunohistochemical staining for the protein, contrasted with the absence of staining in MRT and EpS and the full presence of staining in the nuclei of most other tissues and tumor types. One proposed explanation of the reduced levels of SMARC1B in SS is that incorporation of SS18-SSX into the canonical BAF complex drives ejection/exclusion of the SMARC1B protein, causing SMARC1B instability/degradation. Since that model was described, a new subtype of BAF-family complexes was identified, which brought attention to a potential role in sarcomagenesis for all three major subtypes: GBAF (a.k.a ncBAF), canonical BAF (herein termed CBAF), and polybromo-BAF (PBAF, which provides a second host complex for SMARC1B). As SS18-SSX-mediated synovial sarcomagenesis is robustly recapitulated in the mouse, we determined to test this model through the impact of
genetic manipulation of Smarcb1 on the characteristic SS tissue phenotypes, and to follow up our results with genomic, molecular and biochemical assays.
Results

Genetic silencing of Smarcb1 in mesenchyme induces tumors

To test the tumorigenic potential of Smarcb1 loss alone in mesenchyme, we injected TATCre protein into the paws of mice homozygous for a conditional disruption allele of Smarcb1. Injected Smarcb1/mice developed a limited incidence of paw tumors over latencies consistently longer than 12 months (Fig. 1A, Supplementary Fig. S1A).

To test the hypothesis that SS18-SSX-mediated ejection/loss of SMARCB1 from BAF complexes was a stand-alone mechanism of synovial sarcomagenesis, we disrupted Smarcb1 in a tissue lineage with strong originating potential for SS18-SSX-mediated synovial sarcomagenesis (Supplementary Fig. S1A). Penetrance of tumorigenesis in Myf5Cre;Smarcb1/mice was approximately 40 percent. Expression of SS18-SSX2 (conditionally expressed from the hSS2 allele, Supplementary Fig. S1A) in the same lineage demonstrated 100 percent penetrance of sarcomagenesis. The distribution of tumors in these two groups differed significantly (Fig. 1B).

Increasing expression of SS18-SSX enhances synovial sarcomagenesis

Copy number gain in hSS2 is the only common secondary genetic change present in tumors initiated in Myf5Cre;hSS2 mice. As the hSS2 allele in our mouse model resides at the Rosa26 locus, both Ss18 loci are retained in their native state—unperturbed by chromosomal translocation—likely contributing more SS18 to compete with the fusion for occupancy in BAF-family complexes. To directly assess the impact of hSS2 copy number, we bred mice to be either heterozygous or homozygous for hSS2 at
the Rosa26 locus, then injected TATCre protein in the hindlimb at age 4 weeks.

Homozygous hSS2 mice demonstrated higher (complete) penetrance of tumorigenesis. The resulting tumors grew to similar size at morbidity-determined euthanasia, but in a shorter growth period (Fig. 1C).

**Genetic silencing of Smarcb1 enhances sarcomagenesis when accompanying SS18-SSX expression**

To test specifically for potential competition between SS18-SSX and SMARCB1, we combined variably increased hSS2 copy number and reduced Smarcb1 copy number in several distinct models of synovial sarcomagenesis. First, an assay of tumorigenesis initiated by TATCre limb injections at age 4 weeks compared three genotypes of mice that were all heterozygous for hSS2, and wild type, heterozygous, or homozygous for Smarcb1-floxed. Notably, heterozygosity for Smarcb1 genetic inactivation enhanced tumorigenesis in the presence of conditional fusion expression (Fig. 1D). This was tested again in a more tightly controlled littermates cohort of mice all homozygous for Rosa26hSS2/hSS2 and either heterozygous-floxed or homozygous wild type for Smarcb1. Injection of TATCre at day 8 led to a significantly shorter latency to tumorigenesis in the heterozygous Smarcb1 mice (Supplementary Fig. S1B). These TATCre initiated Rosa26hSS2/hSS2;Smarcb1f/wt tumors did not generally lose heterozygosity at Smarcb1 (Supplementary Fig. S1C), suggesting that Smarcb1 haploinsufficiency or reduced SMARCB1 protein stoichiometry alone may contribute to tumorigenesis. Tumorigenesis and growth rates were also compared after injection at 4 weeks of life into littermates homozygous for hSS2 and either wildtype or homozygous for conditional disruption of
Smarcb1, demonstrating significantly more rapid development and growth of the latter cohort (Fig. 1E).

Expression of the fusion with homozygous conditional disruption of Smarcb1 in the Myf5Cre lineage led to aggressive tumorigenesis at short latency, compared to either genetic manipulation alone. Remarkably, no Myf5Cre;Rosa26hSS2/+/Smarcb1fl/fl mouse survived beyond the age of 1 month, and most developed morbid tumors by age 2 weeks (Fig. 1F and Supplementary Fig. S1D-F). Metastasis was histologically confirmed in the lungs of 5 of the 7 Myf5Cre-initiated combination genotype mice and none of the other two genotypes.

Homozygous silencing of Smarcb1 with or without SS18-SSX expression generates EpS-related histologic features

Histological evaluation of tumors from each of the Smarcb1-loss initiation methods identified EpS features, including necrosis, nuclear pleiomorphism, and paranuclear cytoplasmic inclusion bodies, distinct from the gland forming epithelial cells and spindle cells with monomorphic nuclei that characterize the SSs from SS18-SSX-expressing hSS2 mice (both heterozygous and homozygous at Rosa26, Fig. 1G-H, Supplementary Fig. S1G). We observed no histological difference between hSS2-induced tumors with wild type Smarcb1 or heterozygous inactivation of Smarcb1 (Supplementary Fig. S1H), but homozygous ablation of Smarcb1 accompanying fusion expression initiated by TATCre generated distinct histology that included the same EpS features (Fig. 1G-H).
The histology of tumors with combined homozygous silencing of *Smarcb1* and expression of SS18-SSX initiated by *Myf5Cre* also demonstrated EpS features, although with more of a poorly differentiated, MRT-like appearance overall (Fig. 1G-H).

**Expression of SS18-SSX impacts tumor transcriptomes, and combined loss of Smarcb1 confers additional changes**

The transcriptomes of MRT and SS differ significantly\(^{29}\), whether examining datasets from human tumors or derived cell lines. To explore the transcriptional impact of *Smarcb1* genetic silencing, SS18-SSX expression, or the combination of the two in mice, RNAseq was performed for mouse tumors with either presence or absence of heterozygosity for *hSS2* and wild type or homozygous floxed alleles of *Smarcb1*. Sample tumors included those derived from *Myf5Cre* or TATCre initiation. Tumors of each genotype clustered most closely with themselves in pairwise comparisons of whole transcriptomes and in principal component analysis (Fig. 2A-B). For *hSS2* activation alone, TATCre- and *Myf5Cre*-initiated tumors intermingled. For each of the genotypes that included *Smarcb1* homozygous loss, TATCre and *Myf5Cre* tumors each clustered distinctly, but in adjacent groups. Overall, there was slightly stronger correlation between all transcriptomes that shared *hSS2* expression (with or without *Smarcb1* genetic silencing) than with those that shared *Smarcb1* loss (with or without *hSS2* expression.)

Unsupervised hierarchical (k-means) clustering identified clusters of genes that discriminated between the groups (Fig. 2C). Additional interrogation of the Reactome pathways represented in the differential expression of the first cluster, genes relatively
highly expressed in fusion-expressing tumors with wild type Smarcb1 compared to either genotype with silencing of Smarcb1, indicated involvement of many pathways considered to be characteristic of target genes of the SS18-SSX fusion (Fig. 2D)\textsuperscript{29-31}. These included axon guidance, β-catenin, and Frizzled pathways. The other clusters demonstrated some expected variations between SS and MRT/EpS (Supplementary Fig. S2A). Comparative genomics, using principal component analysis with published human tumor transcriptomes, clustered fusion-only mouse tumors with human SSs and Smarcb1-loss-only mouse tumors with human EpSs, with combination genotype tumors clustering closer to human MRTs (Fig. 2E and Supplementary Fig. S2B). Evaluation of a gene set defined as direct fusion targets by ChIPseq and reduced expression upon knock-down of SS18-SSX in a human SS cell line, as well as increased expression in SSs over other sarcoma types in TCGA\textsuperscript{30} showed differential expression in fusion-only tumors over combination genotype tumors (Fig. 2F), suggesting that even targets of the oncogenic BAF complex may be impacted by genetic loss of Smarcb1, challenging the prior model that held that SMARCB1 is excluded from that complex by inclusion of SS18-SSX.

**BAF-family genomic locations demonstrate variable impacts of fusion expression and Smarcb1 silencing**

In human MRT cancer cell lines, SMARCB1-loss diminishes BAF complex occupancy of many enhancers, alongside relative retention of BAF at super-enhancers\textsuperscript{32}. In human SS cell lines, BAF-family complexes reside at actively transcribed chromatin, and can extend across gene bodies in broader or longer peaks than otherwise typical for BAF localization\textsuperscript{29-31}. 
In order to test how BAF-family complexes distribute across the mouse genome in these different tumor genotypes, we performed ChIPseq in tumor tissues, using antibodies raised against ‘core’ BAF-family subunits shared by all BAF-family subtypes: SMARCC1 (a.k.a. BAF155) and SMARCA4 (a.k.a. BRG1) and conducted comparisons (Supplementary Fig. S3A-B).

The ChIPseq enrichment of SMARCC1 and SMARCA4 across regions defined by intersection of the two in each tumor genotype group was stronger in the fusion-only tumors compared to either tumor genotype that included genetically silenced Smarcb1 (Fig. 3A). While the position of BAF-family peaks with respect to genes was similar between fusion-only and combination genotype tumors, there was an increased prevalence of intronic and decreased prevalence of promoter BAF-family binding sites in Smarcb1-loss-alone tumors (Fig. 3B, Supplementary Fig. S3C). The higher prevalence of combined SMARCA4 and SMARCC1 peaks near transcription start sites (TSSs) in the presence of the fusion may have derived from the extended breadth of BAF peaks in fusion-expressing tumors, regardless of Smarcb1 status, fitting with the previous descriptions from human SS cell lines (Fig. 3C-D)\textsuperscript{29-31}. While there were relative losses of BAF-family enrichment in promoters by the addition of Smarcb1 genetic silencing to fusion expression, the fusion-only tumor-defined distal intergenic SMARCA4 peaks were even more profoundly reduced in the other groups (Fig. 3E).

We next performed ChIPseq for RNA Polymerase II (RNAPOLII) to determine the BAF-family relationship with transcription in each tumor genotype. First, BAF ChIPseq enrichments demonstrated a very similar overall pattern in combination and fusion-only genotype tumors at TSSs across the genome, albeit with reduced enrichment generally
in the combination group (Fig. 3F). When we tested for differential expression of genes annotated by differential enrichment for SMARCC1 and SMARCA4, we found that reduced BAF-family enrichment in a group of tumor genotypes associated with reduced expression of the annotated genes (Supplementary Figure S3D-E).

Focally at TSSs, we observed a striking reduction of BAF enrichment in both groups that bore genetic silencing of Smarcb1—a feature not present in the fusion-only group—visualized as a ‘stripe’ of diminished BAF-family complexes at the TSS (Fig. 3F). Given that PBAF is the BAF-family complex subtype that is described to be specifically abundant at promoters\textsuperscript{33}, we performed another ChIPseq for PBRM1 (a.k.a. BAF180), a PBAF-specific component, in fusion-only tumors—but not in combination tumors—as PBAF was not significantly present on chromatin after genetic silencing of Smarcb1 (Supplementary Fig. S3F-G). PBAF peaks were mostly in promoters, while BAF-family peaks with PBAF omitted localize to distal sites (Fig. 3G). TSSs with strong focal loss of BAF-family ChIP enrichment in combination genotype tumors were also found to have strong promoter focal enrichment for PBAF in fusion-only tumors (Fig. 3H-I).

**SMARCB1 remains present at reduced levels in SS tumors, integrated in BAF-family complexes**

To test if in SS, SMARCB1 might incorporate into PBAF complexes, a subset of BAF complexes that are thought to exclude both native SS18 and the fusion SS18-SSX, but include SMARCB1\textsuperscript{13}, we performed immunoprecipitation (IP) of a human SS cell line, HSSYII, and HEK293T cells as a control, using an antibody against PBRM1 and SMARCB1, then performed Western blots (WBs) for each. These experiments
demonstrated that in SS cells, SMARCB1 protein is present and co-IPs with a PBAF subunit (Fig. 4A). We next tested for SMARCB1 participation in CBAF, using co-IP for DPF2, a CBAF specific component, and found that both proteins were able to co-IP the other from SS cells (Fig. 4B).

Glycerol gradients of nuclear extracts were then utilized to identify the size of SMARCB1-containing complexes, using two human SS cell lines, HSSYII and ASKA, with HEK293T cells as a control. SMARCB1 from SS nuclei co-fractionated with PBRM1, sized appropriately for PBAF, and CBAF-specific components, DPF2 or ARID1A. Another, smaller amount of protein revealed by anti-SMARCB1 western blot appeared in gradient fractions consistent with BAF-family subunit monomers; running at a higher size than the predicted 47kD (Fig. 4C).

The same approaches of co-IP and glycerol gradients of nuclear extracts were tested in mouse tumors, using combination genotype tumors with genetically silenced Smarcb1 as a negative control. Similarly, SS tumors demonstrated strong co-precipitation of SMARCB1 with PBRM1 and DPF2 and the reciprocal co-precipitations, albeit with low levels of SMARCB1 present in the input (Fig. 4D-E and Supplementary Fig. S4A-B). There was a similar distribution of SMARCB1 across both PBAF and CBAF fractions in the mouse gradients. In order to quantify this distribution, appreciating that gradient westerns with different antibodies cannot be compared to each other, but only to the relative distribution of any single protein across the different sized fractions, we compared the relative abundance of SMARCB1 in the CBAF-sized fractions compared to PBRM1 in the same fractions, each normalized against PBAF-peak-sized fractions. In both human SS cell lines and the mouse hSS2-only tumors, SMARCB1 was
significantly more abundant than PBRM1 in gradient fractions 15 through 17, each relative to fractions 19 and 20 (Fig. 4F-G).

In order to test the interaction between SMARCB1 and BAF complexes in SS cells further, we applied short interfering RNA (siRNA) against SMARCB1 to reduce its presence in HSSYII and ASKA cells. Depletion of SMARCB1 by siRNA reduced PBRM1 levels (Fig. 4H), indicating that PBRM1 stability in SS is dependent on SMARCB1 and providing an orthogonal line of evidence that SMARCB1 incorporates into PBAF complexes in SS. SMARCB1 knockdown in SS cells also reduced the stability of the CBAF-specific subunit, DPF2, which only assembles into SMARCB1-containing CBAF complexes. This similarly suggested that SMARCB1 in SS cells without added siSMARCB1 was also incorporating into CBAF complexes.

Glycerol gradients of nuclear extracts were also performed for the ASKA cell line exposed to siSMARCB1 or control scrambled siRNA. These confirmed a drastic reduction of PBRM1 in PBAF sized complexes (Supplementary Fig. S4C-D). Also, SMARCC1 gradient westerns were quantitated as a representative measure of the relative distribution of GBAF, CBAF, and PBAF, since it incorporates into each of these BAF subtypes. Compared to controls, cells or a tumor with forced reduction in SMARCB1 by RNA interference or genetic silencing reduced the relative presence of SMARCC1 in PBAF-sized fractions (Fig. 4I).

**SMARCB1 associates with CBAF complexes that contain the SS18-SSX fusion**

Distributions of SS18-SSX and SMARCB1 in human SS cell lines and mouse hSS2-induced tumors overlapped in CBAF-sized gradient fractions (Fig. 5A). To more directly
test their potential interaction, we performed IP and WB for SMARCB1 and SS18, as well as SMARCC1 and PBRM1. In hSS2-induced tumors, but not those that also had undergone genetic ablation of Smarcb1, anti-SMARCB1 co-precipitated a fusion-sized band on SS18 WB (Fig. 5B). We therefore tested the ability of SMARCB1 and a V5- or HA-tagged SS18-SSX fusion transfected into EXPI293 cells (a HEK293T variant that grows in suspension culture) to reciprocally co-IP with each other. Here, we found clear co-precipitation, although we also observed reduced levels of both SMARCB1 and SMARCC1 proteins in the nuclear extracts (input sample) following expression of the fusion compared to controls that expressed tagged-SS18 (Fig. 5C). Next, we attempted co-IP of SMARCB1 with a CRISPR-HA-tagged endogenous SS18-SSX1 in the human cell line HSSYII30. Indeed, IP of HA-SS18-SSX1 co-precipitated SMARCB1, leading to SMARCB1 depletion from the supernatant, strongly suggesting that the two interact, either directly or within a CBAF complex (Fig. 5D). To test whether these two interact in BAF-family sized complexes, we performed glycerol gradients to size the eluted complexes from the HA-IP. Although the yield of intact complexes was very low (even for SS18-SSX itself), most of the detectable SMARCB1 was found in the same fractions as SS18-SSX, and ran in the fractions predicted for CBAF-sized complexes (Fig. 5E). Again, we noted a faint, SMARCB1-staining band, running at larger size than 47kD in the monomer-sized fractions, possibly indicating SMARCB1 ubiquitylation during incorporation with SS18-SSX in CBAF complexes.

**Synovial sarcomas display reduced abundance of CBAF**

Because SMARCB1 protein levels are reduced in SS, but SMARCB1 is not excluded from CBAF complexes that contain SS18-SSX, we hypothesized that changes in
SMARCB1 abundance could be alternatively attributed to changes in the relative abundance of each BAF-family complex subtype (Fig. 6A). SS18-SSX overexpression led to a reduction in SMARCC1 protein levels in EXPI293 nuclear extracts (Fig. 5C), suggesting that the presence of the fusion leads to an overall reduction in BAF-family complexes in those cells. Further, we developed an analysis algorithm to use SMARCC1 distribution as a measure of the relative abundances of each complex subtype (described in detail in the Supplementary Detailed Methods). For this, we performed glycerol gradients of nuclear extracts, collected in 25 fractions, blotting the higher molecular weight fractions (12 through 24) for BRD9 to define the distribution of GBAF, DPF2 to define the distribution of CBAF, and PBRM1 to define the distribution of PBAF. We calculated the relative abundance of each complex type to explain the overall SMARCC1 distribution across those fractions. On mouse tumors, this algorithm revealed the almost complete obliteration of PBAF in combination genotype tumors, but low abundance of CBAF overall in both hSS2-only and combination tumors (Fig. 6B, compared to the typical predominance of CBAF). To scale up the quantitative use of this algorithm, we performed gradients in triplicate for each of five human SS cell lines and three control cell lines. In SS cells, CBAF relative abundance was significantly reduced (Fig. 6C and Supplementary Fig. S5). Two possible explanations for lower CBAF abundance in SS include: 1) the fusion prevents CBAF assembly, or 2) the fusion promotes CBAF degradation.

Recombinant CBAF complexes assemble avidly, co-incorporating SS18-SSX and SMARCB1
To directly test assembly of CBAFs containing the fusion SS18-SSX, as well as co-assembly with SMARCB1 in a context where degradation would not reduce CBAF levels, we developed a system to co-express multiple CBAF components (including most conserved core subunits) in human EXPI293 cells. These components included SMARCA4, SMARCC1, SMARCC2, 3XFLAG-tagged SMARCD1, SMARCE1, and SS18—either with or without SMARCB1 (Fig. 6D). Anti-FLAG purification of SMARCD1 and associated CBAF sub-complexes from cells co-expressing SMARCB1 identified the position of the SMARCB1 band in SDS-PAGE gels, and revealed its loss when omitted from the expression system (Fig. 6E-F). To test whether SMARCB1 assembly was compatible with the SS18-SSX fusion, we expressed CBAF components (including SMARCB1) and either V5-tagged SS18-SSX or SS18 itself, followed by anti-FLAG enrichment and elution of complexes; this revealed the clear presence of SMARCB1 in both purified complexes (Fig. 6G). A subsequent IP with anti-V5, using the FLAG-purified complexes from each type as the input, demonstrated an equivalent amount of SMARCB1 protein in the purified recombinant CBAF complexes containing SS18-SSX as those with SS18 itself (Fig. 6H). This demonstrates that assembly of SMARCB1 into CBAF was not hampered by the presence of the fusion in this system.

**SS18-SSX incorporation in CBAF complexes leads to their degradation**

To investigate the alternate hypothesis, that degradation of CBAF drives its lower abundance in cells, LICOR quantitative western blots were performed for nuclear extracts from EXPI293 cells transfected with either SS18-SSX or SS18 (n = 5, each), comparing the ratios in abundance of BAF-family components. The core components participate in all three BAF-family subtypes; SMARCB1 and SMARCE1 participate in
both CBAF and PBAF; SS18 or SS18-SSX can participate in both GBAF and CBAF (See Fig. 6A). Strikingly, every component that participates in CBAF was significantly reduced in abundance in fusion-expressing cells (Fig. 7A and Supplementary Fig. S6A). CBAF-exclusive components were among the most significantly reduced in cells expressing the fusion. Westerns of the fractions from glycerol gradients of these two groups demonstrated a drastic reduction of components in all complex-sized fractions, but no significant redistribution of SMARCC1 to PBAF or GBAF fractions (Fig. 7B). As this suggested that the expression of the fusion drives a degradation of CBAF, it was next necessary to test if manipulation of the presence of the fusion in SS cells would also alter the relative abundances of different complex subtypes.

The relative abundances of BAF-family components were compared by quantitative western blots after shRNA depletion for seven days of the fusion or control Renilla in two SS cell lines. Concerned that redistribution due to increased affinity for chromatin could also contribute to a depletion of CBAF in nuclear extracts, we first collected whole cell lysates, harvested with high stringency, instead. These demonstrated significantly increased presence of CBAF-specific components after depletion of the fusion, but also a less-marked decrease of GBAF-specific and PBAF-specific components (Fig. 7C-D, Supplementary Fig. S6B-C).

Density gradient westerns from nuclear extracts for quantitation of complex subtype abundances in the fusion-depleted or control cells also demonstrated a significant increase in the relative abundance of CBAF complexes and decrease in the relative abundance of GBAF and PBAF in week-long fusion-depletion in HSSYII cells (Fig. 7E, Supplementary Fig. S7A-C).
From similarly manipulated cells, the protein remaining bound to the insoluble chromatin fraction following the nuclear extraction protocol demonstrated very little PBAF or CBAF, but nearly as much or more GBAF as that harvested from the nuclear extract (Fig. 7F). This prompted another round of quantitative westerns to compare these components in each of these nuclear "compartments" between fusion depleting and control shRNAs. Despite a relative increase in the chromatin-bound fraction of CBAF in the presence of the fusion, the vast majority of CBAF still partitioned to the nuclear extract. Further, even the absolute level of CBAF in that insoluble chromatin-bound fraction was reduced in control knock-down cells compared to fusion knock-down, suggesting that changes in CBAF localization cannot explain the reduction in CBAF components (Fig. 7G and Supplementary Fig. S7D). Notably, the insoluble chromatin-bound fraction of GBAF was vastly depleted upon knock-down of the fusion, even while the nuclear extract fraction changed less significantly, suggesting that stronger affinity for chromatin in the presence of the fusion profoundly impacts nuclear extract levels of GBAF.

Since no assembly defect was observed, and greater affinity for insoluble chromatin could not account for the depletion of CBAF and increased abundance of GBAF and PBAF in the presence of the fusion, we next tested the hypothesis that if CBAF depletion involved some fusion-promoted degradation mechanism, then this depletion would be blunted at least partly by proteasome inhibition. In the scrambled control knock-down HSSYII cells, in HEK293T cells expressing the fusion, and in a few additional SS cell lines, exposure to MG132, a proteasome inhibitor, decreased the
depletion of CBAF-specific DPF2, contrasted with fusion-depleted HSSYII cells, HEK293T cells expressing SS18, and non-SS cell lines, respectively (Fig. 7H-I).
Discussion

The observation that SS18-SSX fusion oncoproteins interact with BAF-family complexes provided early mechanistic insight into SS\textsuperscript{10-13,24} and has here been corroborated (Fig. 4D-E and S4A) in mice that recapitulate overall fusion-driven sarcomagenesis\textsuperscript{27,28}.

Advancing understanding of the BAF-family of complexes related to SS, MRT, EpS and other cancers, has increasingly ascribed oncogenic and tumor suppressive functions to particular components and BAF-family subtypes. Here, loss of \textit{Smarcb1} in mesenchyme, including the \textit{Myf5Cre} cell lineage with strong origination potential for SS\textsuperscript{27,28}, drove tumorigenesis that instead resembled EpS by histological features and transcriptome (Fig. 1 and 2). This corroborates literature that retracted the briefly held concept in the SS field that SS18-SSX’s only oncogenic function was ejection of SMARCB1 from BAF-family complexes.\textsuperscript{24,29-31}.

Adding \textit{Smarcb1} silencing to SS18-SSX expression synergized in tumorigenesis that only partly recapitulated SS features (Fig. 1, 2, 3). This finding was incompatible with the prevailing model in the field that expression of SS18-SSX renders BAF-family complexes to a SMARCB1-lacking state\textsuperscript{24}. SMARCB1 loss, by itself or added to fusion expression, impacted BAF-family complexes in two ways: it reduced general BAF-family affinity for and distribution across chromatin and functionally obliterated PBAF assembly, altering BAF-family distribution to promoters and TSSs, specifically (Fig. 3F-I and 4H-I).

PBAF is shown here to be prominent and active in SS, but likely disrupted in MRT and EpS. The reportedly recovered distribution of BAF-family complexes to particular TSSs
following SMARCB1 re-expression in MRT cell lines may derive from recovery of PBAF\textsuperscript{34}. In SS, at TSSs, PBAF exerts tumor suppressive function, likely enabling the expression of differentiation genes that affords SS its unique features of both mesenchymal and epithelial differentiation, which is lost upon Smarcb1 silencing/loss. PBAF stability has previously been shown to be compromised by loss of other components\textsuperscript{35}. A role for SMARCB1 and PBAF in TSS-specific binding may indicate their affinity for the H2A.Z-modified histones that reside at the TSS and +1 nucleosome positions\textsuperscript{36}. RSC (the most abundant BAF-related complex in yeast) binds to and ejects H2A.Z-containing nucleosomes, preferentially\textsuperscript{37}.

We find that SMARCB1 incorporates into CBAF in SS (Fig. 4), including CBAF containing SS18-SSX (Fig. 5 and 6). As CBAF is the dominant BAF-family complex type in most cells, alterations in its abundance affect the detectable levels of each component, also impacting overall BAF-family distribution across chromatin. SS18-SSX expression reduces SMARCB1 levels, however SMARCB1 levels are reduced to a less pronounced degree than CBAF-specific components, as the overall abundance of SMARCB1 is rescued partly by an increased presence of PBAF in SS (Fig. 7J).

The near-disappearance of CBAF from nuclear extracts upon expression of the fusion SS18-SSX could derive from one of three mechanisms. First, SS18-SSX could drive a CBAF assembly defect, only subtly different from the SMARCB1 ejection model. Equivalent recombinant complex assembly observed here with SS18 or SS18-SSX (Fig. 6G-H) argues against fusion-mediated CBAF assembly failure, as does the observation in prior literature\textsuperscript{24,29-31} and glycerol gradient westerns presented here (Fig. 4C, 5A,
S4A, and S7A-B) that SS18-SSX-containing CBAFs far outnumber those containing native SS18, though both proteins are similarly expressed.

Second, SS18-SSX could sequester CBAF in other cellular compartments, reducing its detection in nuclear extracts. Indeed, we observed a slightly increased portion of CBAF remaining on insoluble chromatin after nuclear extraction in the presence of the fusion (Fig. 7F), fitting the increased affinity for chromatin conferred by the fusion’s SSX tail. However, the absolute abundance of CBAF associated with chromatin was also depleted (Fig. 7G), due to the fusion eliciting far lower CBAF total levels. In contrast, redistribution to chromatin from SS18-SSX incorporation profoundly impacted GBAF abundance in nuclear extracts. Approximately half of GBAF remains bound to insoluble chromatin following nuclear extraction from SS cells without fusion depletion (Fig. 7F). This argues that BAF-family subtype abundances (based on nuclear extracts to isolate intact complexes) vastly underestimate relative GBAF abundances in SS, specifically.

In the third model, degradation targets SS18-SSX-containing CBAF. This model is supported by the observation that proteasome inhibition blunts CBAF-specific component reductions in the presence of the fusion (Fig. 7H-I). Critically, fully assembled complexes appear to be the target of degradation, because all components involved in CBAF demonstrate some reduction in abundance (Fig. 7A), suggesting that CBAF components are not merely disassembling to be recycled for reincorporation into alternative CBAFs with SS18 (full model in Fig. 7J). The precise mechanism of fusion-mediated CBAF degradation deserves additional investigation. Others have shown that the fusion’s SSXRD domain interacts with a ubiquitin E3 ligase with otherwise established BAF-family component targeting capacity. Here, we further speculate that
the basic tail on SMARCB1\textsuperscript{40}, co-incorporated in a complex with the SSXRD domain on SS18-SSX, may offer tempting substrates for other E3 ligases, as well.

Implications of oncoprotein-mediated CBAF complex degradation are broad. First, it is important to note that this particular effect of SS18-SSX on BAF-family complexes is not likely to be oncogenic directly. Instead, this explains better why expression of SS18-SSX in most cell types is quickly lethal\textsuperscript{27,28}. If cells are rendered CBAF-deficient, most will not be capable of achieving a selectable epigenetic state that rescues general BAF-family function with GBAF and PBAF upregulation. Second, CBAF degradation explains the \textit{BRD9} dependency in SS that has been highlighted recently\textsuperscript{31,41}. BRD9 is the only GBAF component without a paralog (see Fig. 6A). Notably, only drugs degrading BRD9, not functional inhibitors, have impacted SS, making this dependence relationship more about GBAF function than BRD9, specifically. Here, we show that GBAF is by far the more abundant SS18-SSX-containing BAF-family complex on SS chromatin, fitting the hypothesis of Brien et al\textsuperscript{31}, that GBAF is the fusion-bearing complex, rather than Michel et al\textsuperscript{41}, who suggested that GBAF is a SS dependency but not the host complex for the fusion. Importantly, although reduced in abundance, CBAF is not absent from SS chromatin, where it includes the fusion and SMARCB1. Because the mechanism for CBAF depletion relates to the extent of proteasomal degradation, predicted variations in penetrance may provide opportunities for selection of epigenetic states that promote cancer cell survival. In keeping, SS cell lines vary in levels of retained CBAF (Fig. 6C). Our work predicts that therapeutic strategies that target GBAF stability will lead to resistance mechanisms that down-regulate CBAF degradation, as cells strive to promote CBAF maintenance.
In summary, these experiments have demonstrated an essential, defining role for SMARCB1 in synovial sarcomagenesis, revealed its incorporation into both PBAF and SS18-SSX-containing CBAF complexes, and identified CBAF degradation coupled with GBAF and PBAF upregulation as major effects of SS18-SSX expression. This work enhances our understanding of how SS18-SSX disrupts BAF-family complexes and provides additional conceptual support (as well as potential resistance mechanisms tumors will employ) for the targeting of GBAF as a therapeutic strategy in synovial sarcoma. Future efforts will continue to pursue the relative contributions of changes in the abundance, localization, and activity of each BAF-family complex subtype in synovial sarcomagenesis.
Methods

Please see the Supplementary Detailed Methods for more detailed protocols and reagents used (Key Resources Table).

Cell lines

HSSY-II, ASKA, Yamato, SYO1 and Fuji were provided by T. Nielsen. HSSYII-HA-tagged was provided by A. Banito. MoJo was generated in the laboratory. HEK293T was purchased from ATCC and EXPI293 from Thermo. Cells were authenticated by mycoplasma testing and STR DNA profiling every half-year of culture.

Animal Studies

Mouse experiments were approved by the University of Utah animal care committee in accordance with international legal and ethical standards. Smarcb1fl mice were obtained from Dana-Farber Cancer Institute. Rosa26hSS2 and Myf5Cre mice were previously described. Mouse strains were maintained on a mixed C57BL/6 and SvJ background with littermate controls and roughly equivalent distribution of sexes.

Recombinant BAF expression in mammalian biGBac

The pFastBac1-CMV vector was a gift from Dr. Erhu Cao (University of Utah). cDNA’s of each of the BAF subunits were cloned into pFastBac1-CMV, then into pBig1 and pBig2 vectors with custom oligonucleotides. All BAF subunits were assembled into a single pBig2 vector prior to transfection into EXPI293 cells.

RNAi
SS18-SSX specific shRNA expression vectors were provided by A. Banito and delivered by lentiviral transfection (shREN-a = shRen-713, shRen-b =shRen-660, shSSX-a = shSSX-1045 and shSSX-b =shSSX-1274) as previously described. Human SMARCB1 specific siRNAs (Invitrogen) were delivered with Lipofectamine RNAiMAX Transfection Reagent (Thermo).

**Sequencing**

The accession numbers for the RNAseq and ChIPseq data are GSE153856 and GSE153857, respectively.

**Rigor, Reproducibility, and Statistical Analysis**

For every assessment of data that invoked judgment of any kind, samples were randomized in order of assessment and raters were blinded as to the group identity of each sample.

Statistical comparisons between two groups used two-tailed Student’s t-test (unless a paired or heteroscedastic t-test was needed and noted in the figure legend), performed in Graphpad Prism software 7.0, and statistical significance was set at p < 0.05 or 0.01 as indicated in the figure legends. Data are presented as mean ± standard deviation unless otherwise noted. The sample size was determined by power analysis using the results of preliminary experiments to estimate variance and the number (n) is indicated in the figures or figure legends that represents biological replicates. All western blot experiments were repeated to confirm the relationships presented and discussed.
Acknowledgements

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References


Figure Legends

Figure 1. SS18-SSX and Smarcb1 compete in the balance between sarcomagenesis and tumor suppression, driving different phenotypes.

A. Schematic for TATCre protein injection into the paw and gross images of a tumor that developed over a year later in a Smarcb1\textsuperscript{fl/fl} mouse.

B. Schematic of the expression and recombination pattern from Myf5Cre and a mouse with anatomic distribution of Myf5Cre;Smarcb1\textsuperscript{fl/fl} and Myf5Cre;hSS2 tumors from 16 consecutive mice in each group. (2-tailed Fisher Exact test for incidence of tumors in each cohort, p = 0.0002. $X^2 = 17.03$ with 4 degrees of freedom and p = 0.0019 for comparing distributions of tumors in each cohort.)

C. Distribution of tumor size versus time at morbidity among cohorts of hSS2/wt and hSS2/hSS2 mice in which sarcomagenesis was initiated at age 4 weeks by hindlimb injection of TATCre. (2-tailed t-test, p = 6.6 x $10^{-5}$ for time to tumorigenesis.)

D. Kaplan-Meier (KM) plot of the tumor-free fraction among cohorts of hSS2-heterozygous mice bearing each possible Smarcb1-floxed genotype (in magenta) after TATCre injection into the hindlimb at age 4 weeks. (The cohort of hSS2 het alone is data from the cohort depicted in 2A for reference; the other two cohorts are littermate-controlled cohorts: Smarcb1\textsuperscript{fl/wt} compared to Smarcb1\textsuperscript{fl/fl}, log-rank test $z = 4.43$, p<0.001.)
E. Tumor growth by caliper measurements in littermate cohorts of mice homozygous for hSS2 and either wildtype or Smarcb1<sup>fl/fl</sup> genotype, following TATCre injection at age 4 weeks. (2-tailed t-test p = 1.3 x 10<sup>-8</sup> for time to detectable tumor.)

F. KM plot of Myf5Cre-induced combination genotype tumors compared to either hSS2 or Smarcb1<sup>fl/fl</sup> alone. (log-rank tests comparing combination genotype to Smarcb1<sup>fl/fl</sup> alone, z = 4.62, p < 0.0001; to hSS2 alone, z = 8.15, p < 0.0001.)

G. Representative H&E photomicrographs of tumors from Smarcb1<sup>fl/fl</sup>, hSS2 heterozygous, combination hSS2 and Smarcb1<sup>fl/fl</sup>, and hSS2 homozygous mice activated by TATCre limb injection or Myf5Cre (scale bars = 20µm).

H. Graph of the fraction of tumors by each induction method demonstrating each of the histological features (TATCre+Smarcb1<sup>fl/fl</sup>, n = 11; Myf5Cre;Smarcb1<sup>fl/fl</sup>, n = 8; TATCre+hSS2, n = 15; Myf5Cre;hSS2, n = 17; TATCre+hSS2;Smarcb1<sup>fl/fl</sup>, n = 13; Myf5Cre;hSS2;Smarcb1<sup>fl/fl</sup>, n = 8)

Figure 2. Genetic silencing of Smarcb1 added to SS18-SSX expression results in distinct tumor transcriptomes.

A. Euclidean distance of samples heat map of non-hierarchical clustering of transcriptomes from tumors in mice bearing hSS2, homozygosity for Smarcb1-floxed, or combination genotypes, following Myf5Cre or TATCre induction.
B. Two-dimension principal component analysis (PCA) for the transcriptomes of these mouse tumors using the top 500 most differentially expressed genes.

C. Heat map hierarchical clustering of the most differentially expressed genes between the 6 groups of tumors, with K-means clustering.

D. Reactome Pathway analysis of cluster 1 genes, specifically expressed at higher levels in fusion-only expressing tumors, compared to those that have silencing of Smarcb1 alone or in combination the fusion.

E. Two-dimension PCA of transcriptomes of mouse and human SS, MRT, and EpS tumors, after separating principle component 1 (See Supplementary Fig. 2B), which represented the species specific differences between these groups.

F. Differential expression between hSS2-only and combination genotype tumors from mice for homologs of a human gene set defined as direct targets of the fusion in a human synovial sarcoma cell line.

**Figure 3. Smarcb1 loss and SS18-SSX expression drive aberrant BAF-family complexes distributions across chromatin genome-wide.**

A. ChIPseq enrichment plots for SMARCA4 (grey) and SMARCC1 (blue) and centered on intersection peaks for combined SMARCA4 and SMARCC1 enrichment in tumors of fusion-only, Smarcb1-loss-only, or combination genotypes.

B. Distribution of SMARCA4 ChIPseq peaks genome-wide with respect to gene features in each group.
C. Plot of the normalized prevalence of BAF-family peaks of different length in each of the groups after filtering out peaks less than 2kb in length. (Tukey’s range test adjusted p-value for comparing fusion-only to combination genotype tumor BAF-family peak lengths was 0.179, not significant. The comparison of either combination genotype or fusion-only tumors to Smarcb1-loss alone tumor BAF-family peak lengths had p-values below the detectable value.)

D. Example ChIPseq enrichment tracks of lengthened BAF-family peaks in tumors expressing the fusion, contrasted to Smarcb1-loss alone (b1 indicates Smarcb1.)

E. Enrichment plots for log transformed fold-enrichments of SMARCA4 ChIPseq for all three groups at promoter sites enriched in hSS2-only tumors or distal peaks in the same.

F. Heatmaps of ChIPseq distributions around transcription start sites across the whole genome, ordered by enrichment for SMARCA4 in hSS2-only tumors.

G. Distribution of SMARCA4 peaks that coincide with or are independent of PBRM1 peaks in fusion-only tumors with respect to gene annotations across the genome.

H. Enrichment plot of SMARCC1 for fusion-only and combination genotype tumors at TSSs defined as having a steep dip in the combination tumors, with overlaid enrichment plot for PBRM1 in hSS2-only tumors.
I. Example tracks of BAF component enrichments at TSSs (dotted vertical lines) among target genes contrasting hSS2-only and combination hSS2;Smarcb1^f/f tumors.

Figure 4. SMARCB1 at reduced protein levels in SS cells resides in BAF complexes.

A. Western blots (WBs) for reciprocal immunoprecipitation (IP) in human SS cell line, HSSYII, and control HEK293T cells, for PBAF components SMARCB1, and PBRM1.

B. WBs for reciprocal IP in human cells for CBAF components DPF2 and SMARCB1.

C. WBs for BAF-family components in glycerol gradients for human SS cell lines HSSYII and ASKA, as well as control HEK293T cells.

D. WBs for reciprocal IP in fusion-only and combination genotype mouse tumors for PBAF components SMARCB1 and PBRM1.

E. WBs for CBAF component IP in fusion only and control (EA1 = EWSR1-ATF1-induced mouse tumor) tumors.

F. Optical densitometry-quantified gradients of SMARCB1 and PBRM1 depict overlap among the glycerol gradient fractions in two human SS cell lines and a mouse SS tumor.

G. Quantified fraction densities of each protein compared to the mean density of PBAF core fractions 19 and 20 of itself (2-tailed t-test p-values listed above, the three sample sources are HSSYII, ASKA, and mouse SS tumor gradients)
H. WBs for BAF-family components after application of scrambled versus
\textit{SMARC}B1-targeting short interfering RNA (\textit{siSCR} and \textit{siSMARC}B1)

I. Optical densitometry-quantified gradients of SMARCC1 demonstrate shifts
in the relative abundance of PBAF-sized complexes with added disruption
of SMARCB1 in the ASKA cell line or mouse tumors.

\textbf{Figure 5. SMARC}B1 \textbf{associates with SS18-SSX in CBAF complexes}

A. Optical densitometry-quantified glycerol gradients of SS18-SSX and
SMARC}B1 in two human SS cell lines, as well as tumors from fusion-only
and combination genotype mice.

B. WBs for reciprocal IP of SS18 and SMARC}B1, as well as other BAF
components in fusion-only and combination genotype mouse tumors.

C. HEK293 cells transfected with HA-SS18, HA-SS18-SSX, V5-SS18, or V5-
SS18-SSX, followed by tag-IP or SMARC}B1-IP and WB for each.
(Additional contrast-adjusted images demonstrate the clearer presence of
auto-IPed SMARC}B1.)

D. WB for BAF components after elution following IP-HA for SS18-SSX in
HA-tagged HSSYII

E. WBs for SS18 and SMARC}B1 in glycerol gradient fractions (only 20 total
for glycerol 10 to 30\%) for HA-purified complexes

\textbf{Figure 6. Purified mammalian recombinant CBAFs co-incorporate SMARC}B1 with
SS18-SSX.
A. Schematic of BAF-family complex subtypes and their varied componentry in SS. (L1 and L2 refer to the lobes by which SMARCA2/4 translocates DNA around the nucleosome.)

B. The relative abundance of BAF subtypes determined by SMARCC1 distribution in gradients as a product of GBAF, CBAF, and PBAF contributions to each fraction.

C. The fractional abundances of BAF subtypes calculated by optical densitometry-quantified gradients of SMARCC1 overlaid onto the normal distributions of PBAF, CBAF, and GBAF defined by PBRM1, DPF2, and BRD9, respectively, in SS and control cell lines (CRC = colorectal carcinoma; RCC = renal cell carcinoma. (p-values at top are the comparison of each cell line to HEK293T for CBAF fractional abundance, by 2-tailed heteroscedastic t-test).

D. Schematic of canonical BAF components in the variant complexes, demonstrating which components were overexpressed in a mammalian cell-based recombinant system.

E. Coomassie stain of a denaturing polyacrylamide gel electrophoresis of FLAG-purified recombinant CBAF complexes generated with or without the co-expression of SMARCB1. (HSPA* represents the substoichiometric presence of a heat shock protein chaperone.)

F. WBs of purified complexes with antibodies against each overexpressed component in either SMARCB1-including or excluding variants.
G. Coomassie stain of a denaturing gel of FLAG-purified recombinant CBAF complexes generated from expression of SS18 or SS18-SSX.

H. WBs of BAF components in input (FLAG-purified complexes), supernatant, and anti-V5 immunoprecipitation (IP) of complexes from co-expression of V5-tagged SS18 or SS18-SSX.

Figure 7. Expression of SS18-SSX leads to CBAF complex reductions and relative overabundance of PBAFs and GBAFs.

A. Quantitative LICOR WBs of BAF components in nuclear extracts of EXPI293 cells transfected with either SS18-SSX or SS18 (n = 5 each) presenting the log transformed 2-tailed Student’s t-test p-value of the difference between and the ratio of protein in the fusion-transfected versus SS18-transfected cells.

B. WBs of glycerol gradient fractions of EXPI293T cells transfected with SS18-SSX or SS18.

C. LICOR quantitative WBs from whole cell lysates (WCL) collected from HSSYII human SS cells subjected to 7 days of shRNAs (2 sequences each, n = 5 for each sequence of each shRNA) directed against control (Renilla, shRen) or the fusion (SS18-SSX, shSSX), with BAF subunits color-coded by BAF, presented as log-transformed paired 2-tailed t-test p-values and ratios of fusion knock-down over control knock-down.

D. LICOR quantitative WBs of WCLs from SYO-1 human SS cells after knock-down of the fusion or control for 7 days.

E. Fractional abundances of BAF subtypes defined by optical densitometry-quantified gradients of SMARCC1 (as in 6C) for HSSYII cells subjected to
shRNAs against the fusion or control. (p-values from 2-tailed paired t-tests, n = 4 for each shRNA).

F. WBs of nuclear extract (NE) with the paired chromatin fraction (CF, protein that stays with the insoluble chromatin pellet after NE) of proteins after 7 days of fusion or control knock-down.

G. LICOR quantitative WB abundances presented as paired t-test p-values and ratios of fusion over control knock-down in each of the NE and CF components of HSSYII cells after 7 days.

H. LICOR quantitative WB-defined proteins in WCLs presented as the ratios of MG132-treated over DMSO vehicle-treated (veh) cells after week long shRNA depletion of SS18-SSX or control Renilla. (n = 5 for each condition, n = 10 for each group, 2-tailed heteroscedastic t-test comparing the ratios for each protein by knock-down group)

I. LICOR quantitative WB-defined proteins in WCLs presented as of the ratios of MG132-treated over DMSO vehicle-treated (veh) cells from SS (or SS18-SSX-transfected) and control (or SS18-transfected) cell lines (n = 5 each, p-values are from 2-tailed Student’s t-tests comparing each to HEK293T untransfected control cells).

J. Model schematic of the impact of SS18-SSX expression on BAF componentry and relative abundance of BAF subtypes.
Figure 1

A

TATCre

protein injection at age 4 wks.

Smarb1

B

recombination

Myf5Cre,

embryonic
day 11.5

nSS2

Smarb1

C

nSS2/SS2

nSS2/+) (n=9)

no tumor

G

gene expression

D

tumor-free fraction

E

tumor-volume fraction

F

tumor-volume fraction

H

Smadb1-only

nSS2-only

combination

Smadb1

nSS2

combination
Figure 2

A

B

C

D

pathways gene set enrichments for cluster 1 genes

neuronal system
axon guidance
transmission across chemical synapses
semaphorin interactions
deactivation of β-catenin transactivating complex
regulation of FZD by ubiquitination
presynaptic depolarization and Ca²⁺-channel opening
SEMA3A-plexin repulsion signaling by inhibiting integrin adhesion

E

F

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Figure 3

A) hSS2-only tumors, Smarcb1⁻/⁻ tumors, and combination genotype.

B) Promoter, 5' UTR, 3' UTR, and exon regions.

C) hSS2-only, Smarcb1⁻/⁻-only, and combination prevalence.

E) Promoter and distal targets.

F) hSS2-induced tumors, Smarcb1-loss tumors, and combination genotype tumors.

G) Promoter, 5' UTR, 3' UTR, and exon regions.

H) PBRM1, hSS2, and combination enrichment.

I) SMARCC1, SMARCA4, PBRM1, and RNAPOLII.
Figure 6

A

B

C

D

E

F

G

H

Legend:

\[ \text{= 3XFLAG tag} \]

\[ \text{= V5 tag} \]
A role for SMARCB1 in synovial sarcomagenesis reveals that SS18-SSX induces canonical BAF destruction

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